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FABRICATION AND CHARACTERIZATION OF MICROEMULSION BASED ORAL SOLID DOSAGE FORM OF GLIMEPIRIDE

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ABSTRACT: The aim of this investigation was to develop a solid microemulsion of Glimepiride for enhancing its solubility, and its dissolution rate. For this purpose, solubility of Glimepiride was determined in various vehicles. Oil, Surfactant and Cosurfactant were selected based on the solubility. Pseudo-ternary phase diagrams were constructed to identify the microemulsion existing zone. The optimized microemulsion formulation was characterized for its refractive index, % transmittance, pH, viscosity, drug content and particle size. Particle size of the optimized microemulsion formulation was found to be 38.83 nm. Various adsorbents were incorporated in the optimised liquid microemulsion to get solid microemulsion. The solid microemulsion from Aerosil 200 was optimized because of very low amount (1:1) of Aerosil was incorporated in microemulsion as compare to other adsorbent. The prepared solid microemulsions were subjected to characterisation for angle of repose, bulk density, tapped density, Hausner's Ratio, Carr's index, drug content, *in-vitro* drug release study and stability studies. The solid microemulsion with Aerosil 200 showed excellent free flowing property and % compressibility. From *in-vitro* drug release studies, the release of Glimepiride from solid microemulsion prepared from Aerosil 200 (91.37 ± 0.13 %) was found to be higher than the pure drug (56.53 ± 0.10 %). Thus the microemulsion based solid dosage form of Glimepiride can be a promising approach to increase solubility and absorption of the drug to increase its bioavailability for oral drug delivery.

INTRODUCTION¹: More than 40 percent of the drug coming from high-throughput screening are poorly soluble in water compounds with poor solubility are increasingly posing challenges in the development of new drugs, since a large number of drugs coming directly from synthesis or from high throughput screening have a very poor solubility.

It is well known that drug efficacy can be severely limited by poor aqueous solubility, leading to low dissolution rate and thus results in low absorption in the gastrointestinal tract after oral administration hence comprising oral bioavailability. The ability to increase aqueous solubility is thus a valuable aid to increase the efficacy for certain drugs.

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The Biopharmaceutical Classification system divides drugs into four classes depending on *in vitro* and *in vivo* permeability data. Four classes of compound can be distinguished: I (high solubility, high permeability), II (low solubility, high permeability), III (high solubility, low permeability) and IV (low solubility and low

permeability). Class I compounds are typical examples for waiving bioequivalence studies. In the selection process, new chemical compound with low aqueous solubility and low permeability are preferably filtered out since they might pose problems during pharmaceutical development.

For class II drugs dissolution /solubility and for Class III drug permeability limits the oral drug absorption. It is obvious that class II drugs the low ability to dissolve is a more important limitation to their overall rate and extent of absorption than their ability to permeate through the intestinal epithelia. There are several pharmaceutical strategies available to improve the aqueous solubility of poorly soluble drugs: solid dispersion, solubilization using surfactant, the use of co-solvent, reduction of particle size, hydrotrophy and the use of aqueous soluble derivatives or salts. Among all technique microemulsion system has considerable potential to act as a drug delivery vehicle by incorporating a wide range of drug molecules. Microemulsion has got advantage like excellent thermodynamic stability, high drug solubilisation capacity, improved oral bioavailability and protection against enzymatic hydrolysis.

The only problem with microemulsion is poor palatability due to the lipid content leading to the poor patient compliance. Moreover due to their water content, microemulsions cannot be encapsulated in soft gelatin or hard gelatin capsules. All these problems may be overcome by formulating or converting microemulsion into another stable dosage form like conversion of microemulsion containing drug into powder or tablet by adsorbing onto the solid support i.e. adsorbent, or incorporation of microemulsion in gel bases etc. The drug-containing microemulsion comprises a oil soluble drug, and the drug-containing microemulsion is adsorbed onto the solid particle adsorbent and forms a free-flowing, compressible powder.

