Section A-Research paper

FORMULATION AND CHARACTERIZATION OF SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM OFGLYBURIDE THE POORLY WATER-SOLUBLE DRUG

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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 SMEDDS. Pseudoternary phase diagrams diagram was utilized to identify the optimal excipient composition to formulate the SMEDDS system and the area of SMEDDS existence. Glyburide SMEDDS 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 SMEDDS was evaluated the pure drug in pH 7.4 buffers. The chemical stability of glyburide in SMEDDS was determined as per the International Council for Harmonisation (ICH) guidelines.
KEY WORDS: Glyburide; SMEDDS; solubility; stability; SEM; DSC; FTIR

Introduction

According to current estimates, about 40 percent of the novel medication candidates now in development are water insoluble and have low bioavailability. In order to address these issues, several formulation methods have been described, including the use of drug nanoparticles, solid dispersions, micronization, lipids, surfactants, complexation with cyclodextrin, and permeation enhancers, among others.[1]In addition to the requirement for mechanical aids, a complex production process, lengthy order processing, and regulatory complexity, the vast majority of these approaches have their limits[2]. In example, self-microemulsifying drug delivery systems (SMEDDS), which are lipid-based formulation approaches, are well-known for their promise as an alternate strategy for the delivery of hydrophobic medicines, which are linked with poor water solubility and oral bioavailability. In addition to enhancing bioavailability, it is sufficient to raise the solubility and dissolution rate of the drug in the gastro-intestinal fluids[3]. Drug delivery systems which self-microemulsify (SMEDDS) are isotropic combinations of drug, lipids, and surfactants that are typically combined with one or more hydrophilic co-solvents or co-emulsifiers. These systems are able to form fine (oil in water) emulsions in minutes when subjected to moderate agitation followed by dilution with aqueous medium. SMEDDS are often contained in gelatin capsules, which may be either firm or soft[4]. Capsule shell brittleness or softness may be caused by interactions among lipid formulations and the capsule shell. SMEDDS must be changed into Solid SMEDDS in order to solve this issue. Spray chilling, spray drying, adsorption onto solid carriers, melt granulation, melt extrusion, super-critical fluid-based processes, and high pressure homogenization are the primary techniques for converting SMEDDS to S-SMEDDS[5]. After being combined with water and gently stirred for a short period of time, SMEDDSs spontaneously produce oil-in-water (o/w) SMEDDSs. SMEDDSs are isotropic and thermodynamically stable solutions composed of an oil, a surfactant, a cosurfactant (CoS; or solubilizer), and a drug combination. It is the motility of the stomach and intestine that generates the agitation necessary for self-emulsification in vivo As SMEDDS spreads easily across the gastrointestinal system, digestive motility in the stomach and intestines provides the agitation required for self-emulsification to take place[6].

The adsorption method, on the other hand, is simple and requires just the addition of the liquid formulation to solid carriers while mixing in a blender. After being combined with water and gently stirred for a short period of time, SMEDDSs spontaneously produce oil-in-water (o/w)

SMEDDSs. SMEDDSs are isotropic and thermodynamically stable solutions comprised of an oil, a surfactant, a co-surfactant (CoS; or solubilizer), and a drug combination. It is the motility of the stomach and intestine that generates the agitation necessary for self-emulsification *in vivo* As SMEDDS spreads easily across the gastrointestinal system, digestive motion in the stomach and intestines provides the agitation required for self-emulsification to take place[7]. Beyond solubilization, the presence of lipids in the formulation contributes to improved bioavailability through influencing drug absorption. The evaluation of the solubility of the drug in various components, the efficient self-emulsifying region as determined by the phase diagram, and the droplet size distribution of the resultant emulsion following self-emulsification are all important factors in the selection of a suitable self-emulsifying formulation. Improved solubility and dissolution rate of glyburide (GLY), as a result, may either improve clinical effectiveness or decrease the oral dose needed to have the same effect[8]. As a result, we use the SMEDDS formulation, which contains oleic acid as oil, Tween 80 as a surfactant, and propylene glycol as a co-surfactant, in order to increase the solubility and dissolution rate of glyburide (GLY). The formulation was evaluated for its capacity to produce SMEDDSs based on droplet size, zeta potential, and dissolution properties, among other features[9].

MATERIALS AND METHODS:

Materials:

Glyburide (GLY) was kindly provided as a gift sample by Cipla Pharmaceuticals (Mumbai, India).Capryol 90, oleic acid, castor oil, sesame oil, castor oil; surfactants, *i.e.,* Captex-355, Cremophor EL, Tween-80, Labrafil, Tween 20 (all AR grade) were purchased from Merck (Mumbai, India) and Tween-80(SD fine chemicals Private Limited, Gujarat, India); and co-surfactants, *i.e.,* and propylene glycol and poly ethylene glycol 400 (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 (GLY) different oils, surfactants, and co-surfactants:

To determine the solubility of glyburide (GLY) in various oils (Capryol 90, oleic acid, castor oil, sesame oil, castor oil), surfactants (tween 20, tween 60, tween 80, cremophor RH, span 80), and co-surfactants (Propylene glycol, poly ethylene glycol 400), an excess amount of glyburide (GLY) was dissolved in 2 ml of each of the selected oils To achieve equilibrium, the mixtures were continuously agitated for 10 minutes with a vortex mixer and then maintained at 37^0C in a water bath shaker for 78 hours at 37^0C. Equilibrated samples were centrifuged at 3000 rpm for 15 minutes, and the supernatant was filtered through 0.45-micron membrane filters before being diluted with suitable solvent. The amount of drug present was determined by utilising an ultraviolet-visible (UV VIS) spectrophotometer[10].

Factor combination as per the chosen experimental design:

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 SMEEDS. Parameters optimized for the preparation of SMEEDS were the type and concentration of the oil phase, surfactant and co-surfactant [11].

