

FORMULATION, EVALUATION AND CHARACTERIZATION OF GLIPIZIDE NANOEMULSION

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ABSTRACT

The aim of the present study was to formulate, evaluate and characterize the nanoemulsion formulation of Glipizide. Glipizide is a second generation sulphonyl urea drug used in the treatment of noninsulin dependent diabetes mellitus. It has less solubility in water and the half life of the drug is 2-4hrs. Hence by formulating Glipizide nanoemulsion the drug release will be sustained thus dosing intervals will be decreased and it eliminates the variations in the absorption. Solubility studies were conducted to select the oil, surfactant and cosurfactant. Phase diagrams were constructed by aqueous phase titration method. Formulations were selected from the phase diagrams. The prepared nanoemulsions were subjected to different thermodynamic stability tests. The results showed that all the formulations had a good stability. Based on the in vitro drug release studies the formulations were optimized. The optimized formulations were successful in sustaining the drug release for 12hrs. The optimized formulation F9 containing Capryol 90 31.5%, Tween 20 15.76%, Transcutol P 32.46% and water 21.0%) optimized formulation F29 containing Capryol 90 30.57%, Tween 80 11.83%, Transcutol P 35.51% and water 22.10% showed more than 85% of drug release in 12 hrs. The formulations were evaluated for viscosity, pH, percentage transmittance and phase separation. The formulations were also characterized for zeta potential, particle size. The droplet size of the optimized formulation (F9) was found to be 41.6nm and zeta potential was found to be -24.4mV. Pharmacodynamic studies showed that the optimized formulation (F9) reduced blood glucose levels up to 12 hrs.

Keywords: Nanoemulsion, Glipizide, Solubility studies, Phase diagrams, Pharmacodynamic studies.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration (hyperglycemia) caused by insulin deficiency and it is often combined with insulin resistance. Non Insulin Dependent Diabetes Mellitus (NIDDM) represents a heterogeneous group comprising milder form of diabetes that occurs predominantly in adults and vast majority of diabetic patients have non insulin dependent diabetes mellitus.

Glipizide, 1-cyclohexyl -3- [[4- [2- [[(5-methylpyrazin-2-yl) carbonyl] amino] ethyl] -phenyl] sulphonyl] urea, is a potential second generation oral hypoglycemic agent widely used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) by stimulating insulin release from pancreatic β -cells.

Glipizide is an oral hypoglycemic agent, commonly prescribed drug for the treatment of patients with type II diabetes mellitus. Glipizide is a class II drug, it has low solubility and high permeability and the biological half life of Glipizide is 2-4hrs. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm.³ The nanosized droplets leading to enormous interfacial areas associated with nanoemulsions would influence the transport properties of the drug, an important factor in sustained and targeted drug delivery. The attraction of formulating nanoemulsion systems lies in their ability to incorporate hydrophobic drugs into the oil phase thereby enhancing their solubility. The normal dose is 2.5 to 10 mg twice or thrice daily² and frequently prescribed dose is 2.5 mg. For the present study 2.5 mg dose was selected for the development of nanoemulsion formulation. The objective of the present study was to formulate, evaluate and characterize the glipizide nanoemulsion to sustain the drug release, thus decreasing the dosing intervals.

MATERIALS AND METHODS

Materials

Glipizide was a gift sample from Sri Krishna Labs (Hyderabad, India). Diethylene glycol monoethyl ether (Transcutol P) and propylene glycol monocaprylate (Capryol 90) were gift samples from Gattefosse (Cedex, France). Isopropyl myristate, Oleic acid, Propylene glycol,

Tween 20, Tween 80, PEG 400 and Glycerol were purchased from SD fine chemicals Pvt. Ltd., (Mumbai).

Solubility studies

The solubility of Glipizide in various oils (Capryol 90, Isopropyl myristate, Oleic acid, Olive oil, Sunflower oil and Linseed oil), surfactants (Tween 20 and Tween 80) and cosurfactants (Transcutol P, Propylene glycol, PEG 400 and Glycerol) was determined by adding an excess amount of drug in oils, surfactants and cosurfactants separately in stopper vials, and mixed using a cyclic mixer. The mixture vials were then kept at $25 \pm 1.0^\circ\text{C}$ in an Orbital shaker for 72 h to reach equilibrium. The samples were removed after achieving equilibrium and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a $0.45\text{-}\mu\text{m}$ membrane filter. The filtrate was solubilized in suitable solvent, diluted with the pH 7.4 buffer and the concentration of Glipizide was determined using UV-Visible spectrophotometer at 275nm.