Solid microemulsion technique are developing to modify the physicochemical and biopharmaceutical properties of drug, for above mentioned techniques BCS Class II drugs are selected because low solubility and high permeability like antidiabetic

drugs. Antidiabetic drugs to be absorbed must be present in the form of an aqueous solution at the site of absorption for their hypoglycemic activity so solubility enhancement techniques are more essential for drugs like Glimepiride (GMP) 3-ethyl-4-methyl-N-{{2-[4-{{(4methylcyclohexyl) carbamoyl} amino} sulfonyl] phenyl} ethyl, is thiozolidinedione antidiabetic agent that depends on the presence of insulin for its mechanism of action lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cell.

Glimepiride (GMP) is classified under class II according to biopharmaceutical classification system. The drug shows low, pH dependent solubility. In aqueous media, glimepiride exhibits very poor solubility at 37°C (<0.004 mg/ml). In media pH>7, solubility of drug is slightly increased. This poor solubility may cause poor dissolution and unpredicted bioavailability. It is practically insoluble in water and other aqueous media. However, the drawback of this potentially useful hypoglycemic agent is that it is highly hydrophobic and practically insoluble in water. The presented research work thus deals with the techniques of enhancement of solubility as well as dissolution and bioavailability of poorly aqueous soluble drugs like Glimepiride.

MATERIALS AND METHODS:

Glimepiride was a gift sample from Unichem Laboratories Ltd, Goa, India. Capmul MCM, Captex 200, Captex 300 and Captex 355 were a gift sample from Abitec Corporation, Cremophore RH 40, Cremophore EL was gift sample from BASF India Ltd. Mumbai, India. Transcutol P and Labrasol was a gift sample from Gattefosse India Pvt. Ltd., Mumbai, India and Aerosil 200 was gift sample from Unijules, Nagpur, India. All the other chemicals were of the analytical grade.

Solubility study²:

The solubility of Glimepiride in various oils (Oleic Acid, Capryol® 90, Capmul® MCM, Castor oil, olive oil, Captex® 200,), surfactants (Cremophor® EL, Cremophor® RH40, Tween 80), and co-surfactants (, PEG, Ethanol, Transcutol® P) was determined by adding excess amount of GLP to 2 ml of each component placed in screw capped glass

vial. The ingredients were mixed using a magnetic stirrer and then kept on orbital shaker (Remi motors & RIS-24BL) for 72 h at temperature $37 \pm 1.00^\circ\text{C}$. The samples were then centrifuged for 5 min at 37°C . The supernatant was pipette out, diluted with methanol and analysed by UV spectrophotometer (Shimadzu, Japan) at 229 nm for determining drug concentration. The results are shown in Tables 1-3.

TABLE 1: SOLUBILITY OF GLIMEPIRIDE IN VARIOUS OILS

Sr. No.	Oils	Solubility (mg/ml)
1	Mustard oil	1.8 ± 0.20
2	Ground nut oil	1.0 ± 0.11
3	Capmul MCM	4.00 ± 0.15
4	Soyabean	1.00 ± 0.33
5	Oleac acid	1.2 ± 0.21
6.	Captex 200	1.5 ± 0.3
7.	Olive oil	1.2 ± 0.2
8.	IPM	1.0 ± 0.3

Data was expressed as mean \pm S.D. (n=3)

TABLE 2: SOLUBILITY OF GLIMEPIRIDE IN VARIOUS SURFACTANTS

Sr. No.	Surfactants	Solubility (mg/ml)
1	Tween 20	1.0 ± 2.0
2	Tween 80	2.0 ± 0.50
3	Cremophore RH 40	3.0 ± 0.38
4.	Cremophore EL	1.8 ± 0.35

Data was expressed as mean \pm S.D. (n=3)

TABLE 3: SOLUBILITY OF GLIMEPIRIDE IN VARIOUS COSURFACTANTS

Sr. No.	Cosurfactants	Solubility (mg/ml)
1	Ethanol	4.0 ± 0.22
2	PEG 400	1.5 ± 0.17
3	Transcutol P	6.0 ± 0.03
4.	Capryol 90	3.0 ± 0.2

Data was expressed as mean \pm S.D. (n=3)

Construction of pseudo-ternary phase diagrams 3:

Pseudo-ternary phase diagrams were constructed to obtain the suitable components, and their concentration ranges that resulted in a large existence area of microemulsion were preferred. In order to optimize the concentration of oil phase, surfactant and co-surfactant, different batches of varied concentration were prepared and titrated with distilled water till transparency persisted.

