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Original Article

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Self-microemulsifying drug delivery system as carrier for the oral delivery of glimepiride: Formulation development, optimization, *in-vitro* characterization, stability assessment, *ex-vivo* permeation, and *in-vivo* antidiabetic activity in albino mice

Abstract

Aim: The research aimed to design the glimepiride self-micro emulsifying drug delivery system (SMEDDS) for increased oral bioavailability in albino mice by assessing hypoglycemic efficacy.
Materials and Methods: The optimized liquid SMEDDS (L-SMEDDS) prepared by emulsification of Capryol® 90 (oil), Kolliphore® EL (surfactant), and Transcutol® P (co-surfactant) were screened based on their solubility in glimepiride. Adsorption onto Aerosil® 200 Pharma produced solid SMEDDS (S-SMEDDS), and further direct compression was used to manufacture the tablets. The formulations were subjected to droplet size, polydispersity index (PdI), time of emulsification, *in-vitro* drug release, crystallinity nature, surface morphology, thermal behavior, *ex-vivo* permeability, and *in-vivo* hypoglycemic activity in albino mice.
Results: The SMEDDS emulsified in less than 30 sec and had an average droplet size of 22.3 nm with a PdI of 0.296. The solid-state analysis revealed that glimepiride was in a molecular dispersion or amorphous form. *In-vitro* release experiments demonstrated that glimepiride released more efficiently than plain glimepiride. Both L-SMEDDS and S-SMEDDS did not have any significant differences in terms of release. The *ex-vivo* and *in-vivo* studies revealed that SMEDDS possess improved permeability and hypoglycemic activity than plain glimepiride.
Conclusion: The study proved the combination of lipid-based nanosystems with oral dosage forms improved the oral bioavailability of glimepiride SMEDDS.

Keywords: Glimepiride, self-micro emulsifying drug delivery system, adsorption, *in-vitro* release, *ex-vivo* permeation, oral bioavailability

INTRODUCTION

Type II diabetes is first treated with an oral antidiabetic medication. One of the oral sulfonylurea medications of the third generation, glimepiride, is used to treat type II diabetes mellitus (1). It works by enhancing peripheral tissue sensitivity to insulin and lowering blood glucose by stimulating insulin production from beta cells in the pancreas through adenosine triphosphate (ATP) sensitive potassium channels (2). High hypoglycemic effectiveness and low systemic toxicity are two characteristics of glimepiride (1). The biopharmaceutical classification system assigns glimepiride to class II. At 37°C, glimepiride is insoluble in both acidic and neutral aqueous environments (0.004 mg/mL). In fluids with a pH greater than 7, the drug's solubility is slightly increased to 0.02 mg/mL. This poor solubility may result in limited dissolution and unexpected bioavailability. The relatively low water solubility and wettability of glimepiride make it challenging to create effective pharmaceutical formulations for

oral use and have resulted in inconsistent oral bioavailability (3). Glimepiride is marketed as an oral tablet for immediate release under the brand name AMARYL® (Sanofi Aventis US LLC, United States of America, USA) in strengths of 1 mg, 2 mg, and 4 mg, all of which were authorized by the United States Food and Drug Administration (US FDA) on November 30, 1995 (4). The rate at which glimepiride and other poorly water-soluble drugs dissolve in gastrointestinal fluids affects how quickly and how much they are absorbed orally (1). Although many methods, including liposomes, nanosuspensions, and lipid nanoparticles, have been proposed to ensure a proper rate of drug release, one of the most popular is a lipid-based nanosystem called self-emulsifying formulations. These formulations not only solve solubility and bioavailability challenges but they can also alter pharmacokinetics, which improves the drug's safety and efficacy. self-micro emulsifying drug delivery system (SMEDDS) are isotropic multi-component systems composed of synthetic or natural oil, a surfactant, and a co-surfactant. Following gentle agitation, these compounds have the ability to create fine oil-in-water micro- or nano-emulsions, which may then be diluted in an aqueous medium such as gastrointestinal fluid. The formulation provides a considerable interfacial area for drug partitioning between the oil and the gastrointestinal fluid, which self-emulsifies in the stomach and delivers the drugs in small droplets of oil, enhancing drug solubility. Additional benefits include improved drug molecule stability and convenience in using the final product as capsules either a liquid or solid state (5). The goal of the current study is to create and optimize glimepiride's stable SMEDDS in order to increase its oral bioavailability.