S.NO	**Formulation code**	**Drug** Glyburide (GLY)	**Aqueous region %**	**Amphiphilic region %**	**Surfactant : co-surfactant**	**Oil %**
			Water		**Tween80 : Propylene glycol**	**Oleic Acid**
1	**F1**	5mg	50	40	9:1	10
2	**F2**	5mg	48	40	8:2	12
3	**F3**	5mg	46	40	7:3	14
4	**F4**	5mg	44	40	6:4	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

Pseudo-ternary phase diagram study:

Phase diagrams are constructed in order to determine the percentage of components that may result in the greatest amount of SMEDDS existence area. These graphs were constructed using oil, surfactant/co-surfactant, and water, all of which were created at room temperature using the water titration technique[12]. The procedure consisted in preparing solutions with varying weight solutions of surfactant to co-surfactant, such as 1:1, 2:1, 3:1, and so on, and then vortexed for 5 minutes before being heated to 50^0C for one hour to produce an isotropic mixture, which was then dried at room temperature for one hour. The solutions were used to create a mixture of oil and Smix (a mixture of surfactant and co-surfactant) in the following weight ratios: 1:9, 2:8, 3:17, 4:16, 5:5, 6:4, 7:3, 8:2, and 9:1, which was then vortexed for 5 minutes before being baked at 50o C for one hour. After that, all of the mixtures were allowed to sit at room temperature for 24 hours. The presence of water in the mixtures ranging from 5 percent to 95 percent was noticed for their appearance (turbid or clear). The formation of turbidity in the samples indicates the

formation of a coarse emulsion, while the presence of clear isotropic solution indicates the presence of a micro emulsion. The percentage of oil, Smix, and water at which a clear mixture was produced was determined, and the data were utilised to build a ternary phase diagram of the system under consideration[13].

Fourier Transmittance Infra-Red (FTIR):

In order to check the integrity (compatibility) of the drug in the formulations FTIR spectra of formulations along with the pure drug and other excipients were obtained and compared using Shimadzu FTIR spectrophotometer. In the present study potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered KBr crystals. The mixture was compressed to form a disc. The disc was placed in spectrophotometer and spectrum was recorded[14].

FORMULATION OF LIQUID SMEDDS:

From the ternary phase diagram ratio of surfactant to co-surfactant was optimized. Then by varying ratio of oil to Smix, different formulations were prepared. Formulations were prepared by preparing optimized ratio of Smix first, for this surfactant and co-surfactant were accurately weighed and then vortexed for 5-10 mints. After that Smix was placed in oven at 50^0 C for one hour. Oil with different ratio was added to Smix. Then these formulations were vortexed for 5-10 mints and placed in oven at 50^0 C for one hour so that an isotropic mixture was formed. Drug was loaded to these isotropic mixtures at the end and vortexed by vortex shaker until clear solution was obtained[15].

CHARACTERIZATION AND EVALUATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEMS:

Appearance:

The prepared batches of SMEDDS were visually observed for clarity or any signs of settling. The appearance of the SMEDDS formulations was determined by visual inspection of the formulation under light, alternatively on a white and black background, and the turbidity was checked[16]. The test was performed as described in the United States Pharmacopoeia.

Centrifugation

In order to estimate meta stable systems, the optimal SMEDDS formulations were diluted 100 times with distilled water. Centrifuged at 3500 rpm for 30 minutes after passing through two heating-cooling cycles. It is decided which formulations will be used for the freeze thaw stress test based on whether or not there is phase separation[17].

Stress test:

These tests were done to upgrade the best SMEDDS plan under outrageous conditions. Six cycles of stress test were performed at 4°C and 45°C for 48 hours each, followed by 48 hours at

25°C and 21°C for about three cycles of stress testing. Coalescence, cracking, and phase separation were all seen in the instances tested[18].

Micromeritic properties:

Prepared solid- SMEDDS was evaluated for micromeritic properties such as angle of repose, bulk density and tapped density, compressibility index and Hausner's ratio[19].

Thermodynamic stability studies include the following:

The physical stability of a lipid formulation is significant for its performance since it may also be negatively affected by precipitation of the drug in the excipient matrix, which could also result in poor performance. Poor physical stability of a formulation may result in phase separation of excipients, which can have a negative impact on both bioavailability and therapeutic efficacy. In addition, incompatibilities between the formulation and the capsule shell may result in brittleness, softness, and a lack of disintegration or an insufficient release of the drug[20]. For these studies, the following cycles were carried out:

Heating cooling cycle

The improved SMEDDS formulations were diluted 100 times with distilled water to get the desired results. Six cycles were carried out between the chilling temperature (4 ^{o}C) and the heating temperature (45^{o}C), with exposure at each temperature for a total of not less than 48 hours. The centrifugation test was performed on the stable formulation after it had been stabilised[21].

Freeze thaw cycle

An expedited stability testing procedure for a Nano emulsion formulation was carried out in this test. In this study, three freez-thaw cycles of formulations were subjected to temperatures ranging from 21^{o} C to 25^{o}C for a total of not more than 48 hours for each temperature cycle. Six such cycles should be performed for each batch of formulation in order to provide a more accurate estimate of accelerated stability studies. The formulations with the highest levels of stability were chosen for further study and testing[22].

Cloud point measurement:

In a beaker, dilute the formulation 1 ml with 1000 ml of water and put it on a water bath, gradually temperature rises until the diluted formulation becomes hazy or turbid. It provides information on the stability of the micro emulsion at the body temperature in degrees Celsius[23].

Robustness to dilution:

Robustness to dilution was investigated by diluting SMEDDS 50, 100, and 1000 times with water, phosphate buffer pH 6.8, phosphate buffer pH 7.4, and phosphate buffer pH 7.4. Storage of the diluted SMEDDS was carried out for 12 hours, during which time they were checked for indications of phase separation or drug precipitation[24].

Refractive index:

The refractive index of the system was determined by utilising a basic Abbe refractometer and putting 1 drop of self SMEDDS on a glass slide, as described above[25].

Dye-solubility test:

Water soluble dye, methylene blue solution was added to optimized SMEDDS 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 SMEDDS[26].

