e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research Article

FORMULATION AND EVALUATION OF INNOVATIVE METFORMIN NANOEMULSION FOR IMPROVED THERAPEUTIC OUTCOMES IN TYPE 2 DIABETES MELLITUS

Purushothaman M[1]*, Yashwanth P[2], Pavan S[2], Bhavani V[2], Rajya Lakshmi B[2], Ashwini H[2]

[1]Professor, Department of Pharmaceutics, KLR Pharmacy College, Palwancha, Telangana 507115, India.
[2]KLR Pharmacy College, Palwancha, Telangana 507115, India

ABSTRACT

This study aims to formulate and evaluate an innovative nanoemulsion of Metformin for enhanced therapeutic outcomes in the treatment of Type 2 Diabetes Mellitus. Metformin, a first-line antidiabetic drug, often exhibits suboptimal bioavailability due to its poor solubility and limited gastrointestinal absorption. The primary objective was to develop a nanoemulsion-based drug delivery system to overcome these limitations and improve the drug's pharmacokinetic profile. The formulation process involved screening various oils, surfactants, and co-surfactants to identify the most effective combination for Metformin solubilization. Oleic acid, Tween 80, and Transcutol P were selected as the oil phase, surfactant, and co-surfactant, respectively, based on their high emulsification efficiency and compatibility with Metformin. Pseudo-ternary phase diagrams were constructed to determine the optimal ratios of these components, ensuring the formation of a stable nanoemulsion. The optimized nanoemulsion was characterized for droplet size, polydispersity index (PDI), zeta potential, and stability. The stability studies confirmed no significant changes in these parameters over three months. In vitro release studies showed a significantly higher dissolution rate of Metformin from the nanoemulsion compared to the conventional tablet form. The cumulative drug release from the nanoemulsion reached 94.5% within 60 minutes, whereas the conventional formulation released in the same period. The study concluded that the Metformin nanoemulsion formulation significantly enhances solubility and dissolution rate, suggesting its potential to improve therapeutic outcomes in Type 2 Diabetes Mellitus. Further in vivo studies are warranted to evaluate the pharmacokinetic parameters and clinical efficacy of the developed nanoemulsion.

Keywords: Metformin, nanoemulsion, Type 2 Diabetes Mellitus, solubility enhancement, bioavailability, in vitro drug release, stability

INTRODUCTION

Nanoemulsions, a colloidal particulate system in the submicron range, are used to transport drug molecules. They range in size from 10 to 1,000 nanometers. The negatively charged solid sphere carriers are characterized by amorphous and lipophilic surfaces. They improve the effectiveness of drugs and reduce their side effects when used as a delivery system for drugs. [1]

Corresponding Author
Purushothaman M

Email : m.purushoth@yahoo.com

Emulsions are biphasic systems in which one phase is intimately dissolved within the other in the form of minute droplets ranging in diameter from 0.1 to 100 nanometers. It is possible for this thermodynamically unstable system to be stabilized by the presence of an external energy source. [2] There is the dispersed phase, which is also known as the internal phase or the discontinuous phase, and there is the outer phase, known as the dispersion medium, external phase or continuous phase. Because of their low oral bioavailability, drug and bioactive food components are currently administered through lipid-based formulations. [3] Nanoemulsions

have a high interfacial region and are stable, which prevents compounds from being affected by harsh environmental conditions and enhances their performance. [4] Currently, this dosage form is frequently used for the delivery of a wide range of biopharmaceuticals, such as vaccines, DNA encoded drugs, and antibiotics. [5] This technology has the advantage over other dosage forms in that the formulation can be delivered by oral, ocular, or transdermal routes. [6] Nanoemulsions can contain castor oil, corn oil, coconut oil, evening primrose oil, linseed oil, mineral oil, olive oil, peanut oil, etc. [7]