MATERIALS AND METHODS

Glimepiride was graciously provided as a complimentary sample by Alkem Laboratories Limited, India. Capmul® MCM was received as a generous gift from Abitec Corporation, India. Additionally, Labrafil® M 1944CS, Labrafil® 1349 WL, Labrafac® PG, Capryol® 90, Labrafil® M2130CS, Plurol® Oleique CC 497, Transcutol® P, and Transcutol® HP were generously provided by Gattefosse India Private Limited, India. The sources of Kolliphore® RH, Kolliphore® EL, Solutol® HS 15, and Polyplasdone® were BASF India Limited, India. Polysorbate 80, Polysorbate 20, and Polyethylene Glycol 400 were procured from SD Fine Chemicals Limited, India. SuperTab® 21 AN was kindly supplied by DFE Pharma India Limited, India. Avicel® PH 102 was received as a thoughtful gift from FMC Biopolymer India Private Limited, India. Starch 1500® was obtained as a sample from Colorcon Asia Private Limited, India. Aerosil® 200 Pharma was sourced from Evonik Industries Private Limited, India. Talc Luzenac was procured from Imerys Ceramics (India) Private Limited, India. Hyqual® magnesium stearate was generously provided by Mallinckrodt Pharmaceuticals, USA. Oleic acid, Isopropyl Alcohol, Methanol, Ethanol, and glucose powder were purchased from Loba Chemie Private Limited, India. Saline was obtained from Baxter Pharmaceuticals India Private Limited, India. Purified water, 0.1 hydrochloric acid (HCl) phosphate buffers, and tyrode solutions were prepared in-house.

Development and optimization of glimepiride L-SMEDDS

Solubility studies

The saturation solubility of glimepiride in various vehicles (oils, surfactants, and co-surfactants) was determined using the shake flask technique (6,7). Extra glimepiride was blended with 1 g of the specified vehicles in 10 mL clean glass vials using a vortex mixer (Vortex Mixer, Remi Ltd., India) to ensure proper drug-vehicle mixing. The stoppered vials were then shaken in an orbital water bath (Remi Ltd., India) for 72 h at 37°C. To remove the undissolved glimepiride from saturated solutions, all of the samples were centrifuged (Remi Ltd., India) at 12,000 rpm for 15 min after equilibration. Methanol was used to dilute the supernatants, and the concentration of glimepiride was determined using a UV spectrophotometer (Shimadzu UV-1800, Shimadzu, Japan).

Preparation of glimepiride L-SMEDDS and construction of ternary phase diagrams

Formulation components for the liquid SMEDDS (L-SMEDDS) were selected based on their solubility saturation, focusing on the vehicles (oil, surfactant, and co-surfactant) with the greatest solubility for glimepiride. The formulation process involved the active ingredient glimepiride (1 mg) along with Capryol® 90 as the oil phase and a combination of Kolliphor® EL and Transcutol® P. These components were accurately weighed and introduced into sealed glass vials. After achieving the complete dissolution of glimepiride to establish a uniform/monophasic system, the mixture was gently vortexed for 5 min using a vortex mixer (Remi Ltd., India). The vials were then set aside until the mixture was clarified. The resultant formulations were stored at room temperature until further use. For a precise determination of phase boundaries in the phase diagrams, the aqueous titration method was employed. Varying volume ratios of oil and Smix (a combination of surfactant and co-surfactant) were thoroughly combined, ranging from 10:90 to 90:10, within distinct glass vials. Visual assessment was carried out by slowly titrating each volume ratio of oil and Smix with an aqueous phase to observe the formation of transparent, easily flowable oil-in-water nano emulsions. To evaluate the stability of these prepared emulsions, several factors were considered, including spontaneous emulsification, clarity, phase separation, droplet coalescence, drug precipitation, and emulsion integrity after a 48-hour storage period at room temperature. The presence of instability was indicated by incomplete or absent emulsion formation, rapid droplet coalescence resulting in phase separation, and drug precipitation. Furthermore, to investigate the influence of dilution on emulsion stability, mimicking *in-vivo* stomach conditions, stable and clear emulsions from the L-SMEDDS were subjected to incremental dilutions using diverse physiological mediums.

Characterization and evaluation of glimepiride L-SMEDDS

Optical clarity/% transmittance

Spectrophotometric assessments of the optical transparency for glimepiride L-SMEDDS formulations were executed subsequent

to their dilution (7). In brief, a solution containing 1 mg of glimepiride was subjected to a 100-fold dilution in purified water maintained at 37°C. The solution was stirred for 1 min at 100 rpm utilizing a magnetic stirrer (Remi Ltd., India). Measurements of % transmittance and % absorbance were performed at 228 nm using a UV spectrophotometer (Shimadzu UV-1800, Shimadzu, Japan) immediately upon formation, as well as at intervals of 6 h and 24 h after the initial dilution. This assessment aimed to determine the clarity of each dispersion.

Self-emulsification time and dispersibility test

The evaluation of self-emulsification efficacy was conducted employing a conventional dissolution apparatus. A quantity corresponding to 1 mg of glimepiride was introduced into 500 mL of purified water at 37°C, with a rotating paddle set at 50 rpm to ensure gentle agitation. The *in-vitro* emulsification behavior (time) of the L-SMEDDS was visually appraised employing a grading system (8,9). Grade I indicated swift formation with a clear or slightly bluish appearance; Grade II denoted rapid formation with somewhat less clarity or a bluish-white aspect; Grade III signified the creation of a bright white emulsion, akin to the appearance of milk. Furthermore, a specific formulation containing 1 mg of glimepiride was subjected to diverse dilutions (50, 100, and 1000 times) and exposed to various diluting agents (purified water, 0.1 N HCl, and pH 7.5 phosphate buffer). Subsequent visual observations were documented and categorized based on the grading system.

