



DEVELOPMENT AND EVALUATION OF MICROEMULSION FOR GLYBURIDE: BCS CLASS II ANTIDIABETIC AGENT

Swapnil Ghanshyam Dhake¹, Shahzan Husain^{*2}, Pavan Prasanth Manepalli³, Neha Srivastava⁴,
Geeta Rawat⁵, V. Shanmugam⁶, Sandeep Kumar⁷, Kalpana⁸, Prabhakar Vishvakarma^{*9},
Suraj Mandal¹⁰

¹ Assistant Professor, Sandip Institute of Technology and Research Centre, Nashik.

² Research Scholar, Jamia Hamdard University, New Delhi, 110062

³ Clinical Scientific Expert, Novartis healthcare Pvt., Ltd.

⁴ Assistant Professor, SRM Modinagar College of Pharmacy, Srmist Delhi NCR Campus Ghaziabad

⁵ Assistant Professor, SGT University, Haryana, Gurugram

⁶ Professor, MB School of Pharmaceutical Sciences, erstwhile Sree Vidyanikethan College of
Pharmacy, Tirupati

⁷ Assistant Professor, Sanjivani College of Pharmaceutical Science (RUHS)

⁸ Assistant Professor, School of Pharmaceutical Sciences, CSJMU

⁹ Associate Professor, Department of Pharmacy, IIMT College of Medical Sciences, IIMT University,
O-Pocket, Ganganagar, Meerut, 250001, U.P., India

¹⁰ Assistant Professor, Department of Pharmacy, IIMT College of Medical Sciences, IIMT University,
O-Pocket, Ganganagar, Meerut, 250001, U.P., India

Corresponding Author email:

1. Shahzan Husain, Research Scholar, Jamia Hamdard University, New Delhi, 110062, Email:
shahzanhusain2212@gmail.com
2. Dr. Prabhakar Vishvakarma, Associate Professor, Department of Pharmacy, IIMT College of
Medical Sciences, IIMT University, O-Pocket, Ganganagar, Meerut, 250001, U.P., India, Email:
prabhakar.vishvakarma7788@gmail.com

Abstract:

The objective of the present investigation was to develop and evaluate microemulsifying drug delivery system for improving the delivery of a BCS class II antidiabetic agent, glyburide (GLY). The solubility of glyburide in oils, surfactants and co-surfactants (Capmul MCM: Tween80: Span20) was evaluated to identify the components of the microemulsion. Pseudoternary phase diagrams diagram was utilized to identify the optimal excipient composition to formulate the microemulsion system and the area of microemulsion existence. Glyburide microemulsion was characterized by Refractive index, Optical Clarity, Assay, Dye solubility, Viscosity, Surface tension, pH, Drug Content, Polydispersity index, Drug loading, Entrapment efficiency, Particle size, Zeta Potential, Scanning Electron Microscopy (SEM), Differential scanning calorimetry measurements (DSC) and viscosity. The in vitro dissolution

profile of glyburide microemulsion was evaluated the pure drug in pH 7.4 buffers. The chemical stability of glyburide in microemulsion was determined as per the International Council for Harmonisation (ICH) guidelines.

KEY WORDS: Glyburide; microemulsion; solubility; stability; SEM; DSC; FTIR

I. Introduction

Glyburide or glibenclamide is a second generation sulfonylurea used for treat noninsulin-dependent diabetes. It is one of the mostly prescribed long-acting antihyperglycemic agents.^[1] Glyburide is classified as a BCS class II drug, which means that it has high permeability and low water solubility.^[2] The poor solubility of glyburide in water is the reason for its low dissolution rate, which ultimately leads to variable absorption of glyburide. In addition, there are reports that GLY shows large differences in the individual bioavailability and bioequivalence of the marketed products.^[3] Thus, it can be concluded that in vivo performance of glyburide depend on its dissolution rate and the bioavailability. Given this, researchers have attempted different techniques to improve the dissolution rate. Eventually, the in vivo performance of glyburide, solid dispersion, cyclodextrin complexation, and permeation enhancers has been established in improving in vitro and in vivo performance of glyburide. However, to date, the potential of microemulsions has not been established in the delivery of glyburide.^[4] Research also stated that the microemulsion formulation Glyburide, a poorly water-soluble drug, significantly increased oral absorption, irrespective of whether the subject was fed.^[5] Improved absorption from a microemulsion is probably due to the incorporation of the drug into microemulsion droplets. Smaller microemulsion droplets result in an increased specific surface area and enhanced membrane permeability of the drug *via* solubilization of certain membrane components and pore-formation. All these factors lead to enhanced contact with the gastrointestinal tract. Another important factor is the inner polarity of droplets, which is directed by the hydrophilic-lipophilic balance of surfactant used. A change in droplet polarity may affect the form of the drug and surfactant on the droplet interface and alter drug release.^[6] Microemulsions have also been acknowledged as topical,^[7] transdermal,⁸ and parenteral drug delivery systems,^[9] and several studies have reported the use of a microemulsion as an oral drug delivery system.^[10] In this work, different pre-microemulsion and microemulsion. Formulations were prepared to improve glyburide availability. This was done by the selection of the most suitable microemulsion ingredients that reached the highest solubilization of glyburide. Then, the chosen microemulsion formulations were characterized to help in selecting the most suitable formulation.

II. Materials:

Glyburide was kindly provided as a gift sample by Cipla Pharmaceuticals (Mumbai, India). Labrafac CC(Colorcon Asia, Mumbai, India), Capmul MCM (Indchem International, Mumbai, India), cotton seed oil, sunflower oil and soybean oil; surfactants, *i.e.*, Captex-355, Cremophor EL, PEG 400, Labrafil(all AR grade) were purchased from Merck (Mumbai, India) and Tween-80(SD fine chemicals Private Limited, Gujarat, India); and co-surfactants, *i.e.*, *n*-butanol, span-20 and propylene glycol (all

AR grade) were purchased from Merck (Mumbai, India). Hydrochloric acid and potassium dihydrogen phosphate (all AR grade) were purchased from s.d. Fine Chemicals (Mumbai, India).

Solubility studies of glyburide in different oils, surfactants, and co-surfactants:

The solubility of glyburide was examined in different oils, *i.e.*, Labrafac CC, Capmul MCM cotton seed oil, sunflower oil and soybean oil; surfactants, *i.e.*, Captex-355, Cremophor EL, Tween-80, Labrafil and PEG 400; and co-surfactants, *i.e.*, *n*-butanol, span-20 and propylene glycol. The equilibrium solubility method was performed as follows. Briefly, an excess amount of glyburide was added to 10 ml of each solvent (the aforementioned oils, surfactants, and co-surfactants) in 30 ml screw capped vials and the whole mixture was mixed by vortexing. The vials were then shaken at 37°C for 72 h at 100 rpm in a thermostatically controlled water bath shaker (Weiss Gallenkamp, Loughborough, UK). Then, the supernatant layer was separated and subjected to centrifugation at 3,000 rpm for 5 min in order to remove the un-dissolved drug. Samples of these solutions were then collected and the drug concentration was determined spectrophotometrically at 301 nm against a suitable blank of DMSO using an ultraviolet spectrophotometer SP6-550 (Pye Unicam, Cambridge, England). All experiments were performed in triplicate. ^[11]

Oils, surfactants and co-surfactants loading at different percentages in Glyburide microemulsion:

Predetermined amounts of the drug were dissolved in the required quantity of oil. Surfactant and co-surfactant were added to the above mixture as a fixed ratio. Distilled water was added gradually with continuous stirring, which resulted in the formulation of a transparent and homogenous microemulsion. Parameters optimized for the preparation of microemulsion were the type and concentration of the oil phase, surfactant and co-surfactant. ^[11]

