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FORMULATION AND EVALUATION OF GLIBENCLAMIDE NANO EMULSION

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ABSTRACT

Drug molecules are carried by nano emulsions, a colloidal particulate system in the submicron range. The Nano sized droplets, which would result in huge interfacial areas due to the presence of nanoemulsions, would have an effect on the transport characteristics of the drug, which is an essential component of sustained drug delivery. Glibenclamide is a type of hypoglycemic medication that may be taken orally and is a drug that is frequently recommended for the treatment of individuals who have diabetes type II. Nanoemulsions are created using surfactants that are fit for human consumption as well as common food ingredients that have been given the GRAS (Generally Recognized as Safe) designation by the Food and Drug Administration.

Key words: Nano emulsion, Phase diagram, Diabetes Mellitus, Glibenclamide.

INTRODUCTION

Nanoemulsions are transparent dispersions of oil and water that are thermodynamically stable and are stabilized by an interfacial coating of surfactant and co-surfactant molecules. The droplet size of a nanoemulsion is less than 100 nanometers. The Nano sized droplets, which would result in huge interfacial areas due to the presence of nanoemulsions, would have an effect on the transport characteristics of the drug, which is an essential component of sustained drug delivery [1]. The capacity of nanoemulsion systems to incorporate hydrophobic pharmaceuticals into the oil phase, hence increasing the solubility of these drugs, is one of the reasons why their formulation is so appealing.

Nanoemulsions are created using surfactants that are fit for human consumption as well as common food ingredients that have been given the GRAS (Generally Recognized as Safe) designation by the Food and Drug Administration. Compared to creams and ointments, nanoemulsions are more thermodynamically.
stable, and also have a malleable nanostructure that allows them to penetrate the skin more easily [2].

The use of nanoemulsions in a transdermal application has a number of potential benefits, including improved drug solubility, improved thermodynamic stability, and an expanded capacity for transdermal application [3]. The nanoemulsions improve the system's suitability for transdermal distribution by increasing the concentration gradient and the thermodynamic activity toward the skin. Additionally, the nanoemulsions enhance the permeation enhancement activities of the system's components. On the other hand, the low viscosity of nanofluids makes it difficult to use it in the appropriate context. In order to change the rheological behaviour of nanoemulsions, biocompatible gels that have a low level of contact with surfactants have previously been investigated [4].

The use of nanoemulsion as a vehicle has the potential to improve transdermal penetration through a variety of processes [5]. Molecules are dissolved in nanoemulsion, which causes a shift in the thermodynamic behavior of the medication they carry. As a result, the drug's partition coefficient is altered, which increases the likelihood that the drug will be able to pass through the stratum corneum. In addition, the surfactant that is incorporated into these formulations lowers the functional barrier that is posed by the stratum corneum [6].

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Diabetes mellitus is a metabolic illness that is chronic and is defined by high blood sugar levels concentrations (high blood sugar) that is caused by insulin shortage. Diabetes mellitus is frequently associated with insulin resistance. Non-Insulin Dependent Diabetes Mellitus (NIDDM) is a milder form of diabetes that most commonly affects adults. The overwhelming majority of diabetics have non-insulin dependent diabetes mellitus. Non-Insulin Dependent Diabetes Mellitus (NIDDM) is a heterogeneous group comprising a milder form of diabetes [7].

METHODOLOGY

Preparation of stock solution

A stock solution of 20 µg/ml was prepared. 50 mg of glibenclamide was accurately weighed and transferred to a 50 ml capacity volumetric flask. The drug was then dissolved in ethanol and the final volume was made up to the mark. 2.0 ml of this solution was then transferred to a 100 ml capacity volumetric flask and final volume was made up to the mark with alkaline borate buffer pH 9.5.

Preparation of blank solution

A blank solution was prepared similarly as the stock solution avoiding the drug substance in the solution.