Ternary phase diagram was prepared by using a constant ratio of surfactant to co-surfactant. Four ratios of surfactant (Cremophore RH 40) and co-surfactant (Transcutol P) were selected (1:1, 2:1, 3:1 and 4:1). The results are represented in Figure 1.

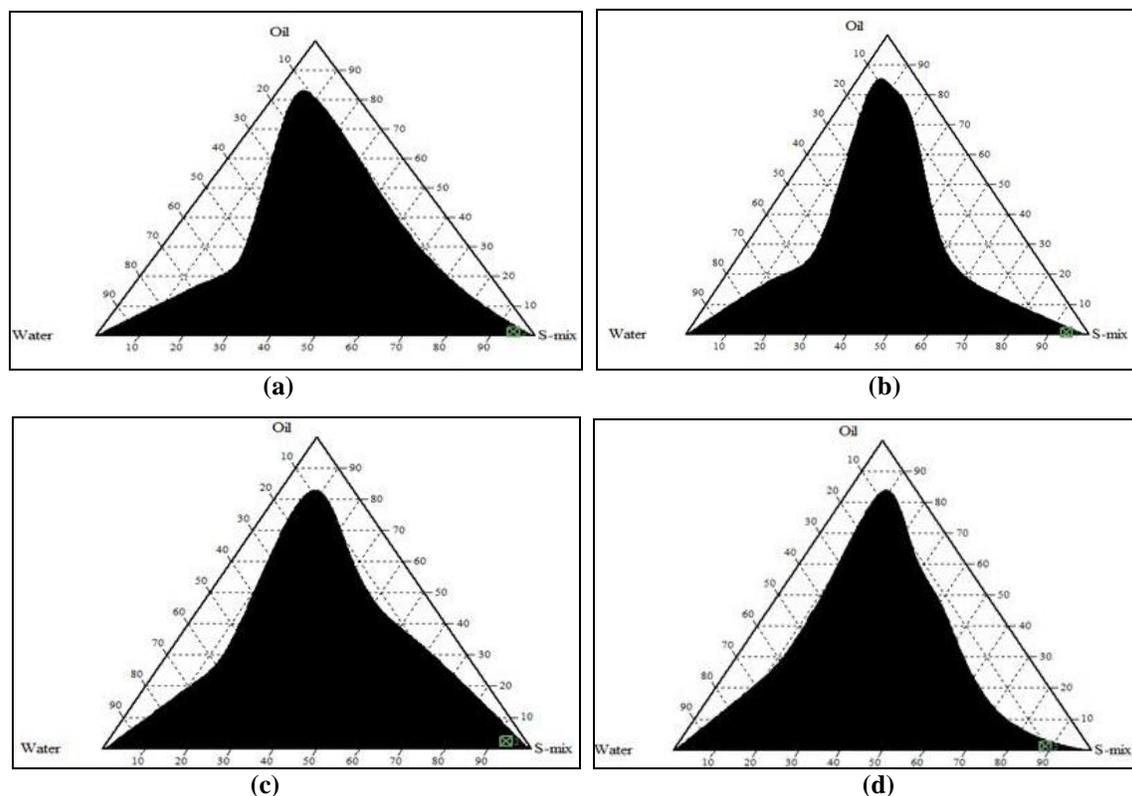


FIGURE 1: PSEUDOTERNARY PHASE DIAGRAM OF MICROEMULSION COMPOSED OF CAMPUL MCM (OIL), WITH CREMOPHORE RH 40(SURFACTANT): TRANSCUTOL P(COSURFACTANT), WATER. (A) 1:1 (B) 2:1 (C) 3:1 (D) 4:1

Preparation of microemulsion by water titration method ⁴:

After the microemulsion regions in the phase diagrams were identified, the microemulsion formulations were selected at different component ratios as described in **Table 8**. In order to prepare the drug loaded microemulsions, the weighed amount of Glimepiride was dissolved into mixture

of oil and surfactant/cosurfactant mixture. Then this mixture was mixed thoroughly using magnetic stirrer until a homogenous dispersion was obtained. The mixture was titrated by adding water drop by drop with constant stirring till the microemulsion was obtained at ambient temperature. The results are depicted in **Table 4**.