Construction of phase diagrams

The pseudo ternary phase diagrams were constructed by aqueous titration method using Capryol 90 as oil, Tween 20 and Tween 80 as surfactants and Transcutol P and Propylene glycol as cosurfactants. Surfactant and cosurfactant (Smix) were mixed in different weight ratios (1:1, 1:2 and 1:3). These Smix ratios were chosen in increasing concentration of cosurfactant with respect to surfactant for study of the phase diagrams needed for nanoemulsion formation. For each phase diagram, oil and Smix were combined in different weight ratios from 1:9 to 9:1 in different glass vials. Slow titration with the aqueous phase was done to each weight ratio of oil and Smix, and visual observations were made for transparent and easily flowable nanoemulsions. The pseudo ternary phase diagrams were constructed using Triplot V4 software (4.1.2. version).

Thermodynamic Stability Studies

Selected formulations were subjected to different thermodynamic stability tests to assess their physical stability.

1. Heating-cooling cycle: Six cycles between refrigerator temperature (4°C) and 45°C with storage at each temperature of not less than 48 h were conducted, and the formulations were examined for stability at these temperatures.

2. Centrifugation test: Formulations were centrifuged at 3500 rpm for 30 min, and examined for phase separation.

3. Freeze-thaw cycle: The formulations were subjected to freeze-thaw cycles between -21°C and +25°C and observed for any phase separation.

Preparation of nanoemulsions

Nanoemulsions were prepared by aqueous phase titration method.^{3,4,7} The composition of the nanoemulsions was chosen according to the pseudo ternary phase diagram. The drug was dissolved in the oil, surfactant and cosurfactant mixture was added in the chosen concentration, and water was added drop wise with continuous stirring until clear nanoemulsion was formed.

Characterization of nanoemulsions

Particle size and zeta potential measurement

The formulation (0.1 ml) was dispersed in 50 ml of water in volumetric flask and gently mixed by inverting the flask. Globule size and zeta potential of the nanoemulsion was determined by particle size analyzer (Horiba) that analyzes the fluctuations in light scattering due to Brownian motion of the particles. Light scattering was monitored at 25 °C at a 90° angle.

Percent Transmittance

The percent transmittance of the nanoemulsion was measured using UV-Visible double beam spectrophotometer keeping distilled water as blank at 560nm.

Viscosity

Viscosity of the samples was measured as such without dilution using Brookfield viscometer LVDV-II+P fitted with an S-34 spindle at 25°C. A sample volume of 10ml was used. The nanoemulsion formulations were subjected to different rpm (5, 10, 20, 30, 50, 60 and 100) and the rheological behavior of the disperse system was examined by constructing rheograms of shear stress vs. shear rate.

In vitro drug release studies

The in vitro drug release of Glipizide from the nanoemulsion formulation was determined by dialysis bag method. 0.1N HCl and pH 7.4 buffer were used as medium for in vitro release studies. 1ml of formulation was placed in the dialysis bag (single dose containing 2.5mg of Glipizide), which was immersed in 50ml of 0.1 N HCl for 2hrs and replaced with pH 7.4 buffer maintained at 37°C and stirred with a magnetic stirrer. Samples were withdrawn at predetermined time intervals. In order to maintain sink conditions, an equal volume of medium was replaced. The samples were analyzed by the UV-Visible spectrophotometer at 275nm to determine the concentration.

Drug - Excipient compatibility studies

Fourier transform infrared analysis (SHIMADZU) was conducted to study the drug excipient interactions. Samples were scanned in the range from 400-4000cm⁻¹.

In vivo studies

Induction of diabetes

Hyperglycemia was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) solution (50 mg/kg in acetate buffer 0.1 M, pH 4.5) to overnight fasted rats. Diabetes was identified by measuring non-fasting plasma glucose levels 48 h after injection of streptozotocin. Animals, which did not develop more than 250 mg/dl glucose levels, were rejected.

Experimental procedure

Wistar albino Rats weighing 180-200gm, fasted over night were used for induction of diabetes. They were housed and maintained in the animal house at room temperature (27°C) and 75% relative humidity. The animals were allowed free access to food and water. In order to perform the pharmacodynamic studies animals were divided into 4 groups. (n=3 per group).

GROUP I - Untreated control group.

GROUP II - Untreated diabetic control group.

GROUP III - Treated with marketed formulation (Semiglyname).

GROUP IV - Treated with optimized formulation (F9).

The optimized nanoemulsion formulation (F9) the gavage tube, the blood drop was collected from the retroorbital plexus using a capillary needle at predetermined time intervals after oral administration. The blood glucose levels were then determined by glucometer (Contour TS). The IAEC approved the protocol for this study bearing number SVCP/IAEC/2012/007.