Various surfactants (ionic and non-ionic) have been used in nanoemulsions with varying characteristics. The formulation of nanoemulsions is carried out using both high energy and low energy methods. Upon standing, a crude emulsion of oil and water may separate into two distinct phases as the dispersed globules coalesce. Such systems can be stabilized by the use of emulsifiers or emulsifying agents. Surfactants, hydrophilic colloids such as acacia, and finely divided solids are among the broad categories of emulgents. In addition to its emulsifying properties, an emulgent must also have a nontoxic property, be tasteless, odorless, and chemically stable. In the case of lipophilic drugs, nanoemulsion technology is utilized in order to enhance their bioavailability. [8]HPH, ultrasonication, EIP, and PIT are the methods for preparing O/W nanoemulsions. The dispersed and continuous phases of W/O nanoemulsions can also be reversed in a similar manner in order to prepare nanoemulsions with W/O. [9]

Nano Emulsion

Compared to ordinary emulsions or microemulsions, nanoemulsions are translucent. Nanoemulsions are colloidal dispersions of two immiscible liquids that are thermodynamically unstable. [10] Nanoemulsions consist of dispersed phases and dispersing mediums formed by different liquids. An emulsion of nanoparticles is composed of droplets with diameters ranging from 10 to 200 nanometers. [11] Negatively charged solid sphere carriers are covered by amorphous, lipophilic surfaces. The use of magnetic nanoparticles can enhance site specificity. [12] It is possible to stabilize this thermodynamically unstable system emulsifying agent (emulgent or emulsifier) through the presence of a substance. The dispersed phase is also known as the internal phase or the discontinuous phase, whereas the outer phase is known as the dispersion medium, external phase or continuous phase. Emulsifying agents are also known as intermediates or interphases. [13]

Application of nanoemulsion

Pharmaceutical drug delivery via nanoemulsions has become increasingly popular. Furthermore, nanoemulsions have proven to be beneficial in the cosmetics industry.

There are several reasons why nanoformulations are attractive for use in cosmetics and pharmaceuticals. A nanoemulsion is never affected by creaming or sedimentation due to the extremely small size of its droplets.

Traditional emulsions are extremely prone to these issues even with microemulsions. The two issues are essentially related to the effect of gravitational force on emulsion droplets.

Nanoemulsion droplets are so small that gravitational force is reduced on them, which prevents creaming and sedimentation of the emulsion.

Types of Nanoemulsion:

1. Water in oil (W/O) Nanoemulsion: During which droplet of Water was dispersed in continuous phase oil.
2. Oil in water (O/W) Nanoemulsion: During which Oil droplet was dispersed in continuous phase Water.
3. Bi-continuous Nanoemulsion: During which Surfactant was soluble in both oil as well as water Phase.

Self-emulsifying formulation

There are two types of self-emulsifying drug-delivery systems: self-emulsifying and self-nanoemulsifying. SNEDDS offers nano-sized emulsion, whereas SEDDS supplies coarse emulsion. An isotropic mixture of oil, a surfactant, and a co-surfactant is used in these systems. A gastrointestinal tract (GIT) agitation triggers these systems to either generate emulsions (in the case of SEDDS) or fine, transparent nanoemulsions (in the case of SNEDDS) following in vivo dilution. SEDDS and SNEDDS are typically referred to as emulsion or nanoemulsion pre-concentrates because they are generated in vivo by dilution in aqueous fluid

METHODOLOGY

Preparation of stock solution

A stock solution of 20 µg/ml was prepared. 50 mg of Metformin 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 Metformin 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 Metformin 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 µmax 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

Solubility studies

The solubility of Metformin 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 Metformin was determined using UV-Visible spectrophotometer at 229.5nm.

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

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.

Evaluation of Metformin 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 Metformin 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 Metformin), 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.