Viscosity:

SMEDDS formulations F1 to F9 shows viscosity value of 110±.51cp to 129±.72cp Low viscosity of the formulation indicates that formulation is o/w type and having Newtonian flow ensures easy handling and packing[27].

Surface tension:

The surface tension data implies water-in-oil SMEDDSs because surface tension amounts of SMEDDS are nearby to oil phase surface tension[28].

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 SMEDDS ranged from 5.2 to 6.8 in Table 6 which corresponds to the normal pH range of GIT fluids[29].

Conductivity measurement:

The results of measuring electrical conductivity are shown in Table. Water is a better conductor of electricity than oil, when the SMEDDS 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:

SMEDDS 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[30].

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 the measurements were performed in triplicate[31]

Morphological analysis of SMEDDS by SEM:

The outer morphological structure of the SMEDDS 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 SMEDDS 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[32].

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[33].

Kinetics of Drug Release:

In vitro dissolution has been recognized as an important element in drug development. To analyze 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. In this by comparing the rvalues obtained, the best-fit model was selected[34].

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 inter-mittently for actual appearance, rheological properties, pH, and rate discharge by UV- Visible spectrophotometer at 301 nm[35].

RESULTS AND DISCUSSION

Pre-formulation Studies:

Standard curves for glyburide in Phosphate buffer pH 6.8 (Indian pharmacopoeia 2017):

A known volume (50 ml) of 0.2 M potassium Di-hydrogen phosphate is placed in a 200 ml volumetric flask. 22.4 ml of 0.2M sodium hydroxide is added and makeup to the volume with distilled water[36].

0.2M potassium Di-hydrogen phosphate:

A known quantity (27.218g) of potassium di hydrogen phosphate is dissolved and diluted to 1000 ml with water[37].

0.2 M sodium hydroxide:

A known quantity (8 g) of sodium hydroxide is dissolved and makeup to 1000 ml with water.

Determination of λmax :

Standard stock solution containing glyburide was prepared by dissolving 100 mg of glyburide in 10 ml of Dimethyl sulphoxide in 100 ml volumetric flask to dissolve the drug. Then the volume was made up to 100 ml using phosphate buffer of pH 6.8 to obtain a concentration of 100µg/ml. the stock solution is further diluted using a phosphate buffer pH (6.8) to prepare 10 µg/ml concentration. The resultant solution was scanned in the range of 200-400 nm in UV spectrophotometer (UV -1700 Shimadzu Corporation, Japan) to get absorption maximum (λ max) using phosphate buffer as blank. The wave length of maximum absorbance considered for further studies[38].

Preparation of standard curves:

From the above prepared stock solution, solutions containing 2 to 10 µg/ml concentrations were prepared using the phosphate buffer pH 6.8 solutions. The absorbance of these solutions is measured at λmax by UV-spectophotometer (UV-1700Sshimadzu Corporation Japan). A standard curve is plotted using concentration on x-axis and the absorbance obtained on Y-axis[39].

S. No	Concentration (µg/ml)	Absorbance(nm)
1	2	0.110
2	4	0.201
3	6	0.325

4	8	0.416
5	10	0.512

Table 2: Data for Calibration curve

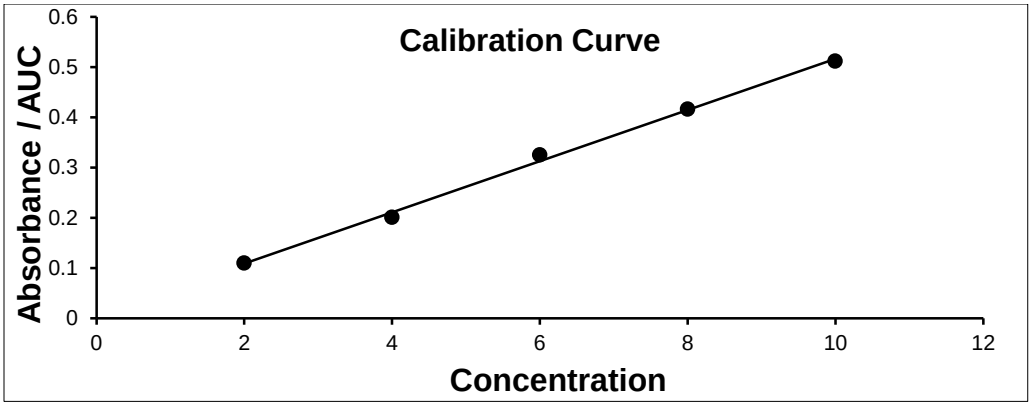

Figure 1: Calibration curve

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-20selected co-surfactant also shows good emulsification with selected oil and Tween 80.

Phase type	**Excipient**	**Solubility (mg/ml)**	**Phase type**	**Excipient**	**Solubility (mg/ml)**	**Phase type**	**Excipient**	**Solubility (mg/ml)**
Oils	Capryol 90	25.423	**Surfactants**	Captex-355	8.823	**Co-Surfactants**	Span 80	4.331
	Oleic acid	39.321		Cremophor EL	7.632			
	Sesame oil	27.445		Tween-80	16.523		Propylene glycol	3.231
	Sunflower oil	22.631		Labrafil	8.310		Poly ethylene glycol 400	7.701
	Castor oil	23.315		Tween 20	10.655			

Table 3: Solubility study of Glyburide (GLY) in Oil, Surfactants and Co- Surfactants