Factor combination as per the chosen experimental design:

| S.NO | Formulation code | Drug (Glyburide) | Aqueous region % | Amphiphilic region % | Surfactant : co-surfactant | Oil % |
|------|------------------|------------------|------------------|----------------------|----------------------------|------------|
| | | | Water | | Tween80 : Span20 | Capmul MCM |
| 1 | F1 | 5mg | 50 | 40 | 1:9 | 10 |
| 2 | F2 | 5mg | 48 | 40 | 2:8 | 12 |
| 3 | F3 | 5mg | 46 | 40 | 3:7 | 14 |
| 4 | F4 | 5mg | 44 | 40 | 4:6 | 16 |
| 5 | F5 | 5mg | 42 | 40 | 5:5 | 18 |
| 6 | F6 | 5mg | 40 | 40 | 6:4 | 20 |
| 7 | F7 | 5mg | 38 | 40 | 7:3 | 22 |

| | | | | | | |
|---|-----------|-----|----|----|-----|----|
| 8 | F8 | 5mg | 36 | 40 | 8:2 | 24 |
| 9 | F9 | 5mg | 34 | 40 | 9:1 | 26 |

Table no 1: Factor combination as per the chosen experimental design

Pseudoternary phase diagram study:

Pseudoternary phase diagrams of oil, water, and surfactant/co-surfactants (S/Cos) mixtures are constructed at fixed surfactant/ co-surfactant weight ratios. Phase diagrams are obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of the monophasic /biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring; the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the microemulsion region of existence. [12]

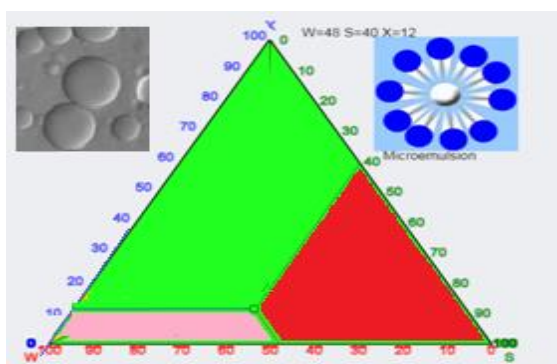


Figure 1: F1 Pseudoternary phase diagram

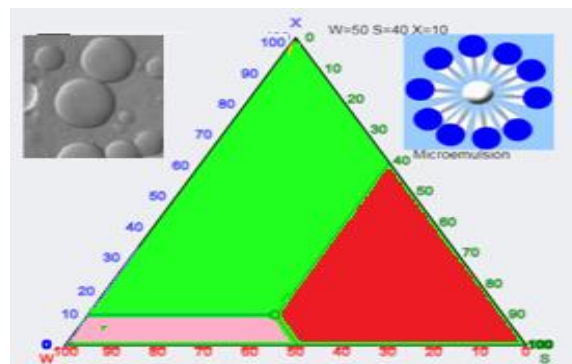


Figure 2: F2 Pseudoternary phase diagram

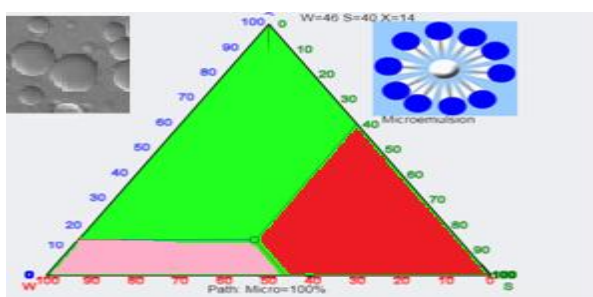


Figure 3: F3 Pseudoternary phase diagram

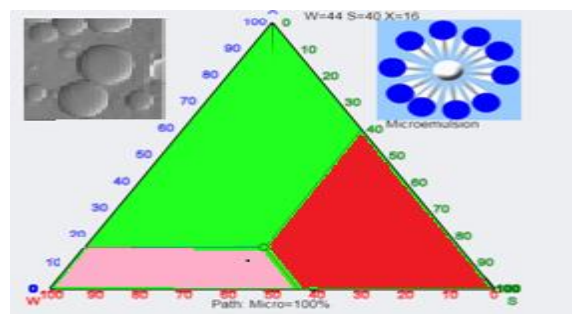


Figure 4: F4 Pseudoternary phase diagram

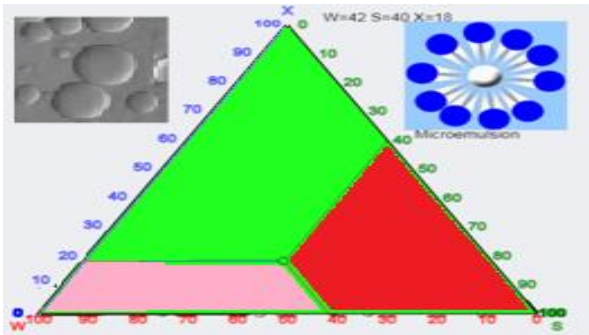


Figure 5: F5 Pseudoternary phase diagram

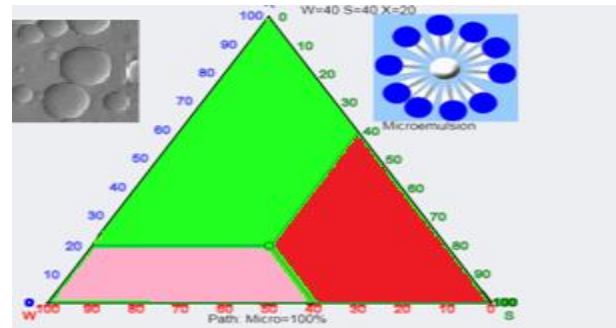


Figure 6: F6 Pseudoternary phase diagram

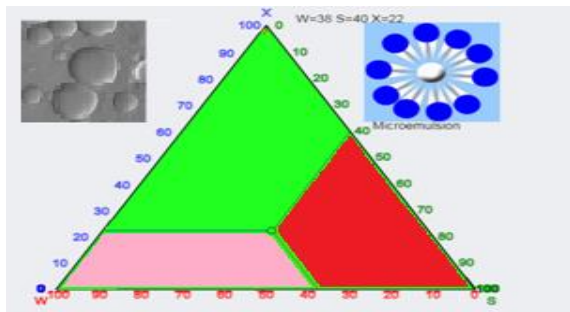


Figure 7: F7 Pseudoternary phase diagram

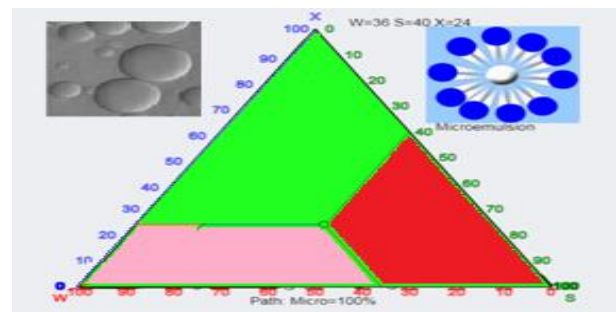


Figure 8: F8 Pseudoternary phase diagram

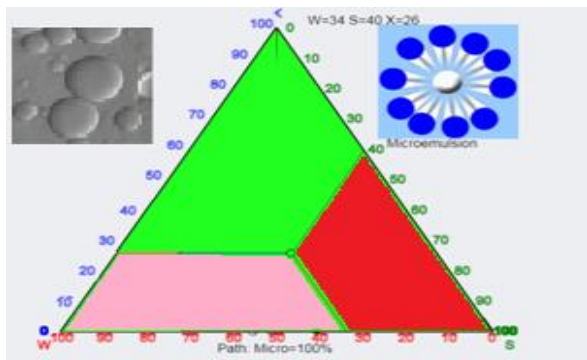


Figure 9: F9 Pseudoternary phase diagram

III. Characterisation study microemulsion:

Appearance:

The prepared batches of microemulsion were visually observed for clarity or any signs of settling. The appearance of the microemulsion formulations was determined by visual inspection of the formulation under light, alternatively on a white and black background, and the turbidity was checked. The test was performed as described in the United States Pharmacopoeia. ^[13]

Centrifugation:

To eliminate metastable systems, selected microemulsions containing the drug were centrifuged (Research Centrifuge, R-24, Remi Instruments Limited, Mumbai) at 4000 rpm for 4 hours. ^[14]

Stress test:

These tests were done to upgrade the best microemulsion plan under outrageous conditions. Stress test was carried out at 4°C and 45°C for 48 hours each for a time of six cycles, trailed by 25°C and 21°C for 48 h for around three cycles. The examples were checked for coalescence, cracking or phase separation. ^[15]

Refractive index:

The refractive index of the system was measured using a simple Abbe refractometer, placing 1 drop of microemulsion on a glass slide. ^[16]

Optical clarity (Percentage transmittance):

Transparency of microemulsion plan and its diluted forms (10 and 100 times with distilled water) was controlled by estimating rate conveyance through ultraviolet spectrophotometer (UV-1601-220x, Shimadzu). Rate conveyance of tests was estimated at 301 nm utilizing filtered water as blank. ^[17]

Assay:

The accurately measured volume (100 µl) of the optimized formulation was diluted with methanol to 100 ml and the absorbance was measured at 301 nm using methanol as a blank. The analysis was carried out in triplicate. ^[18]

Dilutability and dye solubility test:

Dye solubility test done by Water soluble dye, methylene blue solution of 10µl was added to the microemulsion. If the continuous phase water (oil/water emulsion), the colorant will dissolve evenly throughout the system. If the continuous phase is oil (w/o emulsion), the dye will remain as cluster on the surface of the system. ^[19]

Viscosity and Surface tension:

The rheological properties of the microemulsion are evaluated by Brookfield viscometer with spindle SC 3 even if the system is water in oil (w/o) or oil in water (o/w). If system has low viscosity then it is o/w type of the system and if a high viscosity then it is w/o type of the system. The surface tension of microemulsion was measured at 25°C with a Torsion balance. ^[20]

Conductivity measurement:

Conductometer (Equip-Tronics, EQ-664, Mumbai, India) was used for determination of the electrical conductivity of the microemulsion. Conductometer equipped with an inbuilt magnetic stirrer. This was done using a conduction cell (with a cell constant of 1.0) contains two platinum plates separated by a desired distance and having a liquid between the platinum plates acting as a conductor. ^[21]

% Drug content estimation:

A microemulsion containing 100 mg of glyburide was dissolved in 100 ml of 0.1 N. HCl taken in a volumetric flask. Then the solvent was filtered off, 1 ml was taken into 50 ml of a volumetric solution, diluted to the mark with 0.1 N. HCl and analyzed spectrometrically at 301 nm. The concentration of glyburide in mg / ml was obtained using a standard drug calibration curve. Studies of the drug content were carried out in triplicate for each batch of the drug. ^[22]

Droplet size distribution and zeta potential Determination:

Droplet size distribution, polydispersity index (PDI) and zeta potential of the microemulsion was determined by using, Nano Malvern droplet analyzer (UK) and zeta potential analyzer. Poly disperse partials have major hurdles in the drug diffusion they reduce the rate of diffusion of the formulation and also due to polydispersibility, large partials having a low laplas pressure undergoes Ostwald ripening and aggregation of the particle take place in the formulation. Wavelength scattering angle 90° at 25°C, average hydrodynamic diameter of the microemulsion was derived from cumulative analysis by the auto-measure software. ^[22]

pH measurement:

The pH values of all the formulations were measured by immersing the electrode directly into the dispersion using a calibrated pH meter (Digital Potentiometer Model EQ-601 Equip-Tronics). ^[23]

Entrapment efficiency and drug loading:

Glyburide loaded microemulsion entrapment efficiency was evaluated by a centrifugation method. For a short time, 10 µL 0.1 mol/L HCl was added into drug-loaded microemulsion and centrifugation for 10 min at 20,000 rpm. The supernatant was separated and the pellets were washed three times with distilled water. The free drug concentration was determined by UV-visible spectrum photometric

analysis at 301 nm. The entrapment efficiency and drug loading were calculated by formula given below. All the measurements were performed in triplicate. ^[13]

$$\text{Entrapment efficiency (\%)} = [(W_a - W_s) / W_a] \times 100$$

$$\text{Drug loading (\%)} = [(W_a - W_s) / (W_a - W_s + W_l)] \times 100$$

Where W_a is the amount of drug added to formulation, W_s is the amount of free drug, and W_l is the weight of oil phase.

Differential scanning calorimetry measurements (DSC):

DSC measurements were performed with DSC TA Q100 instrument equipped with a refrigerated cooling system. Nitrogen with a flow rate of 50 ml/min was used as purge gas. Approximately 4 to 13 mg of sample was weighed precisely into hermetic aluminum pans. An empty hermetically sealed pan was used as a reference. The samples were cooled from 25 °C to -50°C at a cooling rate of 5°C / min, held for 3 min at -50°C, and then heated to 25°C at a heating rate of 10°C / min. All measurements were performed in triplicate. ^[13]

Morphological analysis of Microemulsion by SEM:

The outer morphological structure of the microemulsion was researched by Scanning Electron Microscopy with a S4800 Type II examining electron microscope (Hitachi high innovations, Japan), working at 15kV. Sample was fixed on a SEM stub utilizing twofold sided adhesive tape and afterward covered with a thin layer of gold. The outer morphological structure of the microemulsion as examined by Scanning Electron Microscopy with a scanning electron microscope (FEI, the Netherlands), working at 15kV. The sample was fixed on a SEM stub utilizing twofold sided adhesive tape and afterward covered with a slim layer of gold. ^[24]

FTIR spectroscopy:

It was determined by Fourier Transform Infrared Spectro-photometer (FTIR, Simadzu Corporation). The sample was scanned over wavelength region of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} by dispersing sample in KBr and compressing into the disc by applying pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in light path and the spectrum was obtained. ^[25]

Kinetics of Drug Release:

In vitro dissolution has been recognized as an important element in drug development. To analysis the mechanism for the release and release rate kinetics of the formulated dosage form, the data obtained from conducted studies was fitted into Zero order, First order, Higuchi matrix, Korsmeyer- Peppas and Hixson Crowell model. (Table 1) In this by comparing the r-values obtained, the best-fit model was selected. [26]

Stability studies of optimized formulation:

Stability studies were completed for advanced detailing for 6 months at 37 ± 2 °C and 04 ± 2 °C as per ICH rule in a controlled chamber. The example was investigated intermittently for actual appearance, rheological properties, pH, and rate discharge by UV-Visible spectrophotometer at 301 nm.

IV. Results and discussion:

Pre-formulation Studies:

Preliminary studies are preliminary studies for understanding physical and chemical properties. Behavior of the new drug and possible obstacles in choosing a dosage form development. It generates auxiliary data for necessary changes in design, develop and evaluate formulations. To find out the wavelength maximum absorption (λ_{max}) of the drug solution of the drug (10 μg / ml) in ethanol was scanned with a spectrophotometer within 400-200 nm wavelength range relative to ethanol as a blank. Absorption The curve shows the characteristic absorption maxima at λ_{max} 301 nm for glyburide. Calibration Curve for Evaluation of the Prepared Product in ethanol, as well as in buffer pH 7.4 as the dissolution media at 301 nm.

| S. No | Concentration ($\mu\text{g}/\text{ml}$) | Absorbance(nm) |
|-------|---|----------------|
| 1 | 2 | 0.110 |
| 2 | 4 | 0.201 |
| 3 | 6 | 0.325 |
| 4 | 8 | 0.416 |
| 5 | 10 | 0.512 |