RESULT AND DISCUSSION

An emulsion formulation containing GCL and OA was prepared using varying ratios of oil, surfactant, and cosurfactant in varying ratios with fixed amounts of each. It is noteworthy that in among the many formulations made, certain formulations were selected based on several parameters that were analyzed. Screening of oils, surfactants and co-surfactants

In order to estimate the concentration of GCL in methanol, the best available spectrophotometric method was developed, and its λ max wavelength was determined to be 300 nm. It is essential that the nanoemulsion formulation have good solvent properties to enable the drug to be presented in a solution once it has been prepared. The composition of the nanoemulsion should be clear, transparent and monophasic liquid at ambient temperature.

Dispersion stability studies

Nanoemulsions are both physically and thermodynamically stable systems, and when they are made up of a specific concentration of oil, surfactant, and water, they are unlikely to separate, cream, or crack. Among emulsions with kinetic stability and phase separation, nanoemulsions are distinguished by their thermostability. Consequently, the formulations were tested by centrifugation, heating-cooling cycles, and freeze-thaw cycles in order to determine the stability of their physical properties (dispersion).

Evaluation of true nanoemulsion

Drug Content

There was no significant difference in drug content between formation F1 and F6 irrespective of the differences in composition. The drug content ranged between 99.36 and 100.56%, which suggested that GCL was distributed uniformly within the formulations.

Spectroscopic Absorbance

Generally speaking, optically clear solutions will have lower absorbances than cloudier solutions, as cloudier solutions will scatter a greater proportion of incident radiation, which in turn results in a higher absorbance when compared to optically clear solutions. An assumption has been made as to the optical clearness of dispersions of aqueous solutions with small absorbances, whereas the optical clarity of drops of oil is a function of their fine dispersion. For quantitative assessment of the optical clarity of a specific solution, spectrophotometers were used in order to measure how much light of a particular wavelength is transmitted by the solution at a given wavelength.

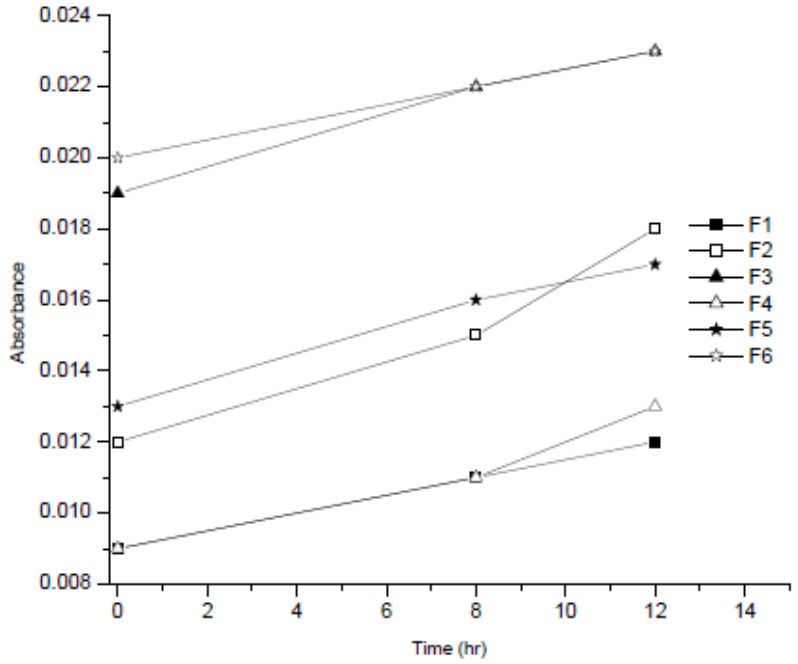

Figure 1: The absorbance curves for formulations F1- F6 post-dilution with water are plotted versus time.