Drug content

The L-SMEDDS formulation containing 1 mg of glimepiride was mixed with methanol and subjected to 5 min of sonication. The resulting solution was then further diluted appropriately and analyzed at a wavelength of 228 nm using a Shimadzu UV spectrophotometer (Shimadzu UV-1800, Shimadzu, Japan). A comparative assessment was performed against established glimepiride standard solutions.

Globule size, size distribution, and zeta potential

A quantity of L-SMEDDS corresponding to 1 mg of glimepiride underwent dilution with 10 mL of purified water and was subsequently subjected to incubation at 25°C. Photon correlation spectroscopy was employed to evaluate parameters such as mean globule size, size distribution, and zeta potential. The measurements were conducted utilizing a Zetasizer ZS90 (Malvern Instruments Ltd., United Kingdom). The process involved monitoring light scattering at a 90° angle using a 50 mV laser, and the temperature was maintained at 25°C throughout the analysis.

Development of glimepiride S-SMEDDS

Adsorption onto a solid carrier: The transformation of Glimepiride L-SMEDDS (F1) into solid SMEDDS (S-SMEDDS) was accomplished by utilizing the solid inert carrier Aerosil® 200 Pharma. This carrier is characterized by its substantial surface area and effective adsorption capabilities. The method employed for this transformation was physical adsorption (10). The procedure involved combining the carrier with the emulsion in a 1:1 ratio within a glass mortar. By adding the carrier in small increments, a non-sticky solid powder was obtained. To ensure the even distribution of droplets, the mixture was homogenized using a glass rod. Subsequently, the produced S-SMEDDS underwent mesh #40 filtration to achieve self-nanoemulsifying granules that possessed consistent free-flowing properties. An evaluation of the granules' micrometric characteristics was conducted as well.

Preparation of tablets: As outlined in Table 1, SuperTab® 24 AN, Avicel® PH 102, Starch 1500®, Polyplasdone®, Aerosil® 200 Pharma, and Luzenac Talc underwent screening through mesh #40. Additionally, Hyqual® magnesium stearate was separately screened through mesh #60. The granular materials were combined with all the components except for Hyqual® Magnesium Stearate using a dual cone blender (Dolphin, India) with an appropriate capacity, operating at 20 rpm for a duration of 10 min. Subsequently, this mixture was lubricated utilizing Hyqual® Magnesium Stearate at the same 20 rpm for 5 min. A rotary compression apparatus (Karnavati, India) was employed to compress the blend into tablets, employing flat-faced punches with an 11 mm round die size. The resulting tablets were subjected to assessments encompassing mean weight, diameter, thickness, hardness, disintegration time, and friability.

Table 1. Unit composition for a tablet of glimepiride S-SMEDDS

Ingredients	% w/w
Glimepiride S-SMEDDS	50.250
SuperTab® 21 AN	10.000
Avicel® PH 102	5.000
Starch 1500®	17.500
Polyplasdone®	2.500
Aerosil® 200 Pharma	12.500
Luzenac Talc	1.125
Hyqual® Magnesium Stearate	1.125

Characterization and evaluation of glimepiride S-SMEDDS

Crystallinity by X-ray powder diffraction (XRD)

The X-ray diffractometer used for characterization was the X: Pert PRO with X-Pert data collector (PAN Analytical, Netherlands). The characterization involved glimepiride, Aerosil® 200 Pharma, and S-SMEDDS, with their respective physical states being the focus. The analysis was conducted by placing the samples in an aluminum cavity at ambient temperature. Monochromatic CuK-radiation at 40 mA and 40 kV was employed, spanning a 2° range from 7 to 80°. The scanning took place at a consistent speed of 4°/min.

Surface morphology by scanning electron microscopy (SEM)

The surface analysis of glimepiride, Aerosil® 200 Pharma, and S-SMEDDS was conducted using the SEM (Phillips XL

30 FEG, Netherlands). An accelerating voltage of 20 kV was applied. For sample preparation, the materials were placed on an aluminum stub fixed with double-sided adhesive tape. Ensuring secure attachment, the sample was evenly dispersed onto the tape-covered surface of the stub. Subsequently, a thin gold layer was sputter-coated onto the sample-bearing stub to enable conductivity enhancement.

Thermal behavior by differential scanning calorimetry (DSC)

The thermal properties of glimepiride, Aerosil® 200 Pharma, and S-SMEDDS were examined using a Perkin Elmer 7 differential calorimeter (PerkinElmer, Inc., USA). The experimental setup included a computerized data station. Each sample was accurately weighed and placed in a sealed pierced aluminum pan. The scanning rate for heating was maintained at 10 °C/min, spanning a temperature range of 30 to 300°C. A nitrogen flow at a rate of 50 ml/min was employed, with an empty pan serving as a reference for comparison purposes.