Table No 2: Data for Calibration curve

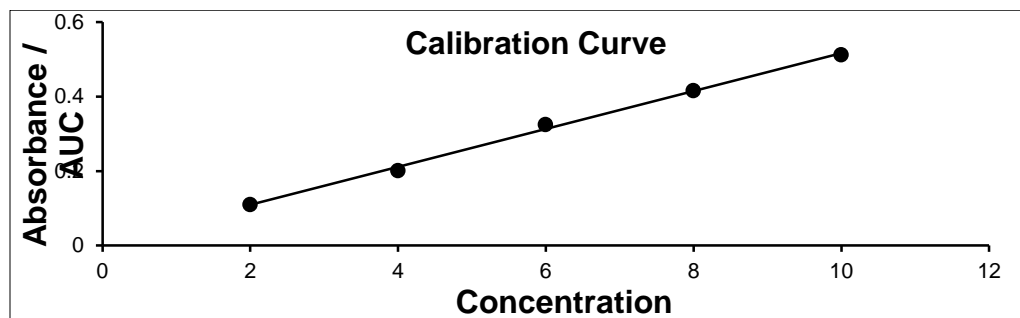


Figure no 10: Calibration curve

Solubility study of Glyburide (GLY) in Oil, Surfactants and Co- Surfactants:

Among all screened oils, the most remarkable solubilisation limit was displayed by Capmul MCM (37.551 mg/ml) was chosen for additional examination. From the results of screening studies, it was observed that, Co Surfactant span-20 found to have very good solubilising capacity compared to Propylene Glycol and *n*-butanol. Span-20 selected co-surfactant also shows good emulsification with selected oil and Tween 80. Aftereffects of solubility are summed up in table and Figure

| Phase type | Excipient | Solubility (mg/ml) | Phase type | Excipient | Solubility (mg/ml) | Phase type | Excipient | Solubility (mg/ml) |
|------------|-----------------|--------------------|-------------|--------------|--------------------|----------------|-------------------|--------------------|
| Oils | Labrafac CC | 32.871 | Surfactants | Captex-355 | 8.871 | Co-Surfactants | <i>n</i> -butanol | 0.331±0.01 |
| | Capmul MCM | 37.551 | | Cremophor EL | 9.551 | | Propylene glycol | 0.231±0.01 |
| | cotton seed oil | 28.744 | | Tween-80 | 14.744 | | span-20 | 7.705±0.02 |
| | sunflower oil | 27.545 | | Labrafil | 8.305 | | | |
| | soybean oil | 30.11 | | PEG 400 | 7.09 | | | |

Table No 3: Solubility study of Glyburide (GLY) in Oil, Surfactants and Co- Surfactants

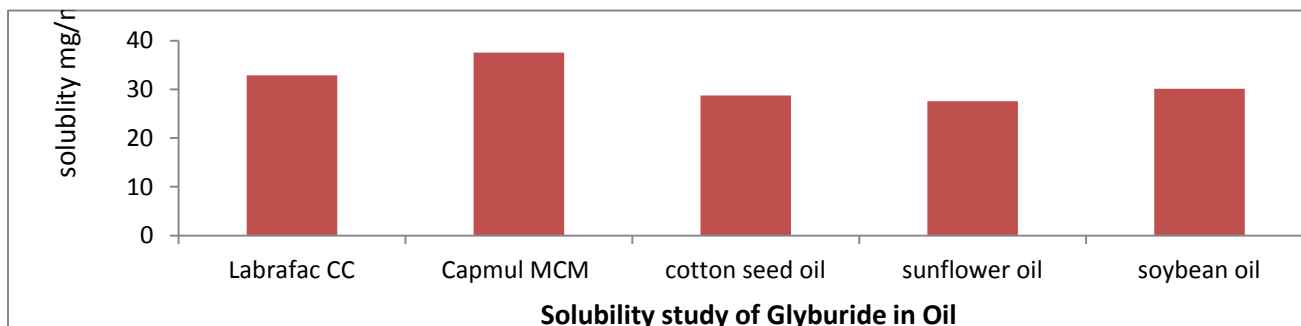


Figure no 11: Solubility study of Glyburide (GLY) in Oil

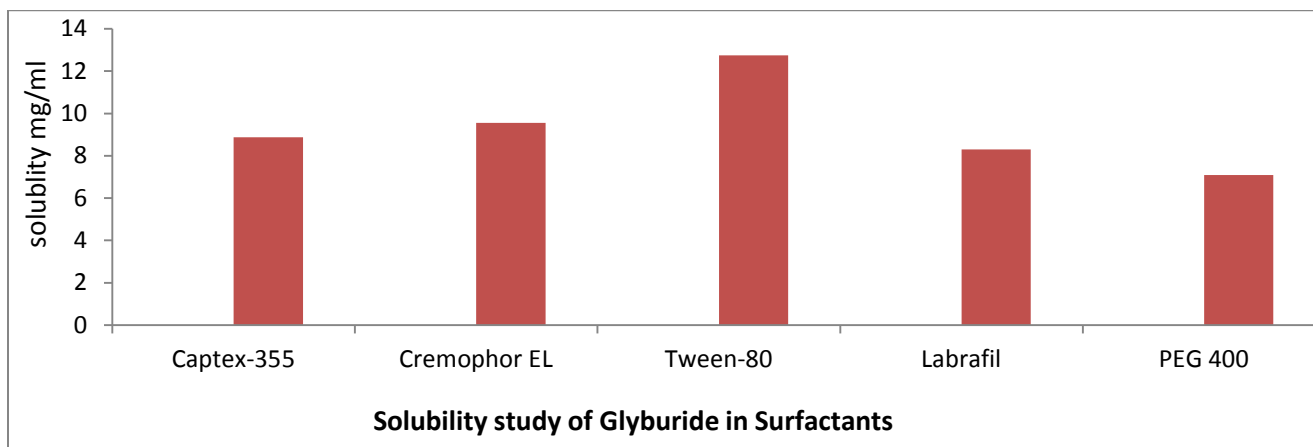


Figure no 12: Solubility study of glyburide Surfactants

FTIR spectroscopy for Drug –polymer interaction:

The spectrum of glyburide showed the following functional groups at their frequencies mentioned. The FT-IR range of the unadulterated medication glyburide was discovered to be like the standard range of glyburide. Further investigation of the similarity of the medication with excipients was explored utilizing FTIR spectroscopy. Pure glyburide shows major peak at IR spectra revealed no considerable change when compared that of glyburide microemulsion formulation prove that there is no drug and excipients interaction. The study of the interaction of excipients with drugs is very important to determine the compatibility of the selected excipients with active drugs. Incompatibility is actually the inactivation of an active drug due to degradation or conversion to a less effective physical or chemical form. When a mixture of two or more active drugs and excipients is mixed together, there is a possibility of interaction in terms of change in appearance, elegance, and, most importantly, the chemical composition of each other. To learn about chemical changes or interactions, chromatographic, spectroscopic, and thermal analyzes are usually preferred.