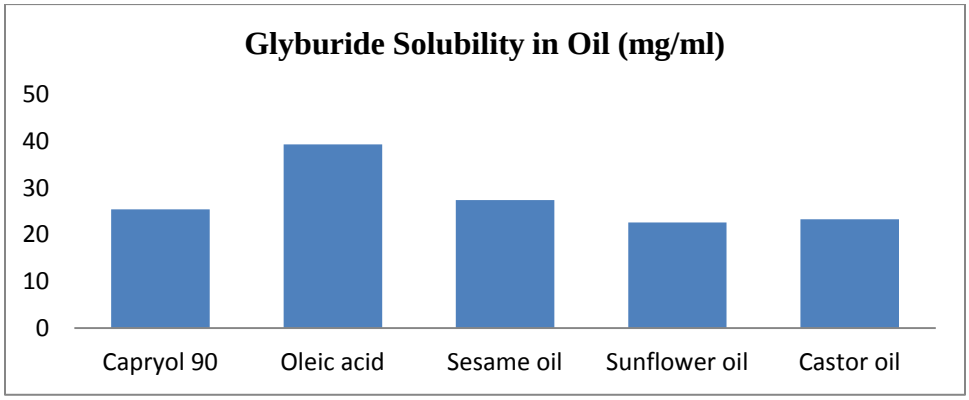

Figure 2: Solubility study of Glyburide in Oil

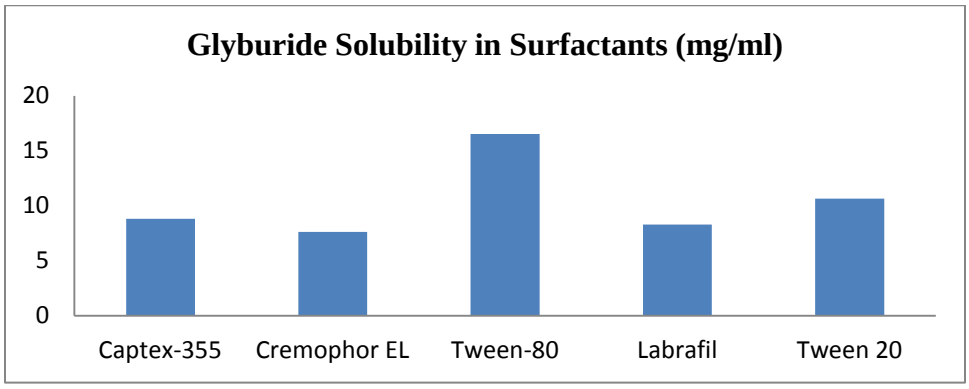

Figure 3: Solubility study of Glyburide in Surfactants

FTIR spectroscopy for Drug –polymer interaction:

In order to check the integrity (compatibility) of the drug in the formulations FTIR spectra of formulations along with the pure drug and other excipients were obtained and compared using shimadzu FTIR spectrophotometer. In the present study potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered KBr crystals. The mixture was compressed to form a disc. The disc was placed in spectrophotometer and spectrum was recorded[40].

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 SMEDDS 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.

S.No	Material	Peak Observation
1	**Glyburide**	3066.82 cm^{-1}, 3003.17 cm^{-1}, 2958.80 cm^{-1}, 1720.50 cm^{-1}, 1490.97 cm^{-1},1219.01 cm^{-1},678.94 cm^{-1}
2	**Oleic acid**	3318.90 cm^{-1}, 2989.66 cm^{-1}, 2881 cm^{-1},1708.93 cm^{-1}, 1462.4 cm^{-1}, 1408 cm^{-1}, 709.8 cm^{-1}
3	**Tween 80**	3599.17 cm^{-1} , 2924.09 cm^{-1} , 1734.01 cm^{-1} , 1111 cm^{-1}
4	**Propylene glycol**	3375.43 cm^{-1} , 2972 cm^{-1}, 2933.73 cm^{-1},1379.^{10} cm^{-1} , 1080.14 cm^{-1} , 1045.42 cm^{-1}