TABLE 4: COMPOSITION OF MICROEMULSIONS

Sr. No.	Formulation code	Campul MCM %v/v	Crephore RH 40 %v/v	Transcutol P %v/v	Purified water %v/v	Observation
1	ME1	60	23.34	11.66	5	μE
2	ME2	55	23.34	11.66	10	μE
3	ME3	50	23.34	11.66	15	μE
4	ME4	45	23.34	11.66	45	μE
5	ME5	15	23.34	11.66	50	E
6	ME6	10	23.34	11.66	55	E
7	ME7	5	23.34	11.66	60	E

μE = Microemulsion, E = Emulsion

Characterisation of microemulsion ⁵:

The prepared Glimepiride microemulsion was inspected for transmittance and visual clarity, centrifugation, pH measurement, refractive index, viscosity and particle size.

Transmittance and visual clarity:

The droplets of the microemulsions being smaller than $\frac{1}{4}$ th the wavelength of visible light, permit white light to pass through the dispersed system making it transparent or translucent. The microemulsion systems were inspected for optical transparency and homogeneity by usual observation against strong light. The system was also checked for the presence of undissolved drug or other solid ingredient. The results are shown in **Table 5**.

Centrifugation:

Physical stability of the microemulsions was studied by centrifugation at 3,000 rpm for 2 hours. After centrifugation the samples were observed for clarity and any phase separation or precipitation. The results are shown in **Table 5**.

pH measurement:

The pH measurement of the microemulsions were determined by using a pH meter which was calibrated before use with standard buffer solutions at pH 4 and 7. The results are shown in **Table 5**.

Refractive index:

The refractive index of medicated formulation was determined using an Abbottype refractometer. The results are shown in **Table 5**.

Viscosity:

The viscosity of the prepared microemulsions was measured using Brookfield viscometer using spindle No. S 64, at 100 rpm. Experiments were carried out in triplicate for each sample, and the results are presented as an average \pm standard deviation in **Table 5**.

Drug content ⁶:

The microemulsion formulation was analyzed for drug content by U.V. spectrophotometer at 229 nm.

Particle size Analysis of formulated microemulsion:

Mean globule size of the optimized microemulsion was determined by photon cross-correlation spectroscopy. Microemulsion was placed in transparent polystyrene cuvette (path length = 1 cm) which was placed in thermostatic sample chamber maintained at 25°C. Sample temperature was set at 25°C and 3 runs of 60s were performed. Detection was carried out at a scattering angle of 90°. From the resulting correlation curves, a 2nd order analysis was performed to calculate the mean

globule size and standard deviation. The results are represented in **Figure 2**.

Preparation of Solid microemulsion ⁷:

The microemulsion was optimised on the basis of the transparency, drug content and particle size. The optimised microemulsion was composed of Campul MCM (55%), Cremophore RH 40(23.34%), Transcutol P (11.66%) and water (10%). After the formulation and evaluation of microemulsion the task ahead was to bring it into solid dosage form. To convert liquid microemulsion into solid, five adsorbing agents were used. They were bentonite, aerosol 200, aluminium hydroxide, magnesium hydroxide and magnesium oxide. The solid microemulsion are prepared by adsorption of microemulsion onto adsorbent. The solid microemulsion dosage form is prepared by adsorbing the liquid microemulsion onto a particulate solid material so as to provide the adsorbent in the form of a powder. The powder can then be made into other suitable solid dosage forms by combination with additional excipients, using appropriate processing. The results are depicted in **Table 6**.

TABLE 6: COMPOSITION OF SOLID MICROEMULSION

Sr. No.	Formulation code	Different Adsorbent	Concentration in % w/w
1	SME 1	Bentonite	1:11
2	SME 2	Aerosil 200	1:1
3	SME 3	Aluminium hydroxide	1:3
4	SME 4	Magnesium hydroxide	1:6
5	SME 5	Magnesium oxide	1:7

Characterization of Solid Microemulsion ⁸:

The prepared solid microemulsion was characterized by angle of repose, bulk density, tapped density, carr's index, hausner's ratio, drug content, *In-vitro* drug release study, stability studies.