***In-vitro* comparative release study**

In-vitro release assessments were conducted on plain glimepiride, L-SMEDDS, an S-SMEDDS tablet, and a commercial tablet using a USP type-II dissolution testing apparatus (DS 8000, Labindia, India). The test was performed with rotating paddles set at 75 rpm, utilizing 500 mL of phosphate buffer at pH 7.8 as the dissolution medium. The temperature was maintained at 37±0.5°C. At predetermined intervals, 5 mL samples were withdrawn and replaced with the same volume of fresh dissolution medium. The withdrawn samples were then filtered through 0.45 µm millipore filters (Merck KGaA, Germany). Subsequent analysis was carried out at 228 nm using a Shimadzu UV spectrophotometer (Shimadzu, Japan).

***Ex-vivo* intestinal permeability study**

A non-everted gut sac model of chicken intestinal (jejunum) segments was utilized to conduct an *ex-vivo* intestinal permeability study (10,11). The segments underwent a thorough cleansing process with purified water to eliminate mucous and luminal contents. Subsequently, they were immersed in a tyrode solution. Sac segments, each measuring 5 cm in length, were created by securely fastening both ends with cotton thread. To enable a comparison, these sacs were individually loaded with drug suspensions in purified water, including a placebo and glimepiride SMEDDS. The prepared sacs were then placed into distinct dissolution baskets, each containing 500 mL of phosphate buffer with a pH of 7.8. These baskets were maintained at a constant temperature of 37±0.5°C and stirred at a speed of 75 rpm. Over specific time intervals, samples were withdrawn from the solution. On each occasion, a 5 mL sample was extracted and immediately replaced with a fresh medium. These samples were subsequently filtered using 0.45 µ millipore filters (Merck KGaA, Germany) and subjected to analysis at a wavelength of 228 nm using a Shimadzu UV spectrophotometer (Shimadzu, Japan). The permeability of glimepiride was assessed throughout a 3 h period.

***In-vivo* glucose level performance of SMEDDS in albino mice**

Six healthy albino mice were housed in polypropylene cages and maintained under standard laboratory conditions, with a constant temperature of 25±1°C and a relative humidity of 55±5%. The animals' care adhered to the guidelines for laboratory animal care and the regulations established by the committee overseeing animal experimentation in India. Each mouse was orally administered (100 µL) with distinct investigation samples, where 1 g of each sample was dissolved in 10 mL of purified water. Blood samples were subsequently collected from the mice's tail veins, and their glucose levels were assessed using a Tyson Bio TB 200 blood glucose meter (Tyson Bioresearch Inc., Taiwan). After a washout period of seven days, the experiment was repeated with various treatments:

Treatment A: Control sample administered with oral saline;
Treatment B: Glucose sample;
Treatment C: Plain glimepiride; and
Treatment D: Glimepiride SMEDDS (1,12).

Stability studies

Stability investigations were carried out to assess alterations in *in-vitro* drug release patterns, drug content, emulsion globule dimensions, and polydispersity index (PdI) while storing the optimized S-SMEDDS for a duration of 30 d. Storage conditions encompassed two temperature and humidity variations: 40°C±2°C/75%RH±5% RH and 25°C±2°C/60% RH±5% RH.

RESULTS

Development and optimization of glimepiride L-SMEDDS

Solubility studies

The initial stage of formulating glimepiride L-SMEDDS involved conducting solubility examinations to identify appropriate oily phases, surfactants, and co-surfactants. It's imperative to consider the drug's solubility when formulating self-emulsifying systems to prevent drug precipitation upon *in-vivo* dilution. To determine a suitable vehicle with optimal drug loading capacity, an assessment of glimepiride's solubility in the screened vehicles was performed. The highest solubility of glimepiride was observed in Capryol® 90 (oil), Kolliphor® EL (surfactant), and Transcutol® P (co-surfactant) (Figure 1). These findings align with previously published literature (13,14).

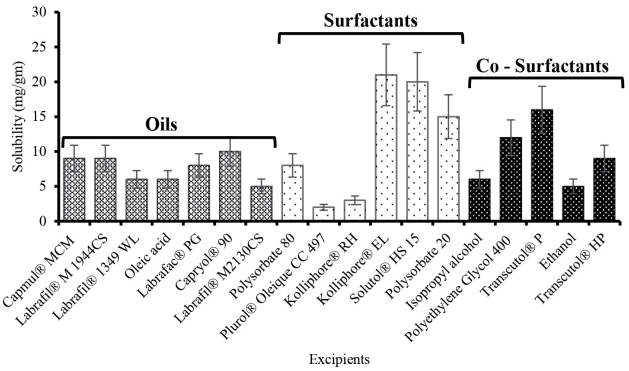

Figure 1. Glimepiride solubility in various vehicles (Mean±SD, n=3)

Preparation of glimepiride L-SMEDDS and construction of ternary phase diagrams

Utilizing the outcomes of initial investigations, ternary phase diagrams were developed for the nine systems to explore the connection between phase characteristics and composition (As shown in Figure 2). The primary aim was to ascertain the concentration range of constituents conducive to producing a nano emulsion. For the construction of phase diagrams, all components were converted to percentage weight/weight percentage (%w/w). The L-SMEDDS exhibited the ability to create a finely dispersed oil-water emulsion with minimal agitation. The selection criteria for composition centered around specific parameters: globule size (<350 nm), PdI (0.350<PdI), and noteworthy physical stability (48 h).