Preparation of the calibration curve

Various dilutions were prepared between the range 2 µg/ml and 12 µg/ml (final volume 10 ml) at an interval of 2 µg/ml from the stock solution. To each dilution 0.1 ml of concentrated hydrochloric acid was added. Addition of this specified amount of hydrochloric acid to dilutions more than 12 µg/ml lead to come out of glibenclamide from the solution possibly due to its limited solubility at such a low pH attained by the solution after addition of concentrated hydrochloric acid because glibenclamide is reported to be dissolved in dilute alkali solutions. The concentrated hydrochloric acid was also added to the blank solution in the similar ratio.

The three out of these dilutions were scanned against the appropriate blank to determine the λmax of the solution and was found to be 229.5 nm. The standard curve was then prepared by plotting absorbance versus concentration plot against the prepared blank solution at 229.5 nm [8, 9].

Solubility studies

The solubility of Glibenclamide 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±1.0°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-µm membrane filter.

The filtrate was solubilized in suitable solvent, diluted with the pH 9.5 buffer and the concentration of Glibenclamide was determined using UV-Visible spectrophotometer at 229.5nm [10].

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 [11].

Preparation of nanoemulsions

Aqueous phase titration method

1. Nanoemulsions were prepared by aqueous phase titration method.
2. The composition of the nanoemulsions was chosen according to the pseudo ternary phase diagram.
3. 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 [12].

Evaluation of Glibenclamide Nano emulsion

Particle size

The formulation (0.1 ml) was dispersed in 50 ml of water in volumetric flask and gently mixed by inverting the flask. Globule size 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.

Zeta potential

The formulation (0.1 ml) was dispersed in 50 ml of water in volumetric flask and gently mixed by inverting the flask. Globule size 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 versus shear rate.

***In vitro* drug release studies**

The *in vitro* drug release of glibenclamide from the nanoemulsion formulation was determined by dialysis bag method.0.1N HCl and pH 9.5 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 glibenclamide), 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} [13].

RESULT AND DISCUSSION

Screening of oils, surfactants and co-surfactants

A spectrophotometric method was developed for the estimation of GCL in methanol and its λ max was found to be 300 nm. The nanoemulsion formulation should be clear, transparent, monophasic liquid at ambient temperature and should have good solvent properties to allow presentation of the drug in solution.

The solubility of GCL in various oils, surfactants and cosurfactants are presented in Figure 1. Upon scanning the λ max of GCL in presence of various oils, surfactants and cosurfactants, it was observed that the λmax of GCL was retained. It can be inferred that selected oil, surfactant and cosurfactant will not interfere with the developed analytical method of the drug.

Furthermore, the results confirm that there is compatibility between the drug and oil, surfactant and co-surfactant used in this study. Among the various oils screened, the maximum solubility of GCL was found in ACC 200 E6 and was selected as oil. GCL also showed good solubility in Cr RH40 and T-80 and was selected as surfactant and co-surfactant respectively.

Phase diagram studies

The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. Ternary phase diagram were constructed for different mass ratios of oil, surfactant and cosurfactant.

Care was taken to ensure that observations were not made on metastable systems; although the free energy required to for an emulsion is very low. After identification of the microemulsion region (clear and transparent area) in the phase diagram (Figure 2), nine formulations were selected at desired component ratios, keeping the concentration of the drug constant.

Dispersion stability studies

Nanoemulsions are thermodynamically and physically stable systems and are formed at particular concentrations of oil, surfactant and water making them stable to phase separation, creaming or cracking.

It is the thermostability that differentiates nanoemulsions fromemulsions with kinetic stability and eventually phases separation. Thus, the formulations were tested for their physical (dispersion) stability by using centrifugation, heating-cooling cycle and freeze-thaw cycle.

Only those formulations which survived dispersion stability tests were selected for further study. Formulations F7, F8 and F9 become turbid during heating-cooling cycle which
Indicates that these formulations were unstable and screened out for further study (Table 1). F1 to F6 were evaluated further.

Evaluation of true nanoemulsion

Drug Content

Irrespective of difference in composition the drug content of formulation F1 to F6 was found in range of 99.36- 100.56% indicating uniform distribution of GCL in formulation (Table II).