Angle of repose:

The fixed funnel and free standing cone method was employed. A funnel that is secured with its tip at a given height, h, which was kept 2 cm above graph paper that is placed on a flat horizontal surface. With r being the radius, of base of conical pile, angle of repose can be determined by following equation:

$$\Theta = \tan^{-1} (h/r)$$

Where, Θ is the angle of repose,

h is height of pile, r is radius of base of the pile.

The results are shown in **Table 7**.

Bulk density:

The solid microemulsion was introduced into the 10 ml graduated cylinder. The volume occupied by the SME was recorded. Bulk density was calculated using following formula:

Bulk density = Mass of powder/Bulk volume, It is calculated in g/ml.

The results are shown in **Table 7**.

Tapped Density:

Solid microemulsion (2 gm) was poured through a glass funnel into 10 ml of graduated cylinder. The cylinder was tapped gently from the height of 2 inches until a constant weight was obtained. The volume occupied by the solid microemulsion after tapping was recorded and tapped density was calculated using following formula:

Tapped density = Mass of powder/Tapped volume

The results are shown in **Table 7**.

Carr's index:

Carr's index can be determined by the following equation,

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

The results are shown in **Table 7**.

Hausner's ratio:

Hausner's ratio can be determined by the following equation,

$$\text{Hausner's ratio} = \text{Tapped Density} / \text{Bulk Density}$$

The results are shown in **Table 7**.

Drug content:

The drug content was calculated by dissolving solid microemulsion equivalent to 1 mg of Glimepiride was transferred to 100 ml volumetric flask and dissolved in minimum amount of methanol; and the volume was made up to 100 ml with phosphate buffer (pH 7.4) and then b solution was filter through 0.45- μm membrane filter paper and assayed for drug content using UV spectrophotometer at 229 nm.

In-vitro drug release study:

Accurately weighed solid microemulsion equivalent to 1 mg of Glimepiride were added to 900 ml of dissolution media (phosphate buffer 7.4) contained in USP dissolution apparatus II and stirred at a speed of 50 rpm at $37 \pm 0.5^\circ\text{C}$. Five milliliter aliquots were withdrawn at 10, 20, 30, 40, 50 60 minute and replaced by 5 ml of fresh dissolution media (37°C). The collected samples were analyzed after suitable dilution at 229.0 nm using UV-visible spectrophotometer against the blank. The dissolution of pure Glimepiride was done similarly. The results are shown in **Figure 3**.

Stability studies:

To assess the stability of drug and formulation, stability studies were done as per ICH guidelines. The formulated solid microemulsion were wrapped in aluminium foil and stored at and $45 \pm 0.5^\circ\text{C}$ for period of one month. After an interval of 15 days the solid microemulsion were tested for physical appearance, angle of repose, bulk density, tapped density, carr's index, hausner's ratio, drug content uniformity. The results are shown in **Table 8**.

RESULT AND DISCUSSION:**Characterisation of microemulsion:**

The formulated microemulsions were characterized for pH, refractive index, centrifugation, transparency/translucency, viscosity, drug content, particle size analysis.

Transparency:

All the microemulsions were transparent and appeared like a homogenous single-phase liquid, when observed for visual clarity against strong light. No traces of undissolved drug or other solid

ingredient were found in all the formulations. This indicated that the drug was completely soluble in the system.

Centrifugation:

None of the microemulsion systems showed signs of phase separation on centrifugation at 3000 rpm for 2 hours. This result provided a rapid and full proof identification of the system as microemulsion.

pH Measurement:

The pH of microemulsion was found to be in the range of 6.40 ± 0.2 to 6.60 ± 0.4 . The range is suitable for oral administration.

Refractive index:

The refractive index was in the range of 1.386 ± 0.03 to 1.412 ± 0.02 . The values of the refractive index of microemulsion showed that the systems were transparent concluding very small particle size of the system.

Viscosity:

The viscosity of the microemulsion was ranged between $126 \pm 2.00\text{cp}$ to $132 \pm 2.00\text{cp}$. The viscosity values indicated the w/o nature of microemulsions.

Drug content:

The drug content of microemulsion formulation was determined. The drug content of ME1, ME2, ME3 and ME4 was found to be 99.18, 99.42, 99.23 and 99.10 respectively. The formulation ME2 was showing good % transmittance, refractive index, viscosity, pH, drug content. Thus ME2 was optimised for further study.