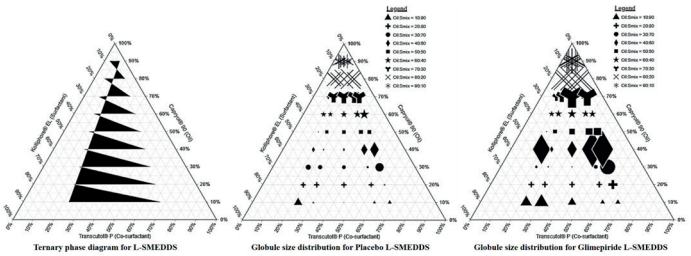

Figure 2. Ternary phase diagram for L-SMEDDS and globule size distribution for placebo L-SMEDDS, and glimepiride L-SMEDDS

Group I (Oil: Smix–10:90)

In group I, the placebo formulations showed globule sizes less than 14–100 nm (PdI less than 0.300) with good physical stability. However, when the glimepiride was loaded into these formulations, the globule size ranged from 125–850 nm (PdI ranging from 0.200–1.000), and all the formulations showed physical instabilities at 48 h. The drug leakage was mainly observed, which may be due to less oil present to solubilize the glimepiride.

Group II (Oil: Smix–20:80)

Group II consists of 20 %w/w Capryol® 90 as oil and 80% w/w Smix. The placebo formulations of this ratio showed 100–200 nm globule size (PdI 0.100-0.400) with grade I emulsions. The glimepiride-loaded emulsions of these ratios showed 100–200 nm globule size (PdI 0.300-0.600). These formulations were significantly unstable over 48 h and showed phase separation and precipitation of glimepiride.

Group III (Oil: Smix–30:70)

In group III, the formulations without glimepiride showed 50–600 nm globule size (PdI 0.100-0.600) and a grade III appearance. Similar results were seen when glimepiride was loaded into emulsions. All the emulsions were grade III with globule sizes of 20–1650 nm (0.100-0.650). Hence, these formulations were disqualified for further evaluation.

Group IV (Oil: Smix–40:60)

The formulations in group IV showed a mean globule size of 30–700 nm (PdI 0.047–0.560) for II and III emulsions. The glimepiride-loaded emulsions showed drug precipitation and leakage with globule sizes of 790–3600 nm (PdI 0.290–0.820).

Group V (Oil: Smix–50:50)

In group V, the oil and Smix were divided into 50%w/w each. Among these formulations, emulsions with higher concentrations of surfactant showed significant physical stability for both placebo and glimepiride emulsions (F1, F2, and F3) (Table 2). Further, decreasing surfactant below 16% w/w showed grade III emulsion with a higher PdI of 0.300-0.700 and various physical instabilities.

Group VI (Oil: Smix–60:40)

In this group, the emulsions with less than 25%w/w of surfactants showed higher globule sizes (more than 300 nm) for both placebo and glimepiride emulsions with a lack of physical stability. The formulation F4 showed less than 200 nm (PdI less than 0.5) with significant stability (Table 2). Moreover, it's worth noting that none of the emulsions exhibited any signs of drug precipitation or leakage. This favorable outcome can be attributed to the elevated oil ratio, which effectively solubilizes glimepiride. But these emulsions showed a grade III appearance after 48 h.

Group VII (Oil: Smix–70:30)

In group VII, the emulsion (F5) with 22.5%w/w surfactant showed good stability at the time of preparation but converted to grade III emulsions after 48 h. All the other combinations in this group showed grade III emulsions of more than 700 nm and 1650 nm for placebo and glimepiride emulsions, respectively.

Group VIII (Oil: Smix–80:20) and group IX (Oil: Smix–90:10)

In both groups, both placebo and glimepiride emulsions showed more than 1000 nm (PdI 1.000) with a grade III appearance. All the emulsions showed phase separation, which is possibly due to a low Smix (less than 20%w/w), which is not sufficient to keep the emulsion thermodynamically stable. Hence, both of these groups were disqualified for further development.

Characterization and evaluation of glimepiride L-SMEDDS

Optical clarity/% transmittance

The transmittance for F1 was 94.20%, which can be seen as visually clear. Further, there was no change in the absorbance levels for F1 due to its better thermodynamic stability. The F2 to F5 showed a slight increase in the absorbance at various intervals, which indicates a change in the globule size and stability (Table 2).