Spectroscopic Absorbance

Lower absorbance should be obtained with optically clear solutions because cloudier solution will scatter more of incident radiation, resulting in higher absorbance. Aqueous dispersions with small absorbance are optically clear and oil droplets are thought to be in a state of finer dispersions. To assess the optical clarity quantitatively, UV-VIS spectrophotometer was used to

measure the amount of light of a given wavelength transmitted by the solution.

Absorbances of formulations F1-F6 upon dilution with distilled water and PB of pH 7.4 at different time intervals are presented in Figure 3 and Figure 4. The result indicated that all the formulations F1-F6 were well stable till 24 hrs as their absorbance values didn't changed at the end of 24 hrs.

Photon correlation spectroscopic studies

Nanoemulsions are characterized by the droplet size in nanometer size range; the droplet size of the emulsion is a crucial factor because it determines the rate and extent of drug release as well as drug absorption. Also, it has been reported that the smaller droplet size of the emulsion may lead to more rapid absorption and improve the bioavailability.

Therefore droplet size analysis was performed to see whether the resultant emulsions are indeed nanoemulsions. Monitoring of change in the size distribution can provide valuable information for optimizing the formulation. Irrespective of the different ratios of oil, surfactant and co-surfactants, no apparent change in mean particle size was observed in different dilution media namely distilled water and phosphate buffer of pH 7.4.

Moreover, no significant increase in mean particle size was observed even after 24 hr post-dilution in different dilution media for the formulation F1-F6.

Evaluation of nanoemulsion gel

Zeta Potential Determination

Zeta potentials of nanoemulsion formulation with and without OA are tabulated in Table II. Data reveals that zeta potential ranges from -6.26 to -14.35 mV for the formulation F1-F6 without OA. The emulsion droplets of these formulations possess negative zeta potential because of the presence of free fatty acids. However, in the presence of OA, as a positive charge inducer, all formulations acquire positive zeta potential, which varied between +15.90 and +24.80 mV, suggesting increased adhesion of the droplets to the cell surface because of electrostatic attraction.

Drug content

Drug content of transdermal nanoemulsion gel (F1-F6) was in the range 99.12- 100.32 % (Table IV) indicating uniform distribution of GCL in formulation. Further it can be inferred that gelling process didn't affect the uniform distribution of GCL.

pH Measurements

pH of 10% w/v of nanoemulsion gel ranged between 5.18 to 5.48 (Table IV). The result indicates that pH of all the nanoemulsion gel (F1-F6) is in close approximation to the skin pH (5.5-6.0), inferring the compatibility of formulation with skin.

Viscosity Determination

The viscosity of the nanoemulsion gel formulations (F1G-F6G) was determined as such without dilution and tabulated in Table 4. Data indicates that at fixed levels of ACC 200 E6, the viscosity decreases from 745.14 to 563.27 as the concentration of Cr RH40 decreases (Table IV). Further, it was observed that as the concentration of ACC 200E6 increases, the viscosity decreases.

Transmission electron microscopy

TEM images of true nanoemulsion and nanoemulsion gel are depicted in Figure 5 and Figure 6 respectively. Figure 5 clearly indicates the spherical nature of true nanoemulsion with no sign of coalescence. Figure 6 depicts the presence of nanoemulsions which are embedded in gel network of carbopol 940. This study clearly indicates that the solubility and droplet size didn't changed while incorporating in gel.

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***In vitro* release studies**

In vitro release studies were performed to compare the release of drug from six nanoemulsion gel formulations (F1G-F6G), and their profiles are depicted in Figure 7. Furthermore, release was also characterized by t50% (dissolution half-life) and percent DE (Table 4). Pharmacopoeias very frequently use these parameters as an acceptance limit of the dissolution test.

Under this pretext, an ideal formulation should be optimized on the basis of maximizing DE and minimizing the t50%.DE of the nanoemulsion gel (F1G-F6G) varied within 45.78- 59.33%, whereas t 50% of these formulations varied between 2.43 to 6.44 hr. It was found that at fixed level of ACC 200E6 (F1G-F3G), t50%. Decreases from 6.44 to 4.21 hr, while DE values increases from 45.78 to 51.57 % (Table 4). This might be because of decrease in viscosity from F1 to F3 (Table 4). Similar trend was observed in F4G-F6G possibly because of decreasing consistency in these transdermal nanoemulsion gels.