TABLE 5: PHYSICOCHEMICAL CHARACTERISTICS OF MICROEMULSIONS.

Sr. No.	Formulations	ME 1	ME 2	ME 3	ME 4
1	pH	6.50 ± 0.02	6.40 ± 0.02	6.40 ± 0.01	6.60 ± 0.02
2	Refractive index	1.3985 ± 0.01	1.3860 ± 0.002	1.3964 ± 0.01	1.4120 ± 0.002
3	Centrifugation	No phase separation	No phase separation	No phase separation	No phase separation
4	% Transmittance	97.0 ± 1.01	99.0 ± 1.03	98.0 ± 1.02	95.0 ± 1.03
5	Viscosity at 100 rpm(cps)	128 ± 3.00	126 ± 1.00	130 ± 1.00	132 ± 2.00
6	Drug content	99.18	99.42	99.23	99.10

Data was expressed as mean \pm S.D. (n=3)

Particle size Analysis of formulated microemulsion:

The particle size of the optimised microemulsion was determined by photon cross-correlation spectroscopy. The particle size of Glimepiride

microemulsion was found to be 38.83nm. The particle size was within the range indicating the microemulsion of very low globule size. The results are represented in **Figure 2**.

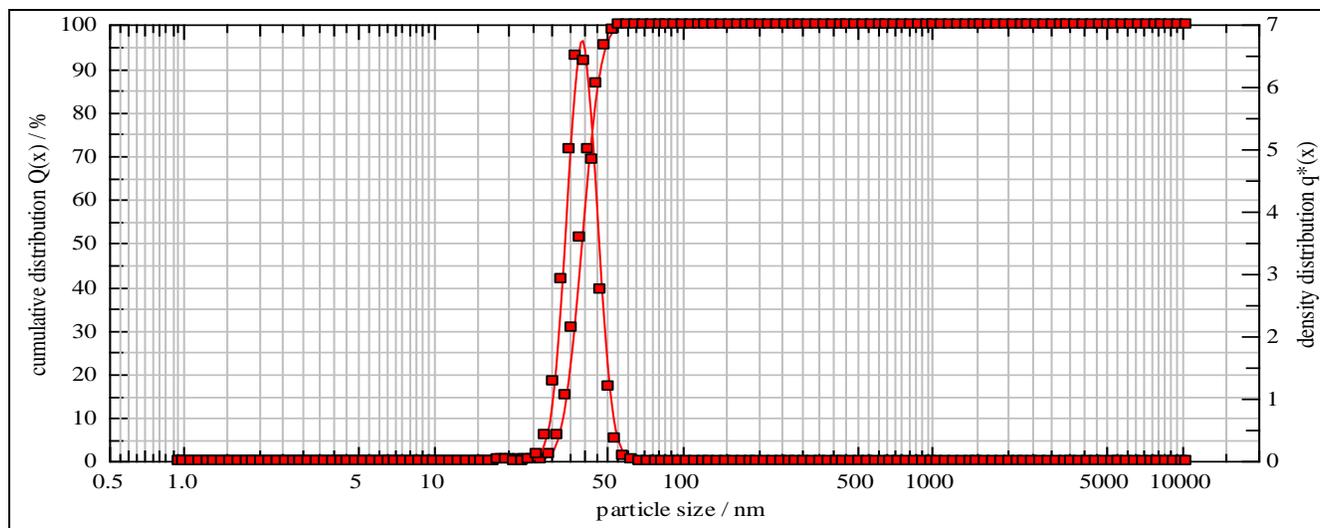


FIGURE 2: PARTICLE SIZE MEASUREMENT OF GLIMEPIRIDE MICROEMULSION

Characterisation of solid microemulsion:

The prepared solid ME were subjected to characterisation for angle of repose, bulk density, tapped density, Hausner's Ratio, Carr's index, drug content, *in-vitro* drug release study and stability studies. The result are revealed in **Table 7**.

Angle of Repose:

The angle of repose was found to be $23.46^{\circ} \pm 0.8^{\circ}$.

Bulk density:

The bulk density was found to be 0.500 ± 0.004 g/cm³.