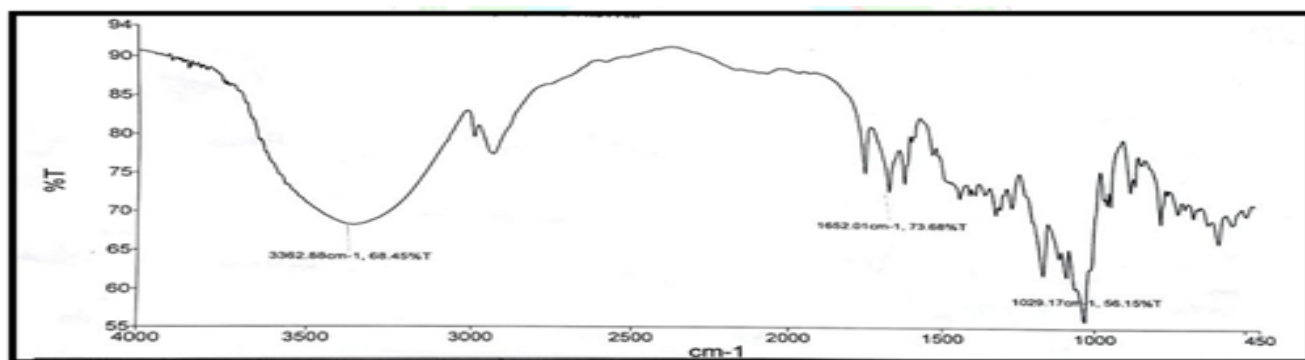


Figure no 13: -FT- IR spectrum of Glyburide

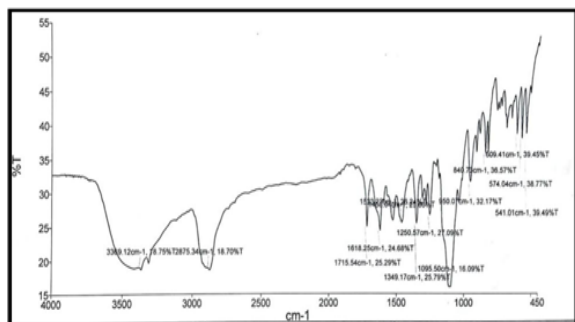


Figure no 14: FTIR spectrum of F3 formulation

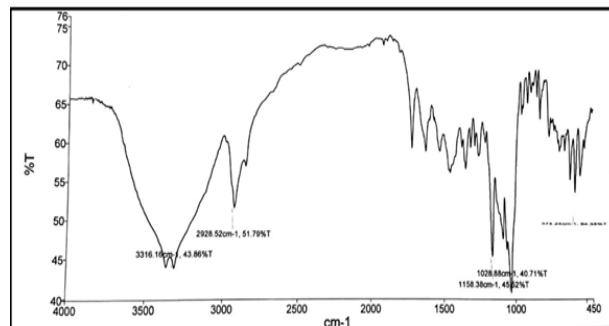


Figure no 15: FTIR spectrum of F5 formulation

V. Pharmaceutical Evaluation:

Physical appearance and Phase separation:

The microemulsions were checked for transparency until they were turbid. Microemulsions remained clear when diluted, due to the presence of oils and surfactants microemulsions look transparent/translucent yellow colored solution.

Centrifugation test and stress tests:

All formulations detected clearly and there is no sign of precipitation. Centrifugation tests showed that the microemulsion formulations F1, F2, F3, F4, F5, F6 and F7 remained homogeneous without any phase separation. According to the following data, formulations passed through various stress conditions, as shown in Table 4. Formulations F1, F2, F3, F4, F5, F6 and F7 passed centrifugation and stress test were stable under all temperature conditions.

| S.NO | Formulation code | Physical appearance | Phase separation | Centrifugation test | Observations of stress tests | | |
|------|------------------|---------------------|------------------|---------------------|------------------------------|-------|------|
| | | | | | 25 to 30 °C | 45 °C | 4 °C |
| 1 | F1 | Clear Liquid | No | Y | ✓ | ✓ | ✓ |
| 2 | F2 | Clear Liquid | No | Y | ✓ | ✓ | X |
| 3 | F3 | Clear Liquid | No | Y | ✓ | ✓ | ✓ |
| 4 | F4 | Clear Liquid | No | Y | ✓ | ✓ | ✓ |
| 5 | F5 | Clear Liquid | No | Y | ✓ | ✓ | ✓ |
| 6 | F6 | Clear Liquid | No | Y | ✓ | ✓ | ✓ |

| | | | | | | | |
|---|----|--------------|----|---|---|---|---|
| 7 | F7 | Clear Liquid | No | Y | ✓ | ✓ | ✓ |
| 8 | F8 | Clear Liquid | No | N | ✓ | X | X |
| 9 | F9 | Clear Liquid | No | N | ✓ | ✓ | ✓ |

✓ - physically and chemically stable, x- physically and chemically unstable

Y= denote maintenance of homogeneity of prepared microemulsion.

N = denote separation of component of microemulsion concentrate

Table No 4: Results of Physical appearance, Phase separation, Centrifugation test and Observations of stress tests

Dye-solubility test:

Water soluble dye, methylene blue solution was added to optimized microemulsion formulations F1 to F9, the dye will dissolve uniformly throughout the system, so the continuous phase was water. Hence the optimized formulations were found to be o/w type of microemulsion.

Viscosity:

Microemulsion formulations F1 to F5 shows viscosity value of $110 \pm .51$ cp to $129 \pm .72$ cp Low viscosity of the formulation indicates that formulation is o/w type and having Newtonian flow ensures easy handling and packing.

Refractive index:

The refractive index of the systems was in the 1.338 to 1.462. It reflects that the microemulsion is almost transparent in the visible spectrum and very little scattering low refractive index.

Optical Clarity:

The % Transmittance of the systems was found to be in range from 96.2 to 99.2.

Surface tension:

The surface tension data implies water-in-oil microemulsions because surface tension amounts of microemulsion are nearby to oil phase surface tension.

pH:

The pH of the composition affects not only the stability of emulsions but also alters the solubility and bioavailability of the drug by emulsion at the point of penetration. The pH of all microemulsion ranged from 5.2 to 6.8 in Table 6 which corresponds to the normal pH range of GIT fluids.

| Formulation code | Refractive index (RI) | Optical Clarity (% Transmittance) | Assay \pm S.D* (%) | Dye solubility | Viscosity(cp) | Surface tension (dynes/cm) | pH of Formulation |
|------------------|-----------------------|-----------------------------------|----------------------|----------------|---------------|----------------------------|-------------------|
| F1 | 1.426 | 96.2 | 96.30 \pm 0.37 | ✓ | 116 \pm .12 | 41.23 \pm 0.61 | 5.2 |
| F2 | 1.462 | 98.2 | 95.81 \pm 0.26 | ✓ | 110 \pm .51 | 43.53 \pm 1.04 | 5.5 |
| F3 | 1.426 | 97.6 | 97.04 \pm 0.14 | ✓ | 115 \pm .23 | 41.62 \pm 0.87 | 6.5 |
| F4 | 1.438 | 96.8 | 91.30 \pm 0.32 | ✓ | 122 \pm .13 | 44.09 \pm 1.53 | 7.3 |
| F5 | 1.396 | 97.2 | 97.67 \pm 0.02 | ✓ | 117 \pm .19 | 43.61 \pm 1.10 | 6.2 |
| F6 | 1.418 | 99.2 | 92.21 \pm 0.19 | ✓ | 129 \pm .72 | 42.80 \pm 1.44 | 5.9 |
| F7 | 1.462 | 96.6 | 88.22 \pm 0.12 | ✓ | 119 \pm .17 | 41.68 \pm 1.22 | 6.1 |
| F8 | 1.338 | 98.4 | 88.34 \pm 0.45 | ✓ | 118 \pm .23 | 40.78 \pm 1.04 | 6.3 |
| F9 | 1.398 | 96.7 | 89.91 \pm 0.12 | ✓ | 113 \pm .01 | 38.67 \pm 1.14 | 6.8 |

✓ - o/w type of microemulsion

Table No 5: Results of Viscosity (cp), Refractive index (RI), Dye solubility, Optical Clarity (% Transmittance), Assay \pm S.D* (%), Surface tension (dynes/cm) and pH

Conductivity measurement:

The results of measuring electrical conductivity are shown in Table no 6. Water is a better conductor of electricity than oil, when the microemulsion contains water in the continuous phase, then the conductivity value is high and it will decrease when the oil is in the continuous phase. ^[27]

Drug Content:

Microemulsion equivalent to 10 mg of glyburide was dissolved in an appropriate amount of ethanol (100 ml). The samples were thoroughly mixed to dissolve the drug in ethanol and analyzed using a Shimadzu 1800A UV-Vis spectrophotometer at 301 nm. During the evaluation, the drug content was found to be in the 90.37 to 99.23% range Table no 6 Optimized lot F3 showed 99.23% drug content.