Compatibility studies

Apart from physical characteristics, compatibility between drug and excipient is a factor in determining the effectiveness of delivery system. Herein to consider compatibility between drug and excipient, we refer to solubility and/or interaction with no alteration in the chemical nature of the drug or the excipient. Because each drug has its own characteristic chemical and physical properties, no delivery prepared from a particular excipient will serve as a universal carrier for all the drugs. The possible drug-excipient interaction was

studied by FTIR and DSC analysis of pure drug, pure excipient, and their PMs and CMs.

Fourier transforms infrared spectroscopic studies

FTIR spectra of pure Glibenclamide, Acconnon 200E6, Cremophor RH40, Tween 80, physical mixtures and co-melts of glibenclamide with various excipients at 1:2 ratio. These characteristic peaks of glibenclamide appearing at the above wavenumbers are also observed in the spectra of PMs and CMs, indicating retention of chemical identity of glibenclamide. However, intensity of peaks corresponding to the drug were sometimes reduced or the peaks were broadened in PMs and CMs, possibly due to the mixing or loss of crystallinity. The FTIR spectrum data confirms that all the excipients do not alter the performance characteristic of the drug indicating their compatibility.

TABLE 1:

Code	ACC 200 E6 (gm)	GCL (gm)	Cr RH40 (gm)	T-80 (gm)	Cr RH40: T-80	OA(gm)
F1	0.012	0.10	0.72	0.18	4:1	0.01
F2	0.012	0.10	0.495	0.495	1:1	0.01
F3	0.012	0.10	0.72	0.72	1:4	0.01
F4	0.012	0.30	0.14	0.14	4:1	0.01
F5	0.012	0.30	0.35	0.35	1:1	0.01
F6	0.012	0.30	0.56	0.56	1:4	0.01
F7	0.012	0.50	0.10	0.10	4:1	0.01
F8	0.012	0.50	0.25	0.25	1:1	0.01
F9	0.012	0.50	0.10	0.40	1:4	0.01

TABLE 2:

Code	Drug Content	Mean Particle Size (nm)			
		Distilled Water		PB (pH 7.4)	
		0 hours	24 hours	0 hours	24 hours
F1	99.73(0.46)	11.60(0.45)	12.65(0.15)	13.58(0.01)	13.89(0.18)
F2	100.56(1.33)	10.08(0.58)	11.47(0.77)	11.46(0.23)	12.56(0.18)
F3	99.36(1.42)	9.25(0.13)	9.28(0.33)	10.63(0.23)	10.98(0.40)
F4	100.12(1.32)	11.68(0.32)	12.70(0.94)	13.19(0.08)	14.26(0.18)
F5	99.81(1.03)	11.33(0.05)	13.13(0.30)	11.26(0.59)	12.82(0.24)
F6	100.32(1.94)	9.60(0.14)	10.18(0.56)	10.43(0.16)	10.75(0.06)

TABLE 3:

Code	Zeta Potential (mV)		Drug Content	pH	Viscosity (mPa)
	Without (OA)	Without (OA)			
F1	5.30(1.30)	5.30(1.30)	99.64 (0.43)	5.48(0.015)	745.14 (2.318)
F2	5.26(0.40)	5.26(0.40)	100.32 (1.24)	5.40(0.021)	721.13 (2.237)
F3	-10.75(0.7)	-10.75(0.7)	99.16 (1.38)	5.18(0.010)	563.27 (1.879)
F4	1.00(0.42)	1.00(0.42)	99.12 (0.86)	5.52(0.010)	610.19 (2.023)
F5	0.19(1.29)	0.19(1.29)	99.12 (0.86)	5.38(0.020)	570.89 (2.011)
F6	4.35(0.34)	4.35(0.34)	100.24 (1.94)	5.20(0.015)	520.58 (1.660)