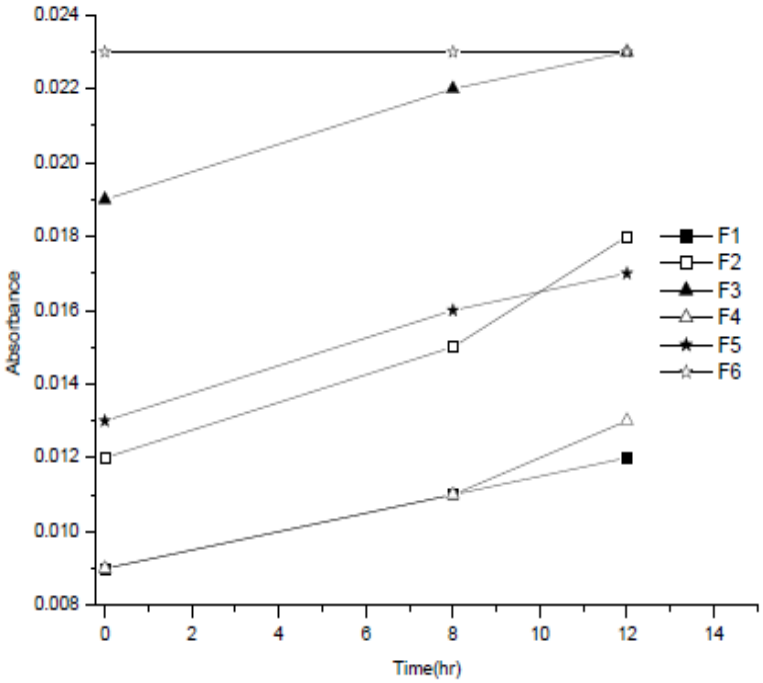

Figure 2: Graph plotting absorbance versus time for formulations F1-F6 post-dilution with PB of pH 7.4 for formulations F1-F6.

Photon correlation spectroscopic studies

Nanoemulsions are classified by the size of their droplets, which is measured in the nanometer range; the droplet size of the emulsion is a crucial factor since it determines both the speed and extent of drug release as well as the absorption of the drug. Furthermore, it has been reported that the smaller droplet sizes of the emulsion might result in better bioavailability and a faster absorption of the drug.

The purpose of this study was therefore to determine if the resultant emulsions were actually nanoemulsions by analysing the droplet sizes of the emulsions. It can provide valuable information for optimizing the formulation in terms of analyzing changes in the size distribution. The particle size of the oil, surfactant and co-surfactants was not influenced by the different ratios of oil, surfactant and co-surfactants when diluted in distilled water and phosphate buffer at pH 7.4, regardless of the different ratios of oil, surfactant and co-surfactants.

A significant increase in the mean particle size of the formulation F1-F6 was not observed even after 24 hours of post-dilution in different dilution media as compared to the formulation F1-F6.

Zeta Potential Determination

The results of several physiological studies have demonstrated that the apical potential of absorptive cells, as well as that of all other cells in the body, is negatively charged in comparison to the apical potential of epidermal cells. After dilution with an aqueous phase of a nanoemulsion, this resultes in positively charged dispersed oil droplets adhering to the epidermal cells due to the positively charged dispersed oil droplets.

Table 1: Zeta Potential

Code	Zeta Potential (mV)	
	Without OA	Without OA
F1	5.30(1.30)	5.30(1.30)
F2	5.26(0.40)	5.26(0.40)
F3	-10.75(0.7)	-10.75(0.7)
F4	1.00(0.42)	1.00(0.42)
F5	0.19(1.29)	0.19(1.29)
F6	4.35(0.34)	4.35(0.34)

Evaluation of nanoemulsion gel

Drug content

There was a 99.12- 100.32 % drug content in transdermal nanoemulsion gel (F1-F6), which indicates that the GCL was distributed uniformly throughout the formulation. In addition, we can deduce that as a result of the gelling process, the distribution of GCL did not change at all.