RESULTS AND DISCUSSION

Solubility studies

The most important criterion for screening of excipients is the solubility of the poorly soluble drug in oil, surfactants, and cosurfactants. The solubility of Glipizide in different oils/surfactants/cosurfactants was determined. As shown in table 1, the solubility of Glipizide was found to be highest in Capryol90 as compared to other oils. Hence, Capryol90 was selected as the oil phase. High drug solubility was found in Tween20 and Tween80 among surfactants and in Transcutol P and Propylene glycol among cosurfactants. Therefore, Tween-80 and Tween20 were selected as surfactants and Transcutol P and Propylene glycol were selected as cosurfactants, respectively, for the phase study.

TABLE 1. SHOWS SOLUBILITY OF GLIPIZIDE IN OILS, SURFACTANTS AND COSURFACTANTS
Mean±SD(n=3)

Oil	Solubility (mg/ml)	Surfactant	Solubility (mg/ml)	Cosurfactant	Solubility (mg/ml)
Capryol 90	40.24±2.14	Tween 20	35.69±1.79	Transcutol P	80.04±2.09
IPM	35.06±1.75	Tween 80	32.18±2.31	PG	29.30±1.89
Oleic acid	32.18±0.98			PEG 400	25.01±2.68
Arachis oil	30.00±2.34			Glycerol	11.34±0.87
Sunflower oil	27.45±1.95				
Linseed oil	21.08±2.86				
Alotic oil	15.30±2.07				
Olive oil	23.65±1.56				

IPM-Isopropyl myristate; PG-Propylene glycol

Construction of phase diagrams:

Pseudo ternary phase diagrams were constructed for 1:1, 1:2 and 1:3 surfactant to cosurfactant ratios. So that nanoemulsion regions could be identified. In construction of phase diagrams Capryol 90 was used as oil, Tween 20 and Tween 80 were used as surfactants and Transcutol P was used as cosurfactant.

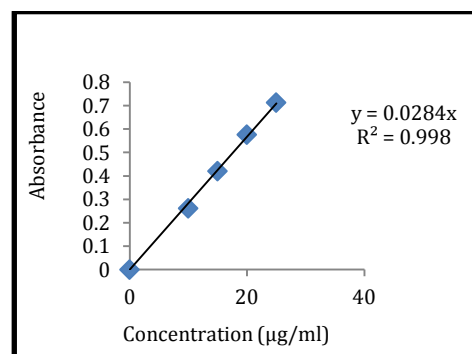


FIG 1: IT SHOWS STANDARD GRAPH OF GLIPIZIDE IN pH 7.4 BUFFER

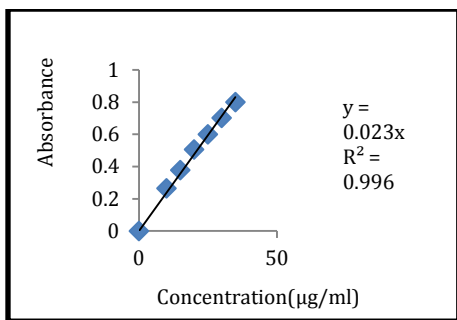


FIGURE 2: IT SHOWS STANDARD GRAPH OF GLIPIZIDE IN 0.1N HCL

Combination I

In combination I (FIG 3, 4, 5) Capryol 90 was used as oil, Tween 20 was used as surfactant and Transcutol P was used as cosurfactant. When the Smix ratio was 1:1, the maximum amount of oil that could be emulsified was found to be 60% (w/w) using 33% (w/w) of Smix. When the proportion of cosurfactant was doubled (Smix 1:2) the nanoemulsion region decreased slightly when compared to Smix 1:1 and the maximum amount of oil that could be emulsified was found to be 64% (w/w) using 38% (w/w) of Smix.

When the proportion of Smix was increased by three times (Smix 1:3) the nanoemulsion region was further decreased and the maximum amount of that could be emulsified was found to be 58% (w/w) using 32% (w/w) of Smix.

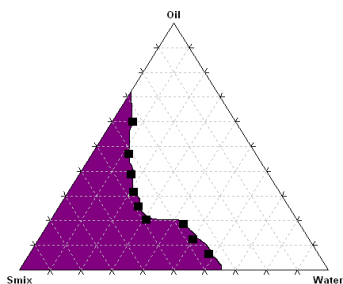


FIGURE 3. IT SHOWS PSEUDO TERNARY PHASE DIAGRAM OF CAPRYOL 90, TWEEN 20, TRANSCUTOL P AND WATER (Smix 1:1)

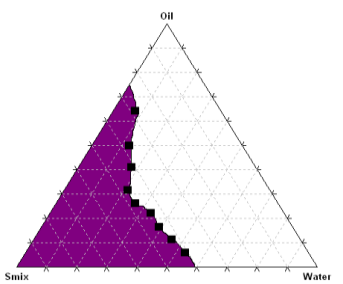


FIGURE4: IT SHOWS PSEUDO TERNARY PHASE DIAGRAM OFCAPRYOL 90, TWEEN 20, TRANSCUTOL P AND WATER (Smix 1:2)