Table 4:

Code	t50% (hour)	% DE
F1	**6.44**	**45.78**
F2	**6.44**	**48.82**
F3	**4.21**	**51.57**
F4	**3.36**	**54.05**
F5	**2.86**	**56.29**
F6	**2.43**	**59.33**

Figure 1: Phase diagram studies

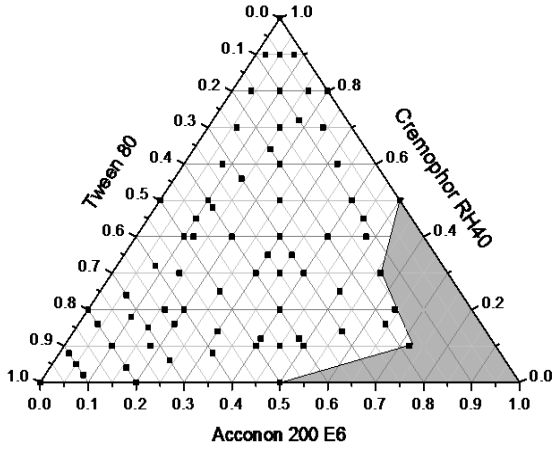


Figure 2: formulations F1-F6 post-dilution with water.

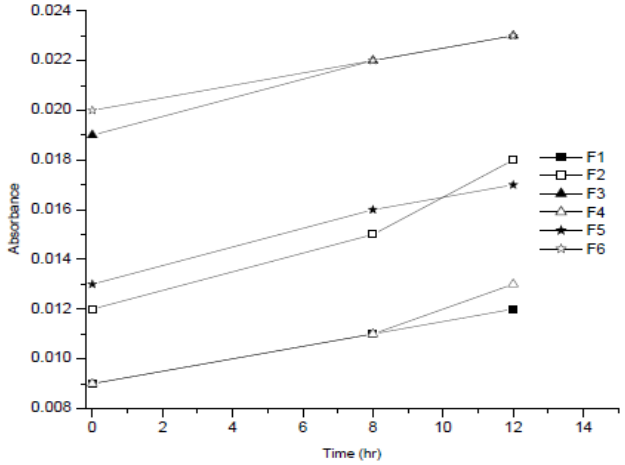


Figure 3: formulations F1-F6 post-dilution with PB of pH 7.4

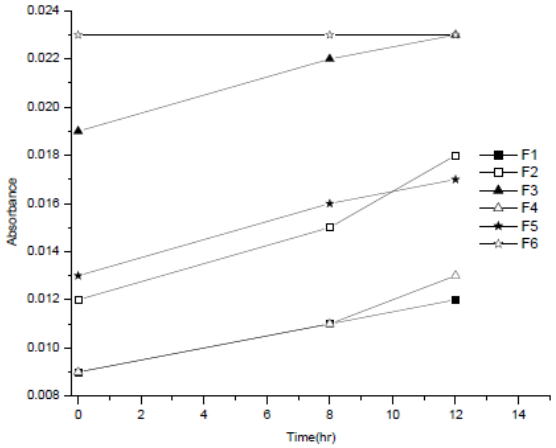


Figure 4: TEM image of true nanoemulsion (F6).

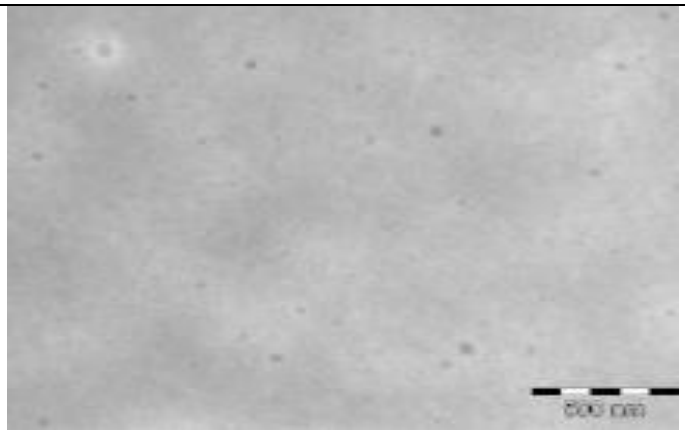


Figure 5: TEM image of nanoemulsion gel (F6)