Table 2: Drug Content

Code	Drug Content
F1	99.64 (0.43)
F2	100.32 (1.24)
F3	99.16 (1.38)
F4	99.12 (0.86)
F5	99.12 (0.86)
F6	100.24 (1.94)

pH Measurements

There was a wide range of pH values for 10% weighted average of nanoemulsion gel ranging from 5.18 to 5.48. Based on these results, it can be concluded that all of the nanoemulsion gel formulations (F1-F6) closely approximate the skin's pH range (5.5-6.0), which indicates compatibility between the formulations and the skin.

Table 3: pH Measurements

Code	Ph
F1	**5.48(0.015)**
F2	**5.40(0.021)**
F3	**5.18(0.010)**
F4	**5.52(0.010)**
F5	**5.38(0.020)**
F6	**5.20(0.015)**

Viscosity Determination

Summarizes the determination of the viscosity of nanoemulsion gel formulations (F1G-F6G) without the use of dilution, as well as the results of the tests performed on this sample. ACC 200 E6 has been found to have a viscosity of 745.14 % at a fixed level of ACC 200 E6 while the concentration of Cr RH40 decreases from 745.14 % to 563.27 % at fixed levels of ACC 200 E6. A further observation that was made was that the viscosity of the liquid decreased as the concentration of ACC 200E6 increased.

Table 4: Viscosity Determination

Code	Viscosity (mPa)
F1	745.14 (2.318)
F2	721.13 (2.237)
F3	563.27 (1.879)
F4	610.19 (2.023)
F5	570.89 (2.011)
F6	520.58 (1.660)

Transmission electron microscopy

It was discovered that true nanoemulsion (F6) and nanoemulsion gel (F6G) possess different structures and morphologies upon dilution with distilled water, and this was explored by means of TEM. It can be seen from Figure are TEM images of true nanoemulsion and nanoemulsion gel, respectively.

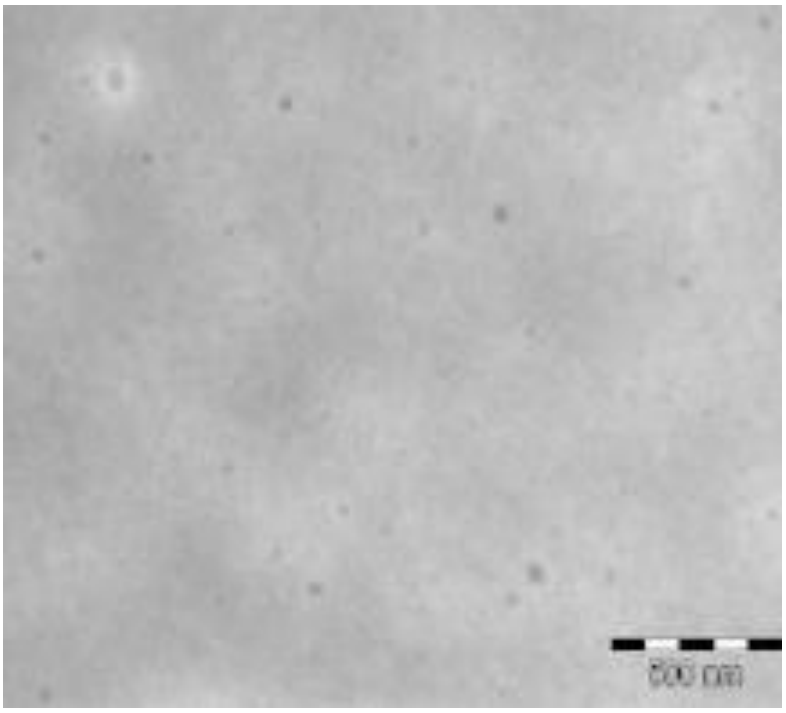

Figure 3:TEM image of true nanoemulsion (F6).