Tapped density:

The tapped density was found to be 0.551 ± 0.02 g/cm³.

Hausner's Ratio:

The Hausner's ratio was found to be 1.102 ± 0.03 .

Carr's index:

The Carr's index was found to be 10.34 ± 0.6 %.

Drug content uniformity

The percentage drug content of SME 2 was found to be 98.56 ± 0.32 %. The solid microemulsion were found to be uniform in drug content.

TABLE 7: PHYSICO-CHEMICAL CHARACTERISTICS OF GLIMEPIRIDE SOLID MICROEMULSION

Sr. No.	Parameters	SME 2
1	Angle of repose	$23.46^{\circ} \pm 0.8^{\circ}$
2	Bulk density	0.500 ± 0.004 g/cm ³
3	Tapped density	0.551 ± 0.02 g/cm ³
4	Hausner's ratio	1.102 ± 0.03
5	Carr's index	10.34 ± 0.6 %
6	Drug content (% w/w)	98.56 ± 0.32 %

Data was expressed as mean \pm S.D. (n=3)

In-vitro drug release studies:

In-vitro drug release studies were carried out using Dissolution apparatus. The *in-vitro* drug release of solid microemulsion was compared with the pure Glimepiride. The outcomes are depicted in **Figure 3**.

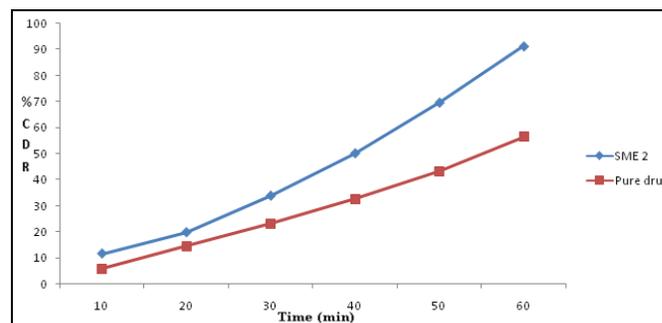


FIGURE 3: CUMULATIVE PERCENTAGE DRUG RELEASE FROM GLIMEPIRIDE SOLID MICROEMULSION

The release of Glimepiride from solid microemulsion prepared from aerosil 200 (91.37%) was found to be higher than the pure drug (56.534%). This may be due to the fact that in the microemulsion system, the solubility of Glimepiride might have increased. Further, the SME released the drug in a fast or manner as compared to pure drug.

Stability studies:

The result of stability studies shown that there were no significant changes in the angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index, drug content of solid microemulsion after storing at a temperature of $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / $75\% \pm 5\%$ relative humidity for one months. These results indicated that drug remain stable after incorporating in the solid microemulsion containing aerosol 200 as adsorbent.

TABLE 8: STABILITY STUDIES OF OPTIMIZED SOLID MICROEMULSION

Formulation	SME 2		
	0	15	30
Periods (days)	0	15	30
Physical appearance	No change	No change	No change
Angle of repose	$23.46^{\circ} \pm 0.8^{\circ}$	$23.46^{\circ} \pm 0.8^{\circ}$	$23.42^{\circ} \pm 0.5^{\circ}$
Bulk density	$0.500 \pm 0.004 \text{ g/cm}^3$	$0.500 \pm 0.004 \text{ g/cm}^3$	$0.507 \pm 0.003 \text{ g/cm}^3$
Tapped density	$0.551 \pm 0.02 \text{ g/cm}^3$	$0.551 \pm 0.02 \text{ g/cm}^3$	$0.561 \pm 0.01 \text{ g/cm}^3$
Hausner's ratio	1.102 ± 0.03	1.102 ± 0.03	1.106 ± 0.013
Carr's index	$10.34 \pm 0.6 \%$	$10.34 \pm 0.6 \%$	$10.52 \pm 0.6 \%$
% Drug content	$98.56 \pm 0.32 \%$	$98.10 \pm 0.22 \%$	$97.60 \pm 0.28 \%$

Data were expressed as mean \pm S.D (n=3).

CONCLUSION: The results of the present work concludes that the antidiabetic drug Glimepiride, which suffers from the poor water solubility issue can be successfully formulated into a solid microemulsion for oral delivery which will improve the solubility and the bioavailability.

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