Droplet size distribution and zeta potential Determination:

Measuring particle size distribution and understanding how it affects products and processes can be critical to the success of manufacturing. It suggested that the zeta potential can serve as a partial indicator of the physical stability of the resulting emulsions. Most prepared microemulsions should preferably achieve high absolute values of the zeta potential (\pm 30 mV) to ensure the creation of a high

energy barrier against coalescence of dispersed droplets. Microemulsion usually has a small particle size (<200 nm) compared to emulsions. [20, 27]

| Formulation code | Conductivity | (%)Drug Content | Polydispersity index (PDI) | %Drug loading | Entrapment efficiency (%) | Particle size (nm) | Zeta Potential (mv) |
|------------------|--------------|-----------------|----------------------------|---------------|---------------------------|--------------------|---------------------|
| F1 | 0.183 | 91.06 | 0.447 | 71.12 ± 0.05 | 55.5±2.4 | 480±78 | 26.20±0.2 |
| F2 | 0.192 | 90.56 | 0.279 | 73.70 ± 0.15 | 73.6±0.8 | 625±55 | 27.80±0.4 |
| F3 | 0.189 | 99.23 | 0.368 | 74.10 ± 0.71 | 85.3±0.8 | 460±42 | 27.90±0.6 |
| F4 | 0.199 | 98.11 | 0.478 | 80.23 ± 0.08 | 84.3±3.6 | 520±76 | 28.12±0.3 |
| F5 | 0.201 | 96.10 | 0.839 | 77.54 ± 0.34 | 85.3±2.9 | 360 ±12 | 28.30±0.1 |
| F6 | 0.176 | 95.97 | 0.575 | 69.64 ± 0.34 | 85.6±1.2 | 710±82 | 29.40±0.7 |
| F7 | 0.180 | 93.38 | 0.478 | 76.30 ± 0.21 | 84.9±1.4 | 814±62 | 31.30±0.4 |
| F8 | 0.179 | 93.71 | 0.612 | 70.44 ± 0.61 | 84.7±3.9 | 650±59 | 28.40±0.3 |
| F9 | 0.191 | 90.37 | 0.536 | 75.51 ± 0.92 | 83.3±4.5 | 580±71 | 27.23±0.6 |

Table No 6: Results of Conductivity, (%)Drug Content, Polydispersity index (PDI), %Drug loading, Entrapment efficiency, (%) Particle size (nm) and Zeta Potential (mv)

Dissolution study of different batches:

The results of in vitro dissolution profiles of various glyburide microemulsion formulations are shown in Table. It is evident from the table that glyburide microemulsion showed more than 90% GLY released. The USP recommends pH 7.4 buffers as a dissolution medium for glyburide.

| S.No | Time in hours | % Drug release of F1 | % Drug release of F2 | % Drug release of F3 | % Drug release of F4 | % Drug release of F5 | % Drug release of F6 | % Drug release of F7 | % Drug release of F8 | % Drug release of F9 |
|------|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1.5 | 32.871 | 37.551 | 36.744 | 27.545 | 29.804 | 34.640 | 38.842 | 30.346 | 36.762 |
| 3 | 3 | 42.555 | 48.709 | 45.395 | 43.501 | 45.868 | 51.390 | 51.540 | 54.735 | 54.550 |
| 4 | 4.5 | 51.303 | 59.746 | 54.541 | 54.695 | 54.387 | 59.010 | 60.093 | 65.858 | 66.856 |
| 5 | 6 | 59.599 | 70.896 | 62.762 | 62.762 | 66.071 | 70.444 | 72.101 | 70.939 | 72.822 |
| 6 | 7.5 | 66.033 | 79.428 | 72.284 | 67.819 | 70.498 | 74.517 | 83.149 | 76.070 | 80.013 |
| 7 | 9 | 71.854 | 84.794 | 82.441 | 78.030 | 80.236 | 82.00 | 87.440 | 83.290 | 91.942 |
| 8 | 10.5 | 77.950 | 90.005 | 86.665 | 82.307 | 87.826 | 84.922 | 93.055 | 91.165 | 94.069 |
| 9 | 12 | 86.742 | 95.062 | 91.189 | 90.902 | 90.759 | 90.328 | 97.931 | 97.767 | 96.708 |

Table no 9: Results of % Drug release of F1 to F9 microemulsion batches

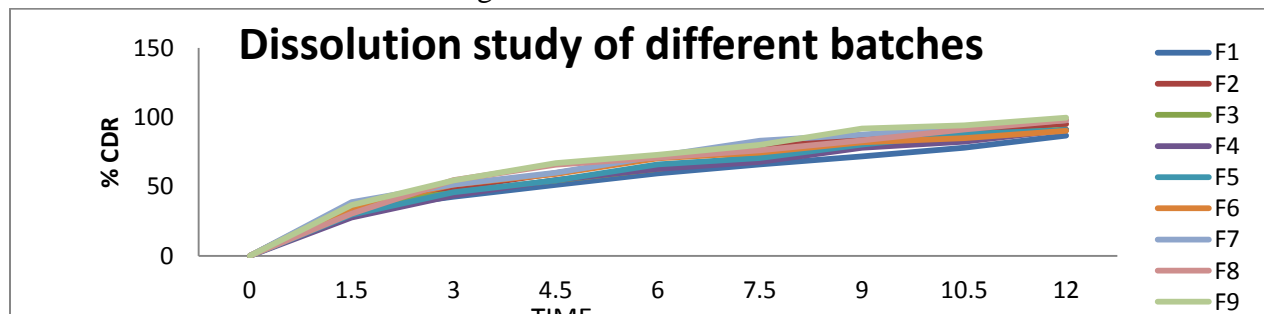


Figure no 16: Dissolution study of F1 to F9 microemulsion batches

Kinetic of Drug release:

To study the release kinetics, data from in vitro drug release studies F3 and F5 series glyburide microemulsion formulations showed Higuchi's model as the most suitable. Using the Korsmeyer and Peppas equations, the n values were 0.551 and 0.543, respectively Table no 10. This value is a characteristic of abnormal kinetics (non-Fick transfer).^[28, 29, 30]

| S.No. | R ² Value | | | | | n Value |
|-------|----------------------|-------------|---------------|--------------------------------|------------------------|---------|
| | Zero order | First order | Higuchi model | Hixson crowell cube root model | Korsmeyer peppas model | |
| F1 | 0.993 | 0.973 | 0.978 | 0.989 | 0.983 | 0.740 |

| | | | | | | |
|----|-------|-------|-------|-------|-------|-------|
| F2 | 0.980 | 0.947 | 0.967 | 0.975 | 0.959 | 0.587 |
| F3 | 0.927 | 0.978 | 0.980 | 0.976 | 0.983 | 0.551 |
| F4 | 0.959 | 0.984 | 0.989 | 0.979 | 0.990 | 0.560 |
| F5 | 0.922 | 0.993 | 0.986 | 0.993 | 0.987 | 0.543 |
| F6 | 0.886 | 0.981 | 0.941 | 0.930 | 0.957 | 0.458 |
| F7 | 0.982 | 0.965 | 0.979 | 0.980 | 0.982 | 0.646 |
| F8 | 0.875 | 0.980 | 0.953 | 0.933 | 0.977 | 0.476 |
| F9 | 0.903 | 0.970 | 0.940 | 0.910 | 0.989 | 0.485 |

Table No 10: Results of Kinetic of Drug release

Morphological analysis of Microemulsion by SEM:

The surface morphology of the glyburide microemulsion was studied using SEM.²¹ The images of F3 and F5 shown in Figure no13 and Figure no 14 shows well-separated particles without agglomeration compared to other batches. Besides particle size, particle shape can also have a significant impact on the performance and handling of many solid particles. Spherical shape particle, without tailing indicates the uniformity of the particle size. In addition, SEM images revealed the absence of crystalline structure of glyburide in microemulsion formulation. [20, 27]