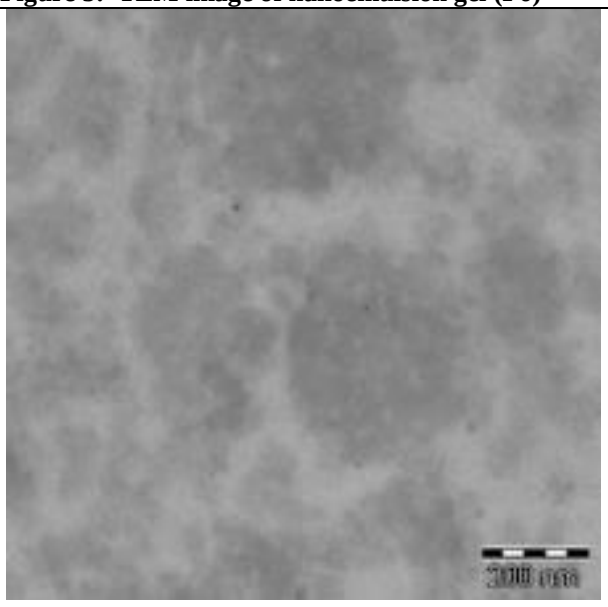


Figure 6: In vitro release studies

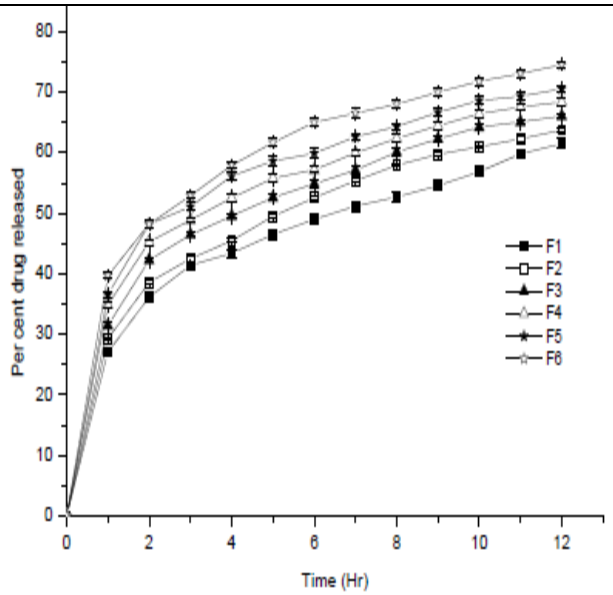


Figure 8: FTIR SPECTRA

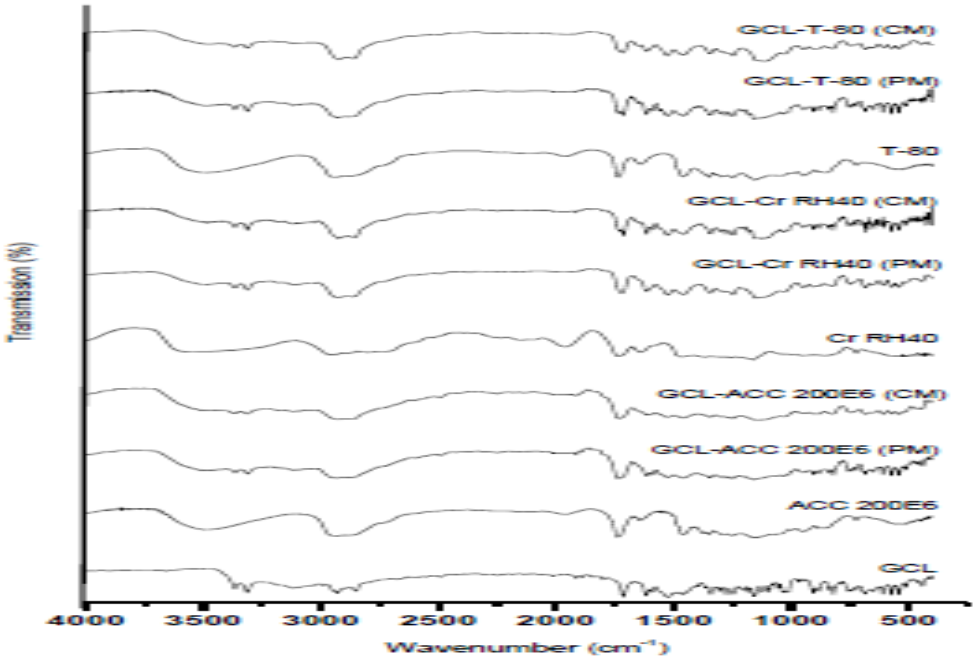
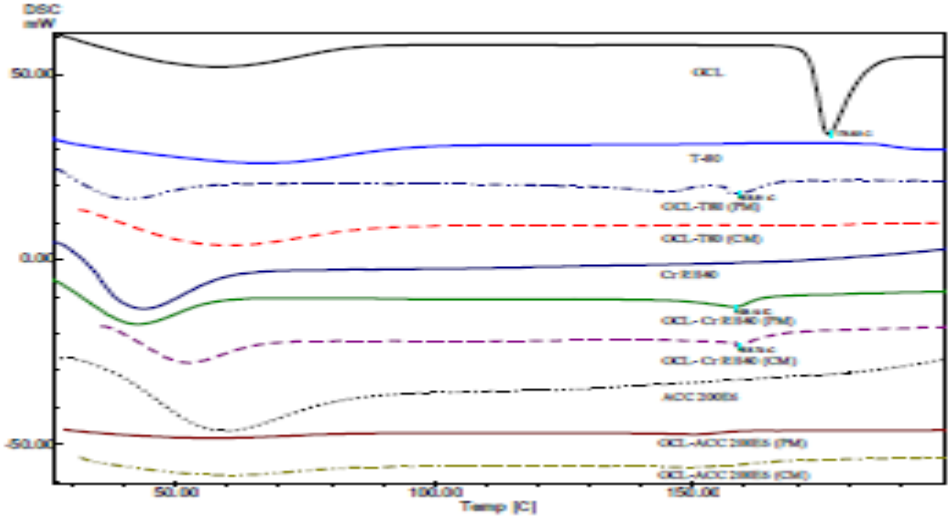


Figure 9: DSC STUDIES



Differential scanning calorimetric Studies

The DSC was used to detect formulation incompatibilities resulting from drug-excipient interactions. The DSC thermograms of the pure glibenclamide, excipients (ACC 200 E6, Cr RH40, and T- 80), their physical mixture and co-melt at the ratio of 1:2 (drug: excipient) are presented in Figure 9.

DSC thermogram of GCL, ACC 200E6, Cr RH40, T80, their PM and CM at 1:2 ratio The sharp endothermic peak of GCL appeared at 176.630C that corresponds to the drug melting point because of its crystalline nature. In thermograms of PM of GCL-T-80, the endothermic peak has shifted to lower melting point (158.81 0C). However in case of CM of GCL-T-80, no endothermic peak corresponding to GCL was present, possibly due to progressive dissolution of GCL during DSC measurement. Similar observations were observed in case of PM and CM of GCL-ACC 200E6.

Peaks corresponding to PM and CM of GCL-Cr RH40, have shifted towards lower temperature (158.19^{o}C and 158.79^{o}C, respectively) because of melting point depression. Similar observations were reported.

CONCLUSION

The Nano sized droplets, which would result in huge interfacial areas due to the presence of nanoemulsions, would have an effect on the transport characteristics of the drug, which is an essential

component of sustained drug delivery. Nanoemulsions are created using surfactants that are fit for human consumption as well as common food ingredients that have been given the GRAS (Generally Recognized as Safe) designation by the Food and Drug Administration.It can be concluded that optimized nanoemulsions can be successfully formulated for the effective treatment of diabetes.

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