Table 4: IR Interpretation: Components used for the formulation of SMEDDS

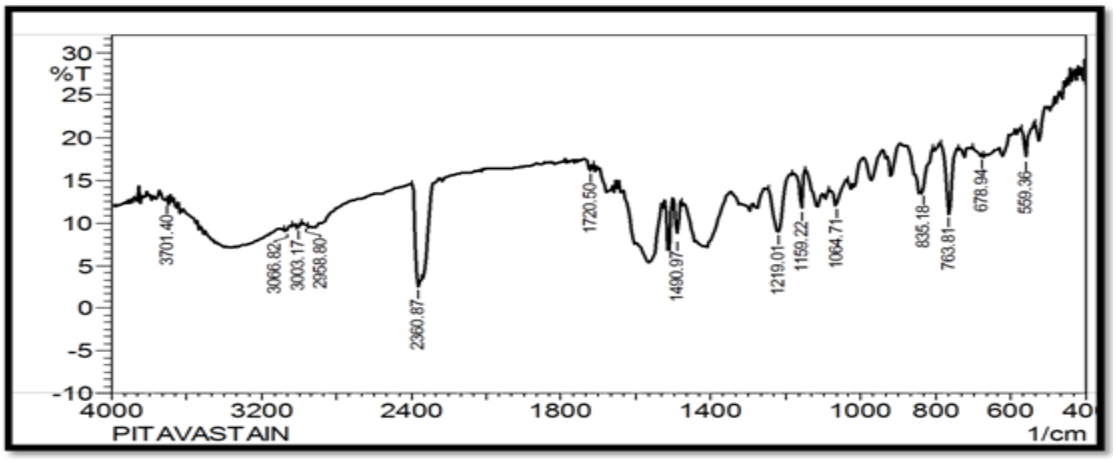

Figure 4: -FT- IR spectrum of Glyburide

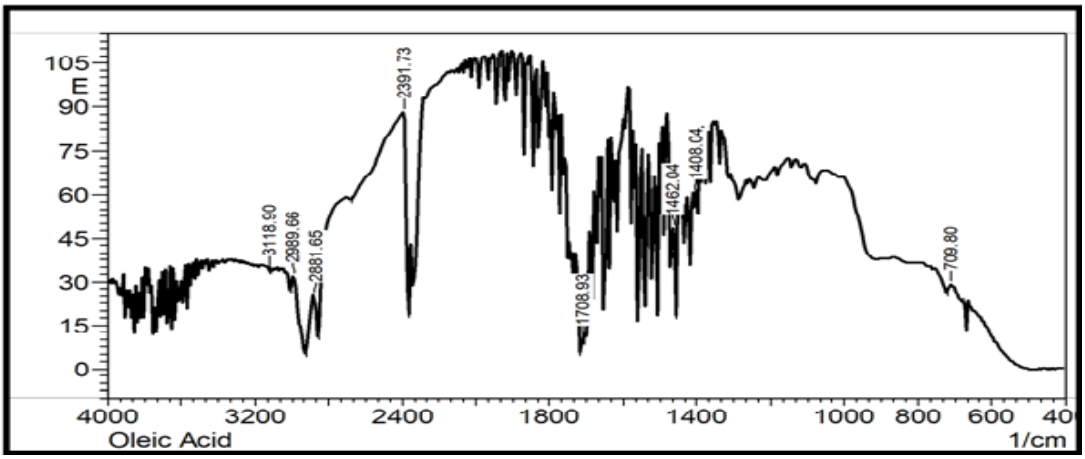

Figure 5: -FTIR spectrum of Oleic Acid

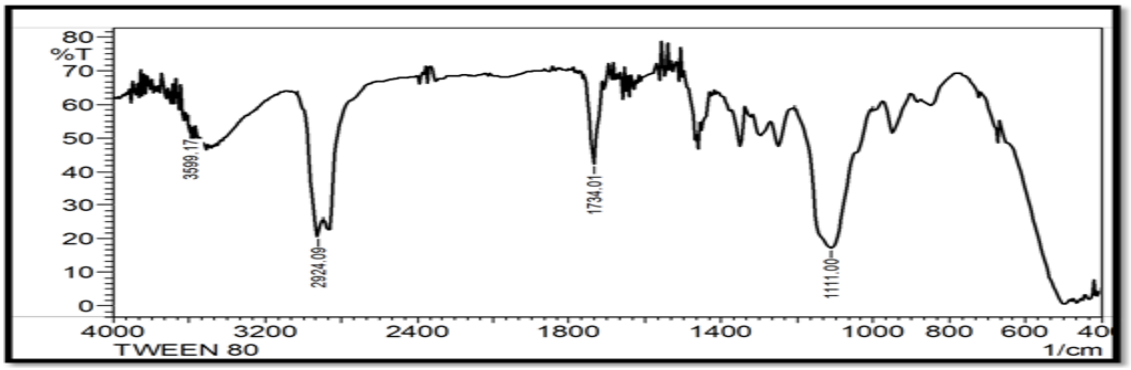

Figure 6:- FT- IR spectrum of Tween 80

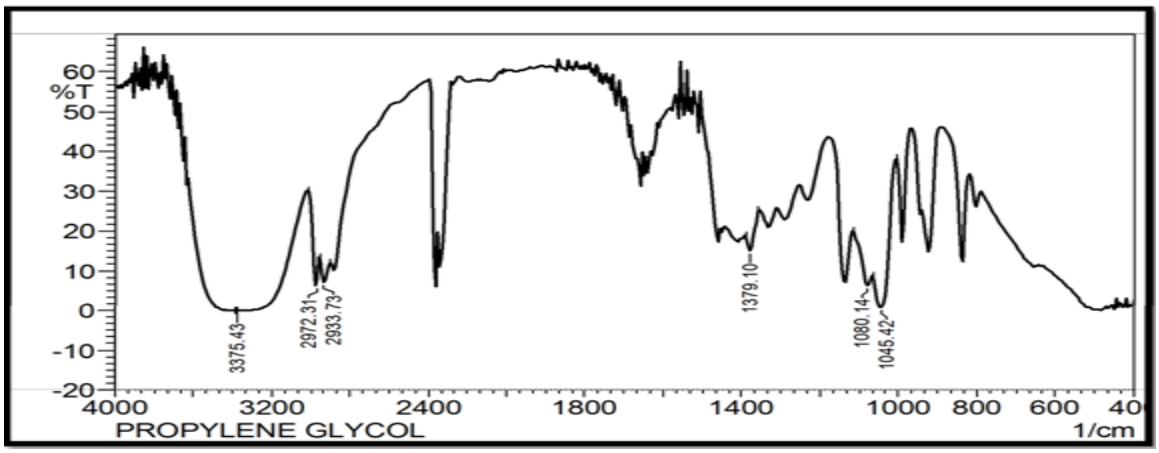

Figure 7:- FTIR spectrum of Propylene Glycol

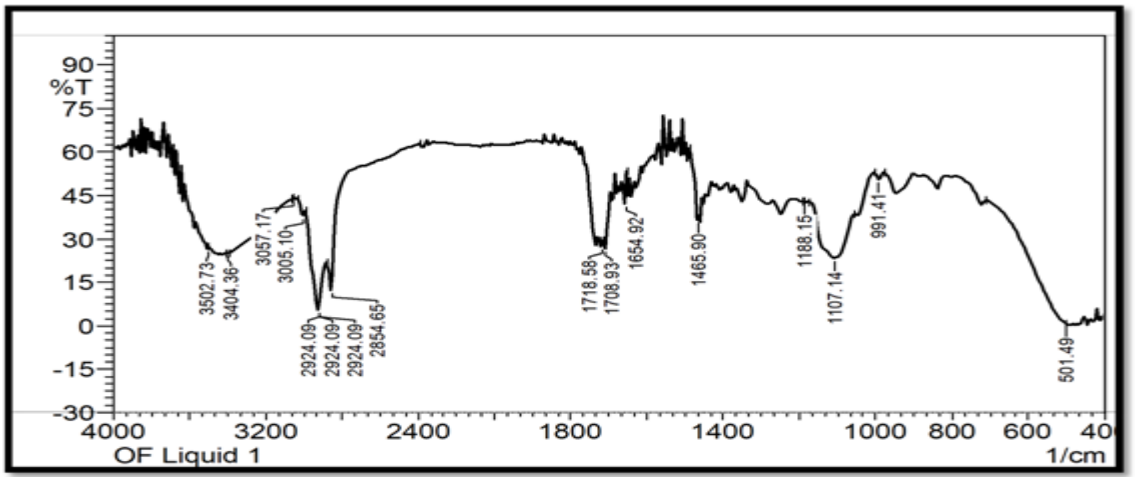

Figure 8: - FTIR spectrum of Glyburide, Oleic Acid, Tween 80, Propylene Glycol

Morphological analysis of SMEDDS by SEM:

The surface morphology of the glyburide (GLY) SMEDDS 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 (GLY) SMEDDS formulation. [20, 27]

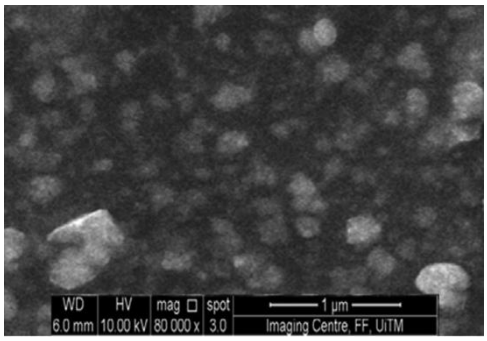

Figure 9: -SEM of OME F4

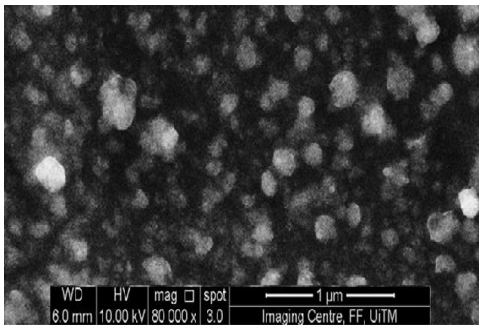

Figure 10:- SEM of OME F5

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 212^0C. 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[41].

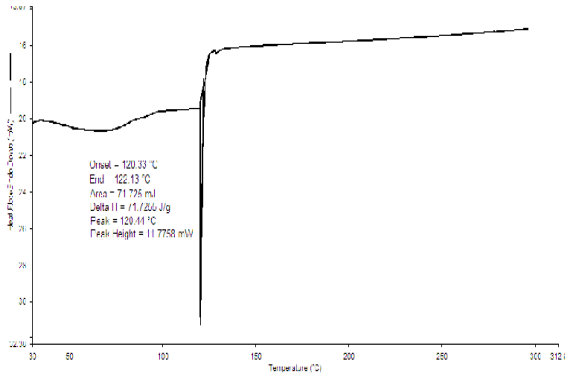

Figure 11: DSC of OME F4

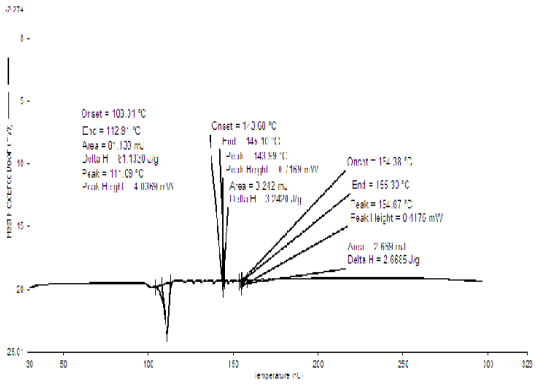

Figure12: DSC of OME F5

Pharmaceutical Evaluation:

Physical appearance and Phase separation:

The SMEDDSs were checked for transparency until they were turbid. SMEDDSs remained clear when diluted, due to the presence of oils and surfactants SMEDDSs look transparent/ translucent yellow colored solution[42].

Centrifugation test and stress tests:

All formulations detected clearly and there is no sign of precipitation.Centrifugation tests showed that the SMEDDS 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. Formulations F1, F2, F3, F4, F5, F6 and F7 passed centrifugation and stress test were stable under all temperature conditions[43].

Code no.	Dispersibility and appearance	Time	Grade
OME F1	Clear	Within 1 mint	A
OME F2	Dull	Within1 mint	C
OME F3	Transparent	Within 2 mints	B
OME F4	Clear and Transparent	Within 1 mint	A
OME F5	Dull	Within1 mint	A
OME F6	Transparent	Within 1 mint	B
OME F7	Clear	Within 3 mints	D
OME F8	Clear	Within 1 mints	D
OME F9	Clear	Within 1 mint	A

Table 5: Result of Dispersibility test and visual assessment of Oleic Acid, Transcutol P, Span 20 containing formulations

CHARACTERIZATION OF MICROMERITIC PROPERTIES

S. No	Formulation	Angle of Repose	Bulk density g/ml	Tapped Density	Carr's index %	Hausner's Ratio
1	**OME F1**	$28^0 12' \pm 0^0 13'$	0.3422± 0.006	0.4021±0.01	15.17 ± 0.001	1.18 ± 0.02

2	**OME F2**	$28^{o}13\pm0^{0}33'$	0.3416 ± 0.006	0.4022±0.01	15.12 ± 0.001	1.11 ± 0.02
3	**OME F3**	$28^{o}23\pm0^{0}15'$	0.3436 ± 0.006	0.4020±0.01	15.18 ± 0.001	1.19 ± 0.02
4	**OME F4**	$28^{o}27\pm0^{0}30'$	0.3425 ± 0.006	0.4023±0.001	15.09 ± 0.001	1.15 ± 0.02
5	**OME F5**	$28^{o}21\pm0^{0}28'$	0.3437 ± 0.006	0.4034±0.01	15.11 ± 0.001	1.17 ± 0.02
6	**OME F6**	$28^{o}26\pm0^{0}24'$	0.3409 ± 0.006	0.4043± 0.001	15.14 ± 0.001	1.14 ± 0.02
7	**OME F7**	$28^{o}22\pm0^{0}18'$	0.3445 ± 0.006	0.4057±0.01	15.18 ± 0.001	1.13 ± 0.02
8	**OME F8**	$29^{o}34\pm0^{0}19'$	0.3423 ± 0.006	0.4045± 0.001	15.02 ± 0.001	1.16 ± 0.02
9	**OME F9**	$28^{o}27\pm0^{0}14'$	0.3427 ± 0.006	0.4041± 0.001	15.16 ± 0.001	1.12 ± 0.02

Table 6: Characterization of micromeritic properties

Thermodynamic stability assessment of Oleic Acid, Transcutol P, Span 20 Formulations:

Formulation	**Heating cooling Cycle**	**Centrifugation**	**Freeze thaw cycle**
OME F1	√	√	√
OME F2	√	√	√
OME F3	×	×	×
OME F4	√	√	√
OME F5	√	√	√
OME F6	×	×	×
OME F7	√	×	×
OME F8	√	√	√
OME F9	√	√	√

√-Passed ×-Failed

Table7: Result of Thermodynamic stability assessment of Oleic Acid, Transcutol P, Span 20 containing formulations.