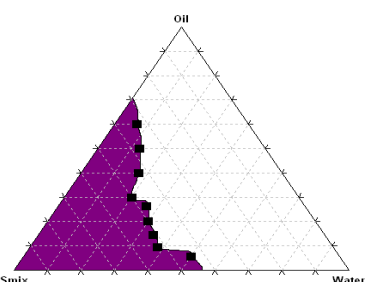


FIGURE 5: IT SHOWS PSEUDO TERNARY PHASE DIAGRAM OFCAPRYOL 90, TWEEN 20, TRANSCUTOL P AND WATER (Smix 1:3)

Combination II

In combination II (FIG 6, 7, 8) Capryol 90 was used as oil, Tween 80 was used as surfactant and Transcutol P was used as cosurfactant. When the Smix ratio was 1:1, the maximum amount of oil that could be emulsified was found to be 59% (w/w) using 33% (w/w) of Smix. When the proportion of cosurfactant was doubled (Smix 1:2) the nanoemulsion region was increased when compared to Smix 1:1 and the maximum amount of oil that could be emulsified was found to be 63% (w/w) using 30% (w/w) of Smix.

When the proportion of Smix was increased by three times (Smix 1:3) the nanoemulsion region decreased when compared to Smix 1:2 and the maximum amount that could be emulsified was found to be 62% (w/w) using 28% (w/w) of Smix. From pseudo ternary phase diagrams, the formulations in which the amount of oil phase completely solubilized the drug and which could accommodate the optimum quantity of Smix and distilled water were selected for the study.

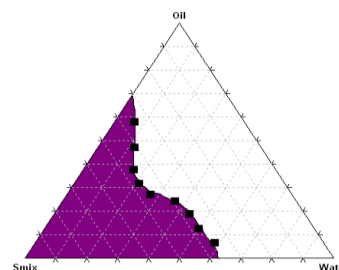


FIGURE 6: IT SHOWS PSEUDO TERNARY PHASE DIAGRAM OF CAPRYOL 90, TWEEN 80, TRANSCUTOL P AND WATER (SMIX 1:1)

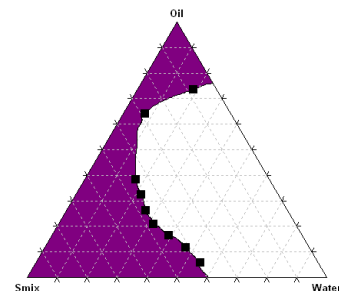


FIGURE 7: IT SHOWS PSEUDO TERNARY PHASE DIAGRAM OFCAPRYOL 90, TWEEN 80, TRANSCUTOL P AND WATER (Smix 1:2)

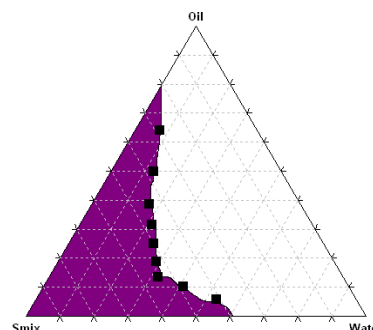


FIGURE 8:IT SHOWS PSEUDO TERNARY PHASE DIAGRAM OFCAPRYOL 90, TWEEN 80, TRANSCUTOL P AND WATER (Smix 1:3)

Thermodynamic Stability Studies

Nanoemulsions are thermodynamically stable and are formed at a particular concentration of oil, surfactant and water, making them stable to phase separation; creaming or cracking. The selected nanoemulsion formulations were subjected to various thermodynamic stability tests, which included heating-cooling cycle,

centrifugation, and freeze–thaw cycle tests. It is the thermostability which differentiates nanoemulsion from emulsions that have kinetic stability and will eventually phase separate. The results showed that all the formulations had a good physical stability.

Globular size analysis:

The globule size analysis of the optimized formulation F9 was done using particle size analyzer (Horiba). The mean globule size was found to be 41.6nm. The particle size distribution of optimized formulation F9 is shown in fig 18.

Determination of zeta potential

Zeta potential of the optimized formulation F9 was determined using particle size analyzer (Horiba). Zeta potential of optimized formulation was found to be -24.4mV. The zeta potential of the optimized formulation (F9) is shown in fig 19.

Viscosity

Viscosity of optimized formulations was measured at different rpm (5, 10, 20, 30, 50, 60 and 100) and at constant temperature of 32°C. This measurement gave viscosity, shear stress and shear rate values. Viscosity of the optimized formulation F9 was found to be 39 cps and formulation F29 was found to be 40cps at 100rpm. Low viscosity is one of the characteristic of the nanoemulsions. The correlation between shear stress and shear rate defining the flow behavior of the optimized nanoemulsions (F9 and F29) is shown in fig 16 and 17. Flow curves of optimized formulations F9 and F29 were straight. So from the results it can be concluded that formulations F9 and F29 were Newtonian liquids.