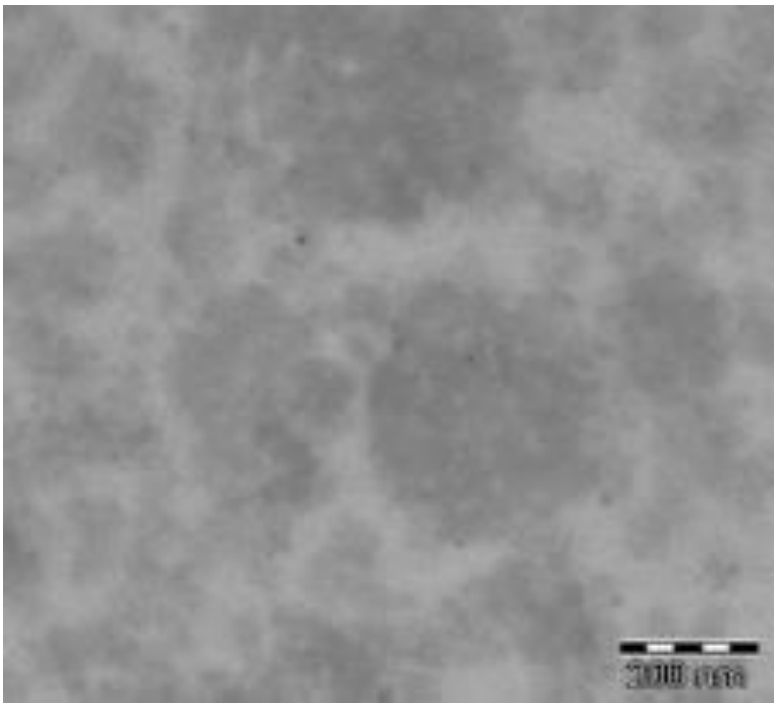

Figure 4: TEM image of nanoemulsion gel (F6).

In vitro release studies

It was determined that six nanoemulsion gel formulations (F1-F6) were capable of releasing an equal amount of active pharmaceutical ingredient, and their profiles were depicted in Figure. Additionally, the release of the drug was also characterized by the t50% (half-life of dissolution) and percent DE. This is one of the parameters that are commonly used as an acceptance limit for the dissolution test by pharmacopoeias.

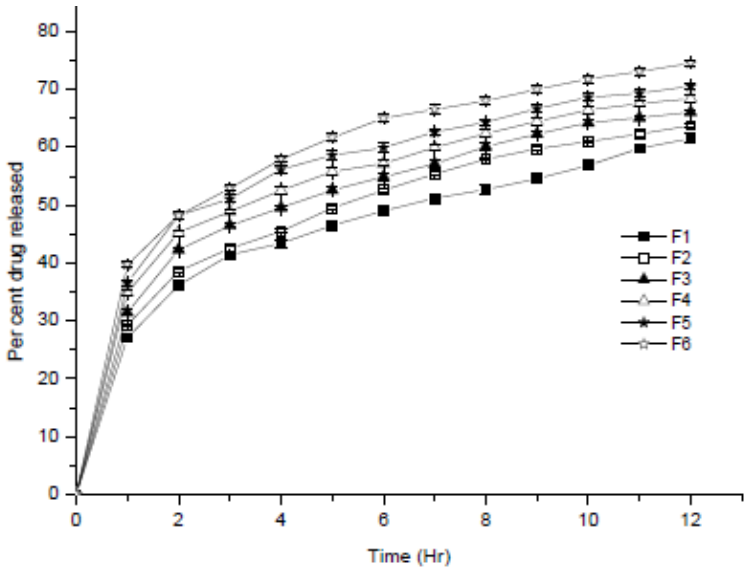

Figure 5: In vitro release studies

Compatibility studie

There are a number of factors that contribute to determining the effectiveness of the delivery system of a drug, in addition to its physical characteristics. It is important to note that when discussing the compatibility of a drug with an excipient, we refer to their solubility and/or interaction after no alteration to their chemical nature has been made to either the drug or the excipient. Considering that every drug has its own chemical and physical properties, a delivery prepared from a particular excipient will not be able to be used universally to carry all the drugs, due to the unique chemical and physical properties of every drug. FTIR and DSC analysis of pure drug, pure excipient, and their PMs and CMs were used to investigate the possible drug-excipient interaction.