S. No	Formulation	Cloud Point (^{0}C)
1	**OME F1**	86.5
2	**OME F2**	86
3	**OME F3**	UNSTABLE
4	**OME F4**	89
5	**OME F5**	88
6	**OME F6**	UNSTABLE
7	**OME F7**	85
8	**OME F8**	82
9	**OME F9**	87

Table 8: Measurement of cloud point of Oleic Acid, Transcutol P, Span 20

S.NO	Medium	Phase Separation								
		OME F1	OME F2	OME F3	OME F4	OME F5	OME F6	OM E F7	OM E F8	OME F9
1	**Distilled water**	No	No	No	No	No	No	No	No	No
2	**Phosphate buffer pH 6.8Ph**	No	No	Yes	No	No	Yes	No	No	No
3	**Phosphate buffer pH7.4**	No	No	Yes	No	No	Yes	No	No	No

Table 9: Results of robustness to dilution Oleic Acid, Transcutol P, Span 20 containing formulations

Refractive index:

The refractive index of the systems was in the 1.338 to 1.462. It reflects that the SMEDDS 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.

Formulation code	Refractive index (RI)	Optical Clarity (%Transmittance)	Dye Solubility	Viscosity(cp)	Surface tension (dynes/cm)	pH of Formulation
OME F1	1.396	93.3	✓	112±0.13	41.62 ±0.87	5.2
OME F2	1.418	95.4	✓	117±0.19	43.61± 1.10	5.5

OME F3	1.462	93.7	✓	126± 0.12	42.80± 1.44	6.3
OME F4	1.331	98.9	✓	110±0.51	41.68± 1.22	6.1
OME F5	1.326	99.2	✓	113±0.23	40.78± 1.04	6.2
OME F6	1.522	94.6	✓	118±0.01	38.67± 1.14	5.9
OME F7	1.426	96.8	✓	164±0.30	44.09± 1.53	6.1
OME F8	1.438	97.2	✓	117±0.13	41.23± 0.61	6.3
OME F9	1.396	93.3	✓	116±0.20	43.53± 1.04	6.8

✓- o/w type of SMEDDS

Table10: Results of Viscosity (mean±SD; n=3), Refractive index (RI), Dye solubility, Optical Clarity (% Transmittance), (%) Assay (mean±SD; n=3), Surface tension (mean±SD; n=3) and pH Oleic Acid, Transcutol P, Span 20 containing formulations

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 SMEDDSs should preferably achieve high absolute values of the zeta potential (±30 mV) to ensure the creation of a high energy barrier against coalescence of dispersed droplets. SMEDDS 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 (%)**	**Zeta Potential (mv)**
OME F1	0.183	91.06	0.839	74.24 ± 0.11	82.2±2.1	-31.30
OME F2	0.192	90.56	0.447	73.24 ± 0.75	80.1±2.2	-28.40
OME F3	0.189	98.11	0.368	72.30 ± 0.21	85.4±2.7	-27.2
OME F4	0.199	99.23	0.268	81.17 ± 0.81	89.6±4.6	-26.2
OME F5	0.201	96.10	0.272	75.24 ± 0.32	89.3±5.6	-27.8
OME F6	0.176	95.97	0.478	74.10 ± 0.01	65.5±2.4	-27.90

OME F7	0.180	93.38	0.612	71.12 ± 0.05	73.6±0.8	-28.12
OME F8	0.179	93.71	0.475	77.54 ± 0.34	85.3±0.8	-28.30
OME F9	0.191	90.37	0.536	69.64 ± 0.34	78.7±1.8	-29.40

Table 11: Results of Conductivity, % Drug Content, Polydispersity index (PDI), %Drug loading(mean±SD; n=3), Entrapment efficiency(mean±SD; n=3), % Particle size (mean±SD; n=3) and Zeta Potential (mean±SD; n=3) Oleic Acid, Transcutol P, Span 20 containing formulations

Dissolution study of different batches:

The results of in vitro dissolution profiles of various Glyburide SMEDDS formulations are shown in Table. It is evident from the table that Glyburide SMEDDS showed more than 90% GLY released. The USP recommends pH 7.4 buffers as a dissolution medium for Glyburide[44].

S.No	Time in hours	% Drug release of **OME** F1	% Drug release of **OME** F2	% Drug release of **OME** F3	% Drug release of **OME** F4	% Drug release of **OME** F5	% Drug release of **OME** F6	% Drug release of **OME** F7	% Drug release of **OME** F8	% Drug release of **OME** 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 12: Results of % Drug release of F1 to F9 SMEDDS batches

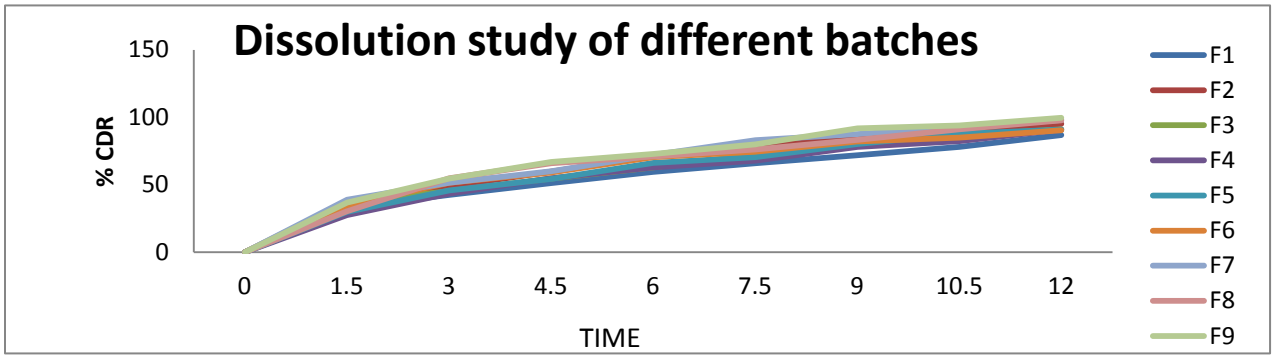

Figure 13**:** Dissolution study of F1 to F9 SMEDDS batches

Kinetic of Drug release:

To study the release kinetics, data from in vitro drug release studies F3 and F5 series glyburide (GLY) SMEDDS formulations showed Higuchi's model as the most suitable. Using the Korsmeyar 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-Fik transfer). [28, 29, 30]

Formulation Code	Zero-order kinetic		First Order Kinetics		Higuchi Model		Korsmeyer-Peppas Model			Hixson Crowel Model	
	R2	$K_0(h^{-1})$	R2	$K_1(h^{-1})$	R2	K_H(h-1/2)	R2	N	K_K	R2	K_{HC}(h-1/3)
OME F1	0.988	0.200	0.827	-0.004	0.952	4.786	0.919	0.368	10.186	0.927	0.008
OME F2	0.998	0.195	0.859	-0.003	0.971	4.690	0.956	0.405	8.394	0.953	0.007
OME F3	0.979	0.189	0.888	-0.002	0.929	4.498	0.930	0.4	7.980	0.904	0.006
OME F4	0.987	0.197	0.887	-0.002	0.967	4.771	0.940	0.486	4.909	0.975	0.006
OME F5	0.992	0.200	0.919	-0.002	0.972	4.821	0.954	0.467	5.610	0.978	0.006
OME F6	0.983	0.171	0.936	-0.002	0.975	4.149	0.968	0.377	9.528	0.969	0.005
OME F7	0.989	0.172	0.891	-0.002	0.944	4.092	0.917	0.401	7.379	0.932	0.005
OME F8	0.991	0.160	0.942	-0.001	0.954	3.824	0.963	0.371	8.689	0.928	0.004
OME F9	0.994	0.199	0.979	-0.002	0.994	4.855	0.993	0.575	2.850	0.997	0.005

Table13: *In Vitro* kinetic release studies Oleic Acid, Transcutol P, Span 20 containing formulations

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± .13 cps and no considerable change in the percentage release, i.e., 95% was observed for 6 months.

Precipitation or phase separation not observed in diluted formulations after they were stored in different dilution mediums. That all formulations were resistant to dilution and pH changes is inferred from this result[45].

Result and Discussion of the Study:

Certain emulsion components, particularly those generated from unsaturated lipids, have the potential to produce undesired degradation products during storage, which may have a negative impact on the stability of SMEDDS. There are two types of products: oxidative products (such as lipid hydroperoxides and aldehydes) and hydrolytic products (such as free fatty acids, mono- and diglycerides, and lyso-phospholipids). It is possible for these degradation products to change the surface property and zeta potential of the emulsion, as well as to disperse in the aqueous phase of the mixture. As fatty acids are formed, the stability of the emulsion may be compromised. To assist in the assessment of stability, these parameters should be closely examined. In light of the fact that the stability research was carried out in accordance with ICH guidelines, conditions may be determined in accordance with the climatic conditions in that specific zone I deall, three distinct conditions should be used to test for stability, according to the recommendation. Will find them as follows[46-54]:

S.No	**Temprature^{0}C/ Relative Humidity**	**Duration**	**Type of Stability**
1	25°C/60% RH	12 months	Long term stability
2	30°C/65% RH	6 months	Intermediate stability
3	40°C/75% RH	6 months	Accelerated study

Table 14: Stability studies of optimized Glyburide SMEDDS

	Period (Month)	**40°C/75% RH**					**30°C/65% RH**				
OMEF4		**Droplet Size**	**Zeta Potential**	**% Transmittance**	**pH**	**% Drug Content**	**Droplet Size**	**Zeta Potential**	**% Transmittance**	**Ph**	**% Drug Content**
	0	15.32± 11.25	-10.4 ±2.0	99.91± 0.17	6.4±0.1	99.45±0 .44	15.32 ±11.4 7	-10.4 ±2.0	99.91± 0.17	6.4±0. 1	99.45 ±0.44
	1	20.24±	-10.25±	99.3±0.2	6.36±	98.93±0	20.15	-1.89±	99.8±0.	6.36±	98.43

		8.90	3.65		0.12	.37	±8.54	1.82	2	0.12	±0.65
	2	17.66± 10.39	-10.67± 1.55	99.7±0.5	6.33±0. 15	97.91±0 .26	17.00 ±5.22	-0.37± 2.01	99.2±0. 4	6.33±0 .15	97.91 ±0.59
	3	19.85± 4.97	-9.43± 3.34	99.1±0.1	6.25 ±0.12	97.25±0 .17	18.65 ±10.2 8	-9.76± 2.78	99.6±0. 3	6.25 ±0.12	98.56 ±0.24
	6	18.65± 3.34	-10.68± 4.97	99.4±0.4	6.27± 0.2	97.12±0 .3	19.23 ±5.89	-0.29± 1.75	99.1±0. 2	6.27± 0.2	98.1± 0.25

Table 15: Stability data of OME F4 Glyburide SMEDDS

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 SMEDDSs. Nine formulations were prepared which should help improve the bioavailability of poorly soluble drugs. Formulations **OME** F4 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 bi-continuous 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 SMEDDS was stable for six months. Thus, despite of effectiveness of SMEDDS 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.

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.

Selection of best formulation:

From the above characterization, the two formulations OME F4 were selected as the best formulation showing,

For OME F4

Refractive index	1.331
% Transmittance	94.9
% Drug content	98.11
Viscosity	110±0.51
PDI	0.268

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