Self-emulsification time and dispersibility test

All the formulations showed rapid emulsification with less than one minute of dilution (Table 2). Increased dilution and changes in diluents did not affect the appearance and did

not show any drug precipitation for F1. This implies that the formulation exhibited resilience against dilution and variations in diluents, thereby preserving its efficacy *in-vivo*. In contrast, the remaining formulations (F2-F6) demonstrated lower levels of emulsification, as indicated in Table 3.

Table 2. Composition and physicochemical data for L-SMEDDS

L-SMEDDS formulations		F1	F2	F3	F4	F5
Composition	Capryol® 90 (Oil) (%)	50.00	50.00	50.00	60.00	70.00
	Kolliphor® EL (Surfactant) (%)	37.50	33.33	25.00	26.60	22.50
	Transcutol® P (Co-surfactant) (%)	12.50	16.67	25.00	13.40	7.50
Appearance		Clear	Clear	Bluish	Bluish	White
Transmittance (%)		94.20	81.60	79.70	80.53	87.76
Absorbance	0 h	0.018	0.029	0.031	0.026	0.040
	6 h	0.020	0.031	0.032	0.025	0.030
	24 h	0.020	0.043	0.046	0.039	0.053
Emulsification time (sec)		18	23	28	29	45
Drug content (%)		95.26	95.92	96.03	97.13	95.45
Placebo SMEDDS	Globule size (nm)	18.2	128.7	139.0	148.0	78.4
	PdI	0.163	0.343	0.262	0.438	0.568
Glimepiride SMEDDS	Globule size (nm)	22.3	175.4	344.3	223.4	330.4
	PdI	0.296	0.293	0.337	0.415	0.280

Table 3. Effect of dilution (media and volume) of glimepiride L-SMEDDS

Diluent/media	Dilution volume (mL)	F1	F2	F3	F4	F5
		Grades				
Purified water	50	I	II	III	III	III
	100	I	II	III	III	III
	1000	I	II	III	III	III
0.1 N HCl	50	I	II	III	III	III
	100	I	II	III	III	III
	1000	I	II	III	III	III
Phosphate buffer, pH 7.5	50	I	III	III	III	III
	100	I	III	III	III	III
	1000	I	III	III	III	III

Drug content

The drug content within the formulation holds immense importance, particularly given the low-dosage nature of glimepiride. It is essential to verify the accurate delivery of the prescribed dose, which leads to the evaluation of drug content. The results indicated that all formulations exhibited drug content exceeding 95%, affirming that there was no substantial drug loss during the formulation process.

Globule size, size distribution, and zeta potential

The globule sizes of the SMEDDS ranged from 20 nm to 350 nm, and the PdI ranged from 0.200 to 0.600, indicating that the globules fell within the nanometric size range (as shown in Table 2). The optimized formulation was selected based on achieving the smallest particle size. A PdI closer to zero indicates greater homogeneity among the droplets. Specifically, formulation F1 exhibited minimal changes in globule size between the placebo and glimepiride emulsions. In contrast, all other emulsions experienced an increase in globule size after incorporating glimepiride. The PdI for F1 was found to be 0.296 (as depicted in Figure 3). Notably, the zeta-potential for F1 measured-13.6 mV, signifying its stability (as demonstrated in Figure 3).

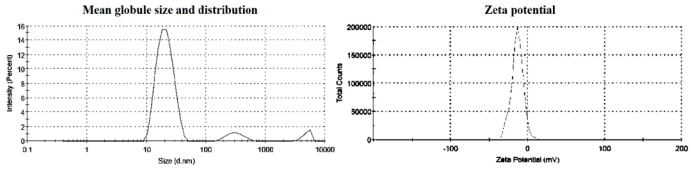

Figure 3. Mean globule size, distribution, and zeta potential of glimepiride L-SMEDDS (F1)

Development of glimepiride S-SMEDDS

Adsorption onto a solid carrier: Adsorbents such as dibasic calcium phosphate, anhydrous lactose, calcium carbonate, and magnesium carbonate displayed limited adsorption capacity. However, Aerosil® 200 Pharma exhibited favorable adsorption characteristics, excellent flow properties, and optimal bulk

density for compression into tablets. The granules demonstrated a bulk density of 0.410 g/mL, a tapped density of 0.520 g/mL, a compressibility index of 21.15%, and a Hausner's ratio of 1.27. The angle of repose was measured at 23.83°.

Preparation of tablets: The tablets were satisfactory with respect to manufacturing feasibility and physical properties. The prepared tablets showed a weight variation of 401.0±3.5, a diameter of 11.00±0.05 mm, a thickness of 3.10±0.05 mm, a hardness of 10–12 kp, and a disintegration time of 20–25 sec. The friability was 0.44±0.07%.