Percentage transmittance

The percent transmittance of the all formulations was measured at 560nm keeping distilled water as a blank. The percentage transmittance of the optimized formulation F9 was found to be 93.4% and formulation F29 was found to be 93.2%. The results of percentage transmittance revealed that all the formulations were nearly transparent.

In vitro drug release studies

Drug release studies were performed for the nanoemulsions using dialysis bag method. Results of Glipizide release from nanoemulsion containing capryol 90(oil), tween 20(surfactant) and transcuto P(cosurfactant) [Smix(1:1), Smix(1:2), Smix(1:3)] are presented in Fig 9,10,11. Drug release from nanoemulsions containing capryol 90), tween 20 and transcuto P(Smix 1:1) was lower than that from the nanoemulsions containing [Smix(1:2), Smix(1:3)]. Drug release was more for the nanoemulsion with Smix 1:2 ratio. The comparison of the release profile of nanoemulsions F1, F2, F3, F4 and F5 containing the same oil (capryol 90), surfactant(tween 20), cosurfactant (transcuto P) with Smix 1:1 with different quantities reveals that the release profile of the nanoemulsion follows the order: F4>F5>F3>F2>F1, the release profile of the nanoemulsion with Smix 1:2 follows the order: F9>F10>F8>F7>F6 and the release profile of the nanoemulsion with Smix 1:3 follows the order: F14>F13>F15>F12>F11. Results of Glipizide release from nanoemulsion containing capryol 90(oil), tween 80(surfactant) and transcuto P(cosurfactant) [Smix(1:1), Smix(1:2), Smix(1:3)] are presented in Fig 12,13,14.

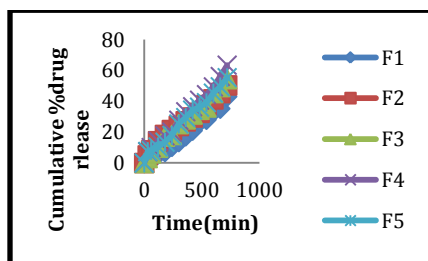


FIGURE 9: IT SHOWS IN VITRO DRUG RELEASE PROFILE OF FORMULATIONS F1-F5

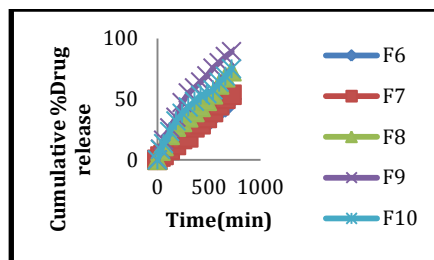


FIGURE 10: IT SHOWS IN VITRO DRUG RELEASE PROFILE OF FORMULATIONS F6-F10

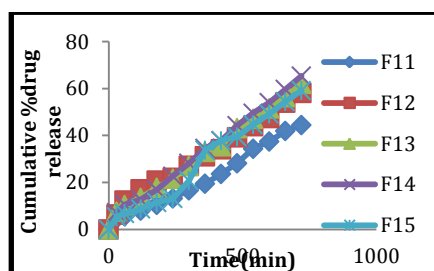


FIGURE 11: IT SHOWS IN VITRO DRUG RELEASE PROFILE OF FORMULATIONS F11-F15

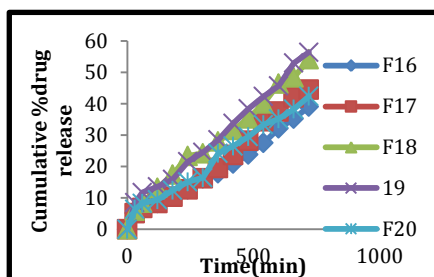


FIGURE 12: IT SHOWS IN VITRO DRUG RELEASE PROFILE OF FORMULATIONS F16-F20

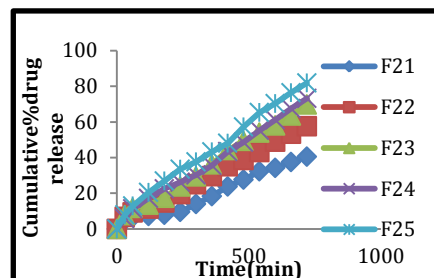


FIGURE 13: IT SHOWS IN VITRO DRUG RELEASE PROFILE OF FORMULATIONS F21-F25

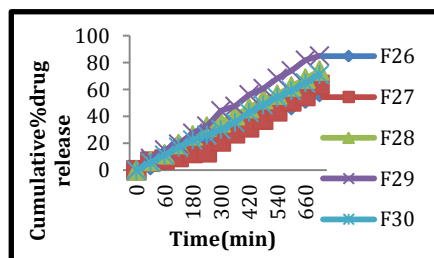


FIGURE 14: IT SHOWS IN VITRO DRUG RELEASE PROFILE OF FORMULATIONS F26-F30

Drug release from nanoemulsions containing capryol 90), tween 80 and transcuto P(Smix 1:1) was lower than that from the nanoemulsions containing [Smix(1:2), Smix(1:3)]. Drug release was more for the nanoemulsion with Smix 1:3 ratio. The comparison of