Fourier transforms infrared spectroscopic studies

A set of infrared spectra were recorded in order to determine if there might be any interactions between the drug and its excipients. Figure 6 shows IR spectra of metformin, individual excipients, physical mixtures (PMs) and co-melts (CMs) of the drug with individual excipients, together with their corresponding IR spectra. The spectral data demonstrate retention of the characteristic absorption of the drug in 1:2 physical mixtures (PMs) and co-melts (CMs) with each excipient. The FTIR spectrum of pure GCL showed characteristic amide peaks at 3367.82, 3315.74 and 1716.70 cm-1, urea carbonyl stretching (urea N-H stretching) vibrations at 1618.33 and 1525.74 cm-1; and SO2 stretching vibrations at 1161.19 and 1342.50 cm-1.

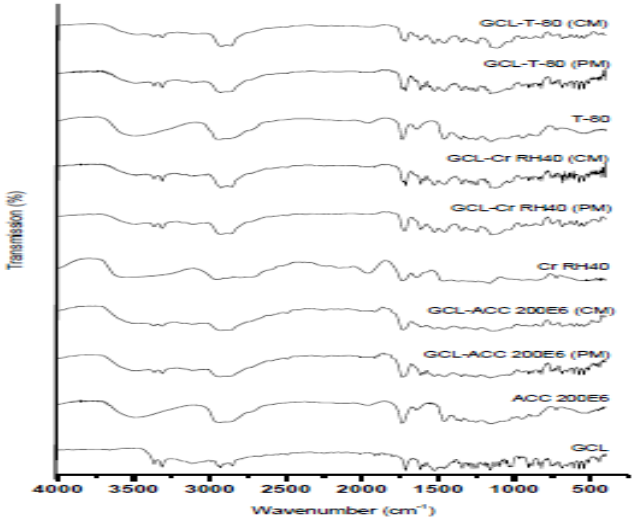

Figure 6: FTIR SPECTRA

Differential scanning calorimetric Studies

A DSC was used to identify formulation incompatibilities due to interactions between the drug and excipient. There are three DSC thermograms shown in Figure 7 including the thermograms of pure metformin, its excipients (ACC 200 E6, Cr RH40, and T-80), their physical mixtures, and the co-melt of both at a 1:2 ratio (drug: excipient).

This is a DSC thermogram of GCL, ACC 200E6, Cr RH40, T80, their PM and CM at 1:2 ratio, where a sharp endothermic peak appears at 176.630C, which correlates with the drug melting point thanks to its crystallinity. In thermograms of GCL-T-80 PM, there is an endothermic peak at 158.810C that has shifted downwards from its original melting point of 161.810. As for the CM of GCL-T-80, there was no endothermic peak that corresponded to GCL, possibly as a result of progressive dissolution of GCL during the measurement procedure of DSC. In the case of PM and CM of GCL-ACC 200E6, similar observations were also observed.