Characterization and evaluation of glimepiride S-SMEDDS

Crystallinity by X-ray powder diffraction (XRD)

Glimepiride exhibited distinct and sharp peaks in its X-ray diffraction pattern, confirming its crystalline nature (as shown in Figure 4). In contrast, Aerosil® 200 Pharma displayed broad and diffuse maxima, suggesting its amorphous character with the absence of intrinsic peaks (as depicted in Figure 4). Notably, the X-ray diffraction patterns of the S-SMEDDS revealed only one broad and diffuse maximum peak, indicative of the amorphous state of the formulation (as shown in Figure 4). These findings align with previously published data (5). The absence of characteristic glimepiride peaks in the S-SMEDDS indicates a transformation in the physical state, transitioning to a non-crystalline, amorphous, or disordered crystalline phase within the molecular dispersion.

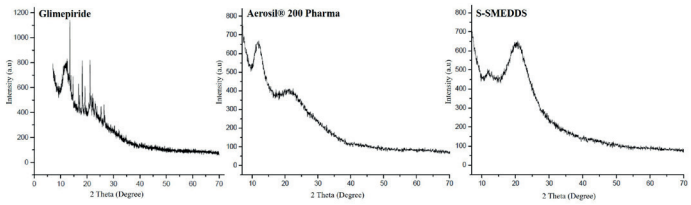

Figure 4. XRD patterns of glimepiride, Aerosil® 200 Pharma, and S-SMEDDS

Surface morphology by scanning electron microscopy (SEM)

Glimepiride displayed smooth, rectangular crystalline structures, as depicted in Figure 5. In contrast, Aerosil® 200 Pharma possesses a highly porous structure capable of adsorbing up to three times its weight in oil. The particles of Aerosil® 200 Pharma, as shown in Figure 5, appear to be spherical and porous, with a size of approximately 10 µ. An important observation from Figure 5 is the effective adsorption onto the solid carrier, as no visible oil droplets are present. This adsorption occurred through physical mixing, with partially covered carriers also visible within the field of view. Notably, in the case of S-SMEDDS, there is an absence of crystalline structures typically associated with glimepiride. This suggests that glimepiride is completely dissolved within the S-SMEDDS. These findings align with existing literature (5), further confirming the dissolution and dispersion characteristics of glimepiride within the S-SMEDDS.

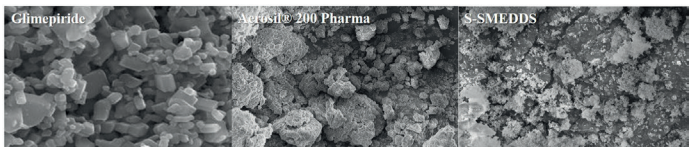

Figure 5. Surface morphology of glimepiride, Aerosil® 200 Pharma, and S-SMEDDS

Thermal behavior by differential scanning calorimetry (DSC)

Glimepiride exhibits a distinct and sharp exothermic peak at 209.06°C, a characteristic of its crystalline nature, as depicted in Figure 6. Notably, there are noticeable endothermic alterations in the DSC curves of Aerosil® 200 Pharma and S-SMEDDS (as shown in Figure 6). These curves reveal a sharp endothermic peak with an onset temperature of 168.33°C, indicative of the formation of a eutectic system between glimepiride and the carrier. In such cases, the lower-temperature peak corresponds to the melting of the eutectic mixture.

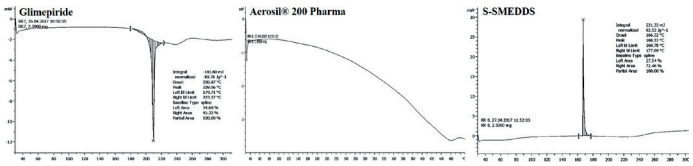

Figure 6. DSC thermogram of glimepiride, Aerosil® 200 Pharma, and S-SMEDDS

In-vitro comparative release study

The formulations demonstrated a release of over 95% of the available glimepiride. However, the release rate and extent of release from SMEDDS formulations were notably faster (less than 60 min) when compared to both plain glimepiride and commercially available formulations (approximately 180 min).

Ex-vivo intestinal permeability study

The *ex-vivo* permeability assessment was conducted alongside the *in-vitro* drug release analysis to gain insights into the permeability characteristics of the formulation. Due to its greater thickness, which makes it suited for experiments comparing permeability, the chicken intestinal sac model was taken into consideration. The glimepiride SMEDDS showed slightly higher permeation of glimepiride than the plain drug suspension.

In-vivo glucose level performance of SMEDDS in albino mice

A variety of pharmaceuticals are frequently evaluated *in-vivo* using pharmacodynamic markers. Glimepiride, an anti-diabetic medication, is known to reduce plasma glucose levels. Therefore, plasma glucose levels were utilized as a reference point for assessing the *in-vivo* efficacy of SMEDDS-containing glimepiride (5,12,14). Following the administration of glucose, plasma glucose levels exhibited a rapid increase. However, the concurrent administration of glucose and pure glimepiride led to a gradual decline in plasma glucose levels following the initial rise, followed by another increase in glucose levels. In contrast, the co-administration of SMEDDS (F1) with glucose resulted in a swift reduction in plasma glucose levels. These observations

suggest that glimepiride SMEDDS is more effective than pure glimepiride in reducing plasma glucose levels, primarily due to the enhanced drug absorption from the SMEDDS formulation (refer to Figure 7). The results of ANOVA indicated that there was a significant difference ($p<0.05$) in plasma glucose level among the four treatments.