the release profile of nanoemulsions F16, F17, F18, F19 and F20 containing the same oil (capryol 90), surfactant(tween 80), cosurfactant (transcutol P) with Smix 1:1 with different quantities reveals that the release profile of the nanoemulsion follows the order: F19>F18>F17>F20>F16, the release profile of the nanoemulsion with Smix 1:2 follows the order: F21>F22>F23>F24>F25 and the release profile of the nanoemulsion with Smix 1:3 follows the order: F14>F15>F13>F12>F11.

The formulations F9 and F29 were optimized based on the drug release studies. The formulation F9 containing oil 31.5%, surfactant 15.76%, cosurfactant 32.46% and water 21.0% showed 89.20% drug release and the formulation F29 containing oil 30.57%, surfactant 11.83%, cosurfactant 35.51% and water 22.10% showed 85.05% drug release in 12 hrs. It was observed that the drug release was decreased with an increasing amount of oil. The oil content of the formulations F9 and F29 was less and Smix ratios was 1:2 and 1:3. Hence maximum amount of the drug release was observed for the nanoemulsion formulations F9 and F29. The drug release curves (Fig 15) reveal that Glipizide nanoemulsions (F9 and F29) exhibit satisfactory sustained drug release behavior compared with that of the market product.

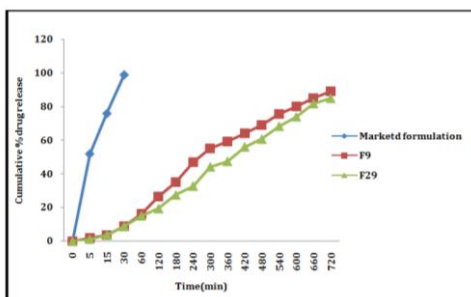


FIGURE 15: IT SHOWS COMPARATIVE DRUG RELEASE PROFILE OF OPTIMIZED FORMULATIONS F9, F29 AND MARKETED FORMULATION (SEMIGLYNAE TABLET)