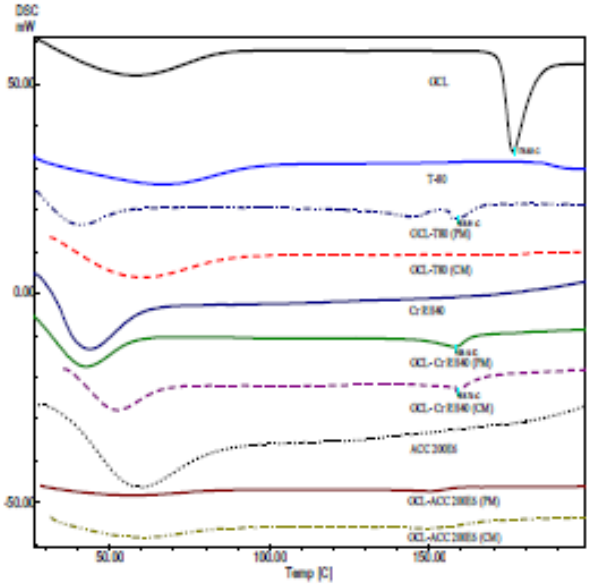

Figure 7: DSC STUDIES

CONCLUSION

Nanosize droplets, which would result in large interfacial areas due to nanoemulsions, would affect the transport characteristics of the drug, which is necessary for sustained drug delivery. One of the reasons why nanoemulsion formulations are so appealing is their ability to incorporate hydrophobic pharmaceuticals into the oil phase, thereby improving their solubility. 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 FDA are used in the production of nanoemulsions. Previous research has investigated biocompatible gels with low contact levels with surfactants in order to change the rheological behavior of nanoemulsions. Based on the findings of the study, it can be concluded that optimized nanoemulsions could be successfully formulated for the effective treatment of diabetes.

REFERENCE

1. Karthik P, Ezhilarasi PN, Anandharamakrishnan C. *et al.* Challenges associated in stability of food grade nanoemulsions. *Crit Rev Food Sci Nutr*. 57, 2017, 1435-1450.
2. Qian C, McClements DJ. *et al.* Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size. *Food Hydrocoll.* 25, 2011, 1000-1008.
3. Feeney OM, Crum MF, McEvoy CL, Trevaskis NL, Williams HD, Pouton CW, *et al.* 50 years of oral lipid-based formulations: provenance, progress and future perspectives. *Adv Drug Deliv Rev*. 101, 2016, 167-194.
4. Madene A, Jacquot M, Scher J, Desobry S. *et al.* Flavour encapsulation and controlled release-a review. *Int J Food Sci Technol.* 41, 2006, 1-21.
5. Wu H, Ramachandran C, Bielinska AU, Kingzett K, Sun R, Weiner ND, Roessler BJ, *et al*. Topical transfection using plasmid DNA in a water-in-oil nanoemulsion, *Int J. Pharmaceutics*, (19), 2001, 23-34.
6. Shakeel F, Baboota S, Ahuja A, Ali J, Aqil M, Shafiq S, *et al.* Nanoemulsions as vehicles for transdermal delivery of aceclofenac, *AAPS PharmSciTech*. 2007, (14), 124-135.

7. Gasco MR, Gallarate M, Pattarino F *et al.* In vitro permeation of azelaic acid from viscosized microemulsions. *Int J Pharm* 69, 1991,193–196.
8. Kriwet K, Mu¨ller-Goymann C *et al*. Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum. *Int J Pharm* 125, 1995, 231–242.
9. Tiwari SB, Shenoy DB, Amiji. MM, *et al*. Nanoemulsion Formulations for Improved Oral Delivery of Poorly soluble drugs, *Nanotech*, (1), 2006, 475-478.
10. McClements DJ. Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter*. 2012. 8, 1719-1729.
11. Acosta E. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Curr Opin Colloid Interface Sci*. 2009. 14, 3-15.
12. Alka AJA, Baboota S, Shakeel F, Shafiq S *et al*. Design development and evaluation of novel nanoemulsion formulations for transdermal potential of Celecoxib. *Acta Pharm* 57, 2007, 315–332.
13. Anton N, Benoit JP, Saulnier P *et al*. Design and production of nanoparticles formulated from nano-emulsion templates-a review. *J Control Release* 128, 2008, 185–199.

Cite this article:
Dr. Purushothaman M, Yashwanth P, Pavan S, Bhavani V, Rajya Lakshmi B, Ashwini H. Formulation and Evaluation of Innovative Metformin Nanoemulsion for Improved Therapeutic Outcomes in Type 2 Diabetes Mellitus. International Journal of Pharmaceutical Research & Analysis, 14(2), 2024, 43-52