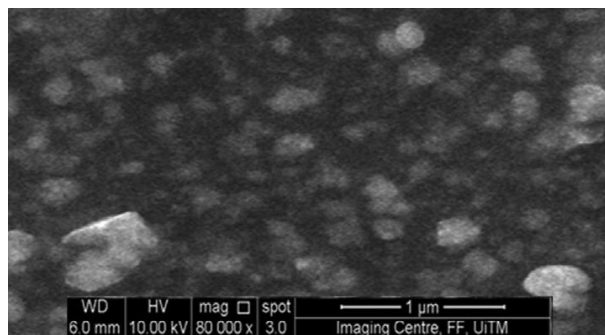


Figure no17: Scanning Electron Microscope of F3

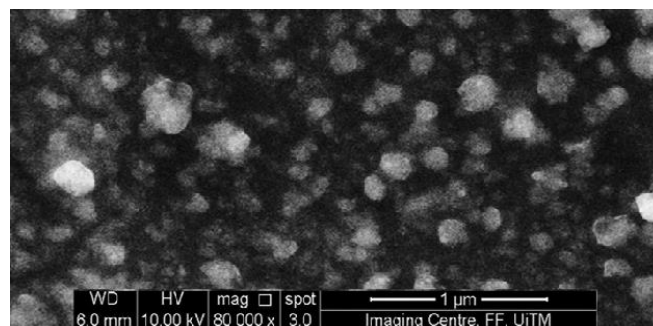


Figure no18: Scanning Electron Microscope of F5

Differential scanning calorimetric study (DSC):

Formulation F5 showed endotherms at 123.5°C and 257°C. Placebo composition F5 showed endotherms at 128.6°C and 254.2°C. Tween80 showed an endothermic effect at 212°C. F3 endotherm at 247 ° C and placebo endotherm at 254.2°C was associated with the presence of Tween80 in the formulations. No drug peak was observed in F3 and F5 indicating that the drug was completely dissolved in the formulation.

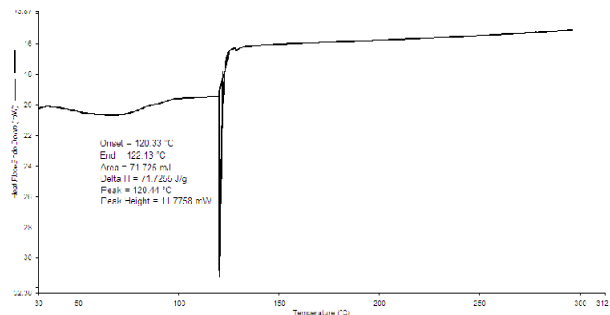


Figure no19: DSC of F3

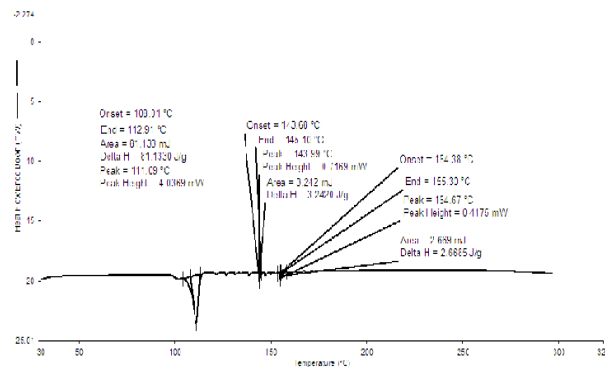


Figure no 20: DSC of F5

Stability studies:

Stability studies were completed for advanced detailing for 6 months at 37 ± 2 °C and 04 ± 2 °C as per ICH rule in a controlled chamber. The example was investigated intermittently for actual appearance, rheological properties, pH, and rate discharge by UV-Visible spectrophotometer at 301 nm. The physical appearance of the preparation was good without any phase separation or turbidity. The average pH of 5.6, viscosity of $122 \pm .13$ cps and no considerable change in the percentage release, i.e., 95% was observed for 6 months.

V. Conclusion:

Mixing different concentrations of oils significantly increases the solubilizing capacity for poorly water-soluble drug glyburide. This interesting observation was explained by the hypothesis of non-ideal mixing of oils and their penetration into the surfactant layers. The phase diagrams suggest that the composition for administration can be formulated as an oil / surfactant mixture and water-in-oil microemulsions. Nine formulations were prepared which should help improve the bioavailability of poorly soluble drugs. Formulations F3 and F5 containing Capmul MCM, tween 80, Span20 and distilled water selected as best formulation which is a transparent and low viscosity system, with a particle size 360 ± 12 . There is no sign of drug and polymer interaction studied by FTIR. Conductivity studies have revealed structural changes from w/o to o/w through the bicontinuous phase. For the selected compositions, centrifugation test, Stress test, Dye solubility, refractive index, ph, particle size, viscosity, % transmission, zeta potential were studied. F5 were optimized DSC stability studies showed that the formulation was stable. The stability studies confirmed that the optimized microemulsion was stable for six months. Thus, despite of effectiveness of microemulsion based delivery system for improvement of solubility and bioavailability of glyburide, benefits to the risk ratio of the developed

formulation via clinical investigation will only decide its suitability in the actual clinical practice. To find out the pharmacokinetic and pharmacodynamic parameters of the optimized drug, additional *in vivo* testing is required.

VI. References:

1. Groop L, Wahlin-Boll E, Totterman KJ, Melander A, Tolppanen EM, Fyhrqvist F. Pharmacokinetics and metabolic effects of glibenclamide and glipizide in type 2 diabetes. *Eur J Clin Pharmacol* 1985;28:697–704.
2. Wei H, Lobenberg RL. Biorelevant dissolution media as a predictive tool for glyburide a class II drug. *Eur J Pharm Sci* 2006;19:45–62.
3. Chalk JB, Patterson M, Smith MT, Eadie MJ. Correlations between *in vitro* dissolution, *in vivo* bioavailability and hypoglycaemic effect of oral glibenclamide. *Eur J Clin Pharmacol* 1986;31:177–82.
4. Yogeshwar G, Bachhav and Vandana B. Patravale. “SMEDDS of Glyburide: Formulation, *In Vitro* Evaluation, and Stability Studies”. American Association of Pharmaceutical Scientists, Vol. 10, No. 2, June 2009 /0200-0482/0
5. Itoh K, Tozuka Y, Oguchi T, Yamamoto K. Improvement of physicochemical properties of N-4472. Part I: formulation design by using self microemulsifying system. *Int J Pharm.* 2002; 238:153-160.
6. Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolizedglycerides for improving *in vitro* dissolution and oral absorption of lipophilic drugs. *Int J Pharm.* 1994; 106:15-23.
7. García-Celma MJ, Azemar N, Pes MA, Solans C. Solubilization of anti-fungal drugs in water/POE(20) sorbitan monoleate/oil systems. *Int J Pharm.* 1994; 105:77-81.
8. Thevenin MA, Grossiord JL, Poelman MC. Sucrose esters/cosurfactant microemulsion systems for transdermal delivery: assessment of bicontinuous structures. *Int J Pharm.* 1996; 137:177-186.
9. Corsawant CV, Thoren P, Engstrom S. Triglyceride based microemulsion for intravenous administration of sparingly soluble substances. *J Pharm Sci.* 1998; 87:200-208.
10. Li L, Nandi I, Kim KH. Development of an ethyl laurate- based microemulsion for rapid-onset intranasal delivery of diazepam. *Int J Pharm.* 2002; 237:77-85.