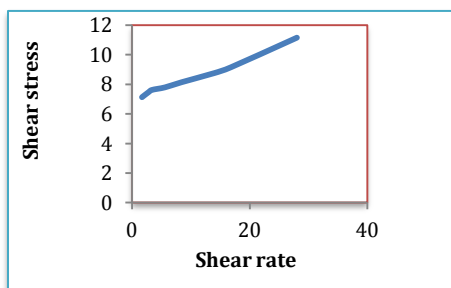


FIGURE 16: SHEAR RATE VS SHEAR STRESS PROFILE OF OPTIMIZED FORMULATION F9

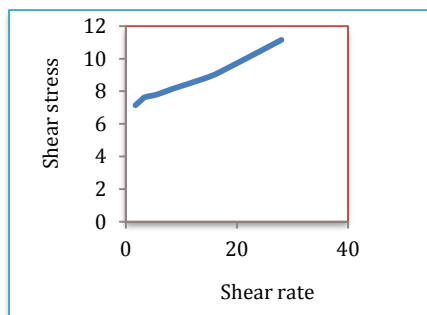


FIGURE 17: SHEAR RATE VS SHEAR STRESS PROFILE OF OPTIMIZED FORMULATION F9

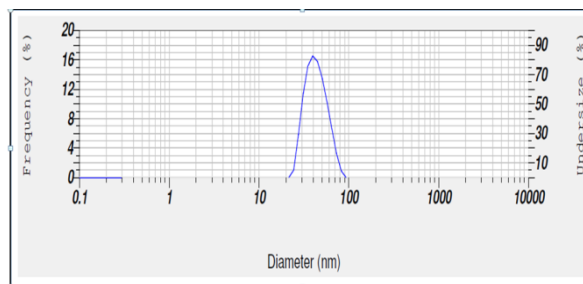


FIGURE 18: IT SHOWS PARTICLE SIZE DISTRIBUTION GRAPH OF OPTIMIZED FORMULATION F9

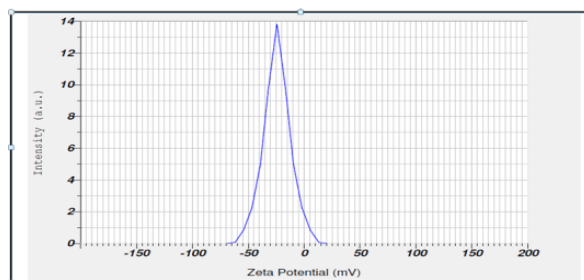


FIGURE 19: IT SHOWS ZETA POTENTIAL OF OPTIMIZED FORMULATION F9

Drug - Excipient compatibility studies

The FTIR spectra of drug and optimized formulations were recorded by FTIR spectrophotometer. The IR spectrum of the pure Glipizide, optimized formulation F9 and F29 recorded by FTIR spectrometer has shown in fig 20,21 and 22. The absorption peaks of Glipizide were found at 3325.39 cm⁻¹ (N-H), 2854.74 cm⁻¹ (C-H), 1716.70 cm⁻¹ (C=O), 1247.99 cm⁻¹ (C-N) and 1651.12 cm⁻¹ (C=C). The characteristic peaks of the optimized formulations followed the same trajectory as that of the drug alone with minor differences. Thus there were no drug-excipient interactions.

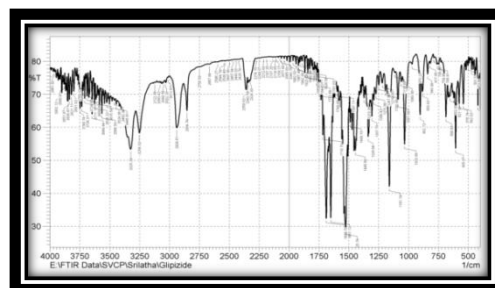


FIGURE 20: IT SHOWS FTIR SPECTRA OF GLIPIZIDE

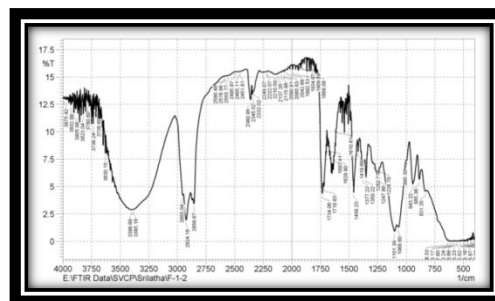


FIGURE 21: IT SHOWS FTIR SPECTRA OF FORMULATION F9

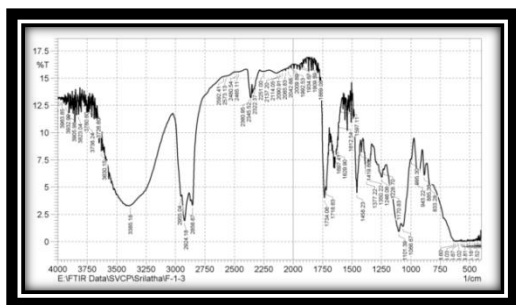


FIGURE 22: IT SHOWS FTIR SPECTRA OF FORMULATION F29

In vivo studies

Pharmacodynamic studies were conducted in male wistar rats. A previously optimized streptazotisin dose of 45mg/kg was successfully induced diabetes in rats. Mean blood glucose levels in non-diabetic control group varied from 73 to 76 mg/dl. In diabetic control group, blood glucose levels varied from 307 to 314 mg/dl. The percentage reduction in blood glucose levels in marketed formulation was observed up to 4hr only. There was an increase in blood glucose levels after 4 hrs. Whereas the optimized formulation (F9) showed reduced blood glucose levels up to 12 hrs. The blood glucose levels in all the groups are shown fig 23.

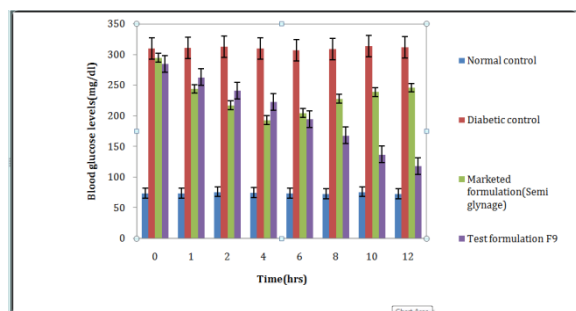


FIGURE 23: IT SHOWS BLOOD GLUCOSE LEVELS IN NORMAL CONTROL GROUP, DIABETIC INDUCED GROUP, MARKETED FORMULATION AND OPTIMIZED FORMULATION (F9) ADMINISTERED GROUP

CONCLUSION

On the basis of the in vitro drug release studies the formulation F9, which contained Capryol90 31.5%, Tween 20 15.76%, Transcutol P 32.46% and water 21.0% was optimized. The optimized formulation showed low particle size, low viscosity and high percentage transmittance. The in vivo studies revealed a decreasing blood glucose levels up to 12hrs as compared to marketed formulation. From in vitro and in vivo data it can be concluded that optimized nanoemulsions can be successfully formulated for sustained delivery in the effective treatment of diabetes.

ACKNOWLEDGMENT

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REFERENCES

- 1.V.Devarajan and V.Ravichandran. Nanoemulsions: as modified drug delivery tool. International journal of comprehensive pharmacy, 2011; 4.
2. Gorus FK, Schuit FC, Intveld PA, Interaction of sulfonylureas with pancreatic beta cells-A study with Glipizide. Diabetes, 1988; 37; 1090-1095.
3. Nithin shama, Mayank bansal, Sharad visht, PK. Sharma, GT. Kulkarni. Nanoemulsion: A new concept of delivery system. Chronicles of young scientists, 2010; 2: 2-6.

4. Sheikh Shafiq, Faiyaz Shakeel, Sushma Talegaonkar, Farhan J. Ahmad, Roop K. Khar, Mushir Ali. Development and bioavailability assessment of ramipril nanoemulsion formulation. European Journal of Pharmaceutics and Biopharmaceutics, 2007; 66: 227-243.
5. Vikas Bali, Mushir Ali, Javed Ali. Study of surfactant combinations and development of a novel nanoemulsion for minimizing variations in bioavailability of ezetimibe. Colloids and Surfaces B: Biointerfaces, 2010; 76: 410-420.
6. Adnan Azeem, Farhan J. Ahmad, Roop K. Khar and Sushama Talegaonkar, Nanocarrier for the Transdermal Delivery of an Antiparkinsonian Drug. AAPS PharmSciTech, 2009;10.
7. Regina G. Kelmann, Gislaïne Kuminek, Helder F. Teixeira, Let'cia S. Koester. Carbamazepine parenteral nanoemulsions prepared by spontaneous emulsification process. International Journal of Pharmaceutics, 2007; 342: 231-239.
8. Faiyaz Shakeel, Sanjula Baboota, Alka Ahuja, Javed Ali, Mohammed Aqil, and Sheikh Shafiq. Nanoemulsion as Vehicles for Transdermal Delivery of Aceclofenac. AAPS PharmSciTech, 2007; 8.
9. Sanjula Baboota, Faiyaz Shakeel, Alka Ahuj Javed Ali, Sheikh Shafiq. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta. Pharm., 2007; 57: 315-332.
10. Hussein O. Ammar, H. A. Salama, M. Ghorab, and A. A. Mahmoud. Nanoemulsion as a Potential Ophthalmic Delivery System for Dorzolamide Hydrochloride. AAPS PharmSciTech, 2009; 10.
11. P. Schalbart, M. Kawaji, K. Fumoto. Formation of tetradecane nanoemulsion by low-energy emulsification methods, International journal of refrigeration, 2010; 33: 1612-1624.
12. Sheikh Shafiq and Faiyaz Shakeel. Enhanced Stability of Ramipril in Nanoemulsion Containing Cremophor-EL: A Technical Note. AAPS PharmSciTech, 2008; 9.
13. Sheikh Shafiq-un-Nabi, Faiyaz Shakeel, Sushma Talegaonkar, Javed Ali, Sanjula Baboota, Alka Ahuja, Roop K. Khar et al. Formulation Development and Optimization Using Nanoemulsion Technique: A Technical Note. AAPS PharmSciTech, 2007; 8.
14. Poluri Koteswari, Sistla Rama Krishna, Veerareddy, Prabhakar Reddy, Lakshmi M Narasu. Formulation and preparation of felodipine nanoemulsions. Asian journal of pharmaceutical and clinical research, 2011; 4 Suppl 1.
15. Narasimhareddy D, Srinath MS, Sudharani MV. Glipizide and Valdecoxib combination mucoadhesive tablets: Formulation, in vitro and in vivo evaluation. International journal of institutional pharmacy and life sciences, 2012; 2.
16. Adolfo Andrade Cetto, Helmut Wiedenfeld, Ma Cristina Revilla, Islas A. Sergio. Hypoglycaemic effect of Equisetum myriochaetum aerial parts on streptozotocin diabetic rats. Journal of Ethnopharmacology, 2000; 72: 129-133.
17. Kanokporn Burapapadha, Mont Kumpugdee-Vollrath, Doungdaw Chantasarnd, Pornsak Sriamornsak. Fabrication of pectin-based nanoemulsions loaded with itraconazole for pharmaceutical application. Carbohydrate Polymers, 2010; 82: 384-393.
18. Yomesh Patel, Aditi Poddar, Krutika Sawant. Formulation and characterization of cefuroxime axetil nanoemulsion for improved bioavailability Pharm Bioall Sci, 2012; 4: 4-5.