11. Vandana Patel, Hirenkumar Kukadiya, Rajshree Mashru, Naazneen Surti and Surjyanarayan Mandal. Development of Microemulsion for Solubility Enhancement of Clopidogrel. Iranian Journal of Pharmaceutical Research (2010), 9 (4): 327-334
12. V. Wilson, X. Lou, D.J. Osterling, D.F. Stolarik, G. Jenkins, W. Gao, G.G.Z. Zhang, L.S. Taylor, Relationship between amorphous solid dispersion in Vivo absorption and in Vitro dissolution: phase behavior during dissolution, speciation, and membrane mass transport, J. Contr. Release 292 (2018) 172–182.
13. Podlogar F, Gasperlin M, Tomsic M, Jamnik A, Rogac MB. Structural characterisation of water-Tween 40/Imwitor 308-isopropyl myristatemicro emulsions using different experimental methods. Int J Pharm. 2004;276(1-2):115-28.
14. Lavanya N, Aparna C and Umamahesh B. “Formulation and evaluation of glipizide microemulsion”. Int J Pharm Pharm Sci, Vol 8, Issue 8, 171-176
15. Vishal Yadav, Prakash Jadhav, Kishor Kanase, Anjali Bodhe and Shailaja Dombe. “Preparation and evaluation of microemulsion containing antihypertensive drug”. Int J App Pharm, Vol 10, Issue 5, 2018, 138-146
16. Podlogar F, Gasperlin M, Tomsic M, Jamnik A, Rogac MB. Structural characterisation of water-Tween 40/Imwitor 308-isopropyl myristatemicro emulsions using different experimental methods. Int J Pharm. 2004;276(1-2):115-28.
17. Shailendra Singh Solanki et al. “Microemulsion Drug Delivery System: For Bioavailability Enhancement of Ampelopsin”. International Scholarly Research Network, ISRN Pharmaceutics. Volume 2012, Article ID 108164
18. Pavithra DC, Sivasubramanium L. New spectrophotometric determination of Raloxifene HCl in tablets. Indian J Pharm Sci 2006;68:375-6.
19. Divya A, Ch Praveen Kumar, K Gnanaprakash, M Gobinath. Design, formulation and characterization of tenofovir microemulsion as oral drug delivery. Int J Pharm Rev Res 2014;4:1-5.
20. Alany RG, Tucker IG, Davies NM, Rades T. Characterizing colloidal structures of pseudo ternary phase diagrams formed by oil/water/amphiphile systems. Drug Dev Ind Pharm. 2001;27(1):31-8.
21. Eskandar Moghimipour, Anayatollah Salimi, Soroosh Eftekhari. Design and Characterization of Micro emulsion Systems for Naproxen. Adv Pharm Bull. 2013;3(1):63-71.
22. Kale and Deore. “Solubility Enhancement of Nebivolol” .Journal of Young Pharmacists, Vol 8, Issue 4, Oct-Dec, 2016

23. Ghosh PK, Murthy RS. Microemulsions: A potential drug delivery system. *Curr Drug Deliv* 2006;3:167-80.
24. Hintzen F, Perera G, Hauptstein S, Müller C, Laffleur F, Bernkop-Schnürch A. *In vivo* evaluation of an oral self-micro emulsifying drug delivery system (SMEDDS) for leuprorelin. *Int J Pharm.* 2014;10-472(1-2):20-6.
25. Henri LR, John LC, David LC, James HW. Microemulsions: a commentary on their preparation. *J Soc Cosmet Chem* 1988;39:201-9.
26. Shah BM, Misra M, Shishoo CJ, Padh H. Nose to brain micro emulsion-based drug delivery system of rivastig mine: formulation and ex-vivo characterization. *Drug Deliv.* 2015;22(7):918-30.
27. Zhu Y, Zhang J, Zheng Q, Wang M, Deng W, Li Q *et al.* *In vitro* and *in vivo* evaluation of capsaicin-loaded micro emulsion for enhanced oral bioavailability. *J Sci Food Agric.* 2015;95(13):2678-85.
28. Tandel H, Raval K, Nayani A, Upadhay M. Preparation and evaluation of cilnidipine micro emulsion. *J Phar m Bio allied Sci.*2012;4(Suppl 1):S114-5.
29. Hosny KM, Hassan AH. Intranasal *in situ* gel loaded with saquinavir mesylate nano sized micro emulsion: preparation, characterization, and *in vivo* evaluation. *Int J Pharm.* 2014;475(1-2):191-7.
30. Shah BM, Misra M, Shishoo CJ, Padh H. Nose to brain micro emulsion-based drug delivery system of rivastig mine: formulation and ex-vivo characterization. *Drug Deliv.* 2015;22(7):918-30.
31. Pal N, Mandal S, Shiva K, Kumar B. Pharmacognostical, Phytochemical and Pharmacological Evaluation of *Mallotus philippensis*. *Journal of Drug Delivery and Therapeutics.* 2022 Sep 20;12(5):175-81.
32. Singh A, Mandal S. Ajwain (*Trachyspermum ammi* Linn): A review on Tremendous Herbal Plant with Various Pharmacological Activity. *International Journal of Recent Advances in Multidisciplinary Topics.* 2021 Jun 9;2(6):36-8.
33. Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. *Plant Arch.* 2021;21:1345-54.
34. Mandal S, Shiva K, Kumar KP, Goel S, Patel RK, Sharma S, Chaudhary R, Bhati A, Pal N, Dixit AK. Ocular drug delivery system (ODDS): Exploration the challenges and approaches to improve ODDS. *Journal of Pharmaceutical and Biological Sciences.* 2021 Jul 1;9(2):88-94.

35. Ali SA, Pathak D, Mandal S. A REVIEW OF CURRENT KNOWLEDGE ON AIRBORNE TRANSMISSION OF COVID-19 AND THEIR RELATIONSHIP WITH ENVIRONMENT. *International Journal of Pharma Professional's Research (IJPPR)*. 2023;14(1):1-5.
36. Shiva K, Mandal S, Kumar S. Formulation and evaluation of topical antifungal gel of fluconazole using aloe vera gel. *Int J Sci Res Develop*. 2021;1:187-93.
37. Vishvakarma P, Mandal S, Verma A. A REVIEW ON CURRENT ASPECTS OF NUTRACEUTICALS AND DIETARY SUPPLEMENTS. *International Journal of Pharma Professional's Research (IJPPR)*. 2023;14(1):78-91.
38. Ali S, Farooqui NA, Ahmad S, Salman M, Mandal S. CATHARANTHUS ROSEUS (SADABAHAR): A BRIEF STUDY ON MEDICINAL PLANT HAVING DIFFERENT PHARMACOLOGICAL ACTIVITIES. *Plant Archives*. 2021;21(2):556-9.
39. MANDAL S, JAISWAL DV, SHIVA K. A review on marketed Carica papaya leaf extract (CPL) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. *International Journal of Pharmaceutical Research*. 2020 Jul;12(3).
40. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: An Analysis Of The Medicinal Properties Of The Plant. *Journal of Pharmaceutical Negative Results*. 2023 Jan 1:1595-600.
41. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. *Journal of Pharmaceutical Negative Results*. 2022 Dec 31:9189-98.
42. Mandal S, Pathak D, Rajput K, Khan S, Shiva K. THROMBOPHOB-INDUCED ACUTE URTICARIA: A CASE REPORT AND DISCUSSION OF THE CASE. *International Journal of Pharma Professional's Research (IJPPR)*. 2022;13(4):1-4.
43. Mandal S, Shiva K, Yadav R, Sen J, Kori R. LEIOMYOSARCOMA: A CASE REPORT ON THE PREOPERATIVE DIAGNOSTIC CRITERIA. *International Journal of Pharma Professional's Research (IJPPR)*. 2022;13(4):1-4.
44. Mandal S, Vishvakarma P, Mandal S. Future Aspects And Applications Of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. *European Journal of Molecular & Clinical Medicine*.;10(01):2023.
45. Chawla A, Mandal S, Vishvakarma P, Nile NP, Lokhande VN, Kakad VK, Chawla A. Ultra-Performance Liquid Chromatography (Uplc).

46. Mandal S, Raju D, Namdeo P, Patel A, Bhatt AK, Gupta JK, Haneef M, Vishvakarma P, Sharma UK. DEVELOPMENT, CHARACTERIZATION, AND EVALUATION OF ROSA ALBA L EXTRACT-LOADED PHYTOSOMES.
47. Mandal S, Goel S, Saxena M, Gupta P, Kumari J, Kumar P, Kumar M, Kumar R, Shiva K. Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat.
48. Shiva K, Kaushik A, Irshad M, Sharma G, Mandal S. EVALUATION AND PREPARATION: HERBAL GEL CONTAINING THUJA OCCIDENTALIS AND CURCUMA LONGA EXTRACTS.
49. Vishvakarma P, Mohapatra L, Kumar NN, Mandal S, Mandal S. An Innovative Approach on Microemulsion: A Review.