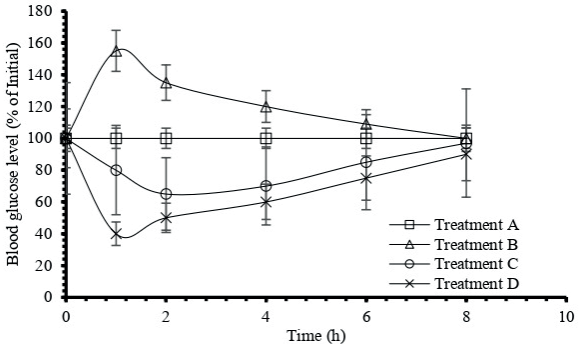

Figure 7. The antidiabetic activity of treatment A (control sample treated with oral saline), treatment B (glucose sample), treatment C (plain glimepiride), and treatment D (glimepiride SMEDDS) in albino mice. The data are expressed as the mean (n=3) ($p<0.05$)

Stability studies

Samples collected after a duration of three months displayed no noteworthy alterations in *in-vitro* drug release assessments (as depicted in Figures 8A and 8B), drug content, emulsion globule size, and PdI. This signifies a strong resemblance in the dissolution release profile before and after undergoing stability investigations, as outlined in Table 4. As a result, it can be concluded that the glimepiride S-SMEDDS tablets remained stable under both accelerated and extended storage conditions.

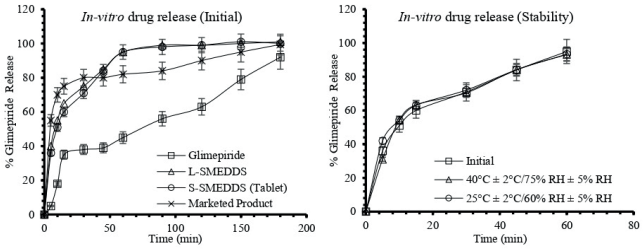

Figure 8. *In-vitro* drug release patterns of L-SMEDDS and a tablet of S-SMEDDS compared to those from a plain and marketed product (tablet) of glimepiride; Stability of S-SMEDDS for 30 days The data are expressed as the mean (n=3)

Table 4. Stability data for glimepiride L-SMEDDS and S-SMEDDS

Test parameter	Initial	40°C±2°C/75% RH±5% RH for 30 d	30°C±2°C/65% RH±5% RH for 30 d
Glimepiride L-SMEDDS			
Physical observation	Clear	Clear	Clear
Globule size (nm)	22.3	25.7	24.9
PDI	0.296	0.279	0.291
Zeta potential (mV)	-13.6	-14.1	-12.7
Glimepiride S-SMEDDS			
Drug content (%)	95.26	96.13	95.57
Drug released (%) at 60 min	94.91	93.12	93.42

DISCUSSION

SMEDDS create a nano emulsion in the gastrointestinal tract (GIT), which is patient-acceptable but indicates globule size in the nanoscale range due to formulations' isotropic nature or transmittance percentages that are closer to 100%. Since the SMEDDS droplets measure less than one-fourth the wavelength of visible light, they allow white light to pass through the dispersed system, imparting a translucent or transparent quality to it. The emulsion's droplet size plays a pivotal role in its self-emulsification process, impacting both drug release and absorption. Consequently, the formulation holds promise for enhancing drug absorption and, consequently, increasing oral bioavailability (15). It is of paramount importance that the newly formed nano emulsion within the gastrointestinal tract does not undergo precipitation following phase separation due to infinite dilution by digestive fluids. This concern becomes particularly pronounced when dealing with drugs characterized by low water solubility or nano emulsions that undergo phase transitions (7). While loosely packed liquid crystal (LC) phases are very quickly disturbed, resulting in the creation of finer oil in water droplets, rigid, compactly packed LC phases do not spontaneously emulsify. A characteristic of the surfactant, surfactant combination, and surfactant concentration is the development of such an LC phase (16). To avoid such a situation, dispersibility tests in different diluents were performed. The rate and extent of glimepiride release and absorption are influenced by both the globule size and PdI of the L-SMEDDS, as glimepiride diffuses more rapidly from smaller globules with higher surface area. Additionally, the drug's ability to dissolve within lipid formulations during dilution and gastrointestinal digestion is a critical factor affecting its subsequent dispersion and absorption (17–21). Traditional emulsions facilitate the spontaneous creation of an interface between emulsion globules and water due to the relatively low free energy required for emulsion formation. Rapid self-emulsification with small globule sizes and the long-term stability of the resulting emulsion is notably influenced by variations in the fatty acid carbon chain lengths of the oil, the surfactant, and their degree of unsaturation. The magnitude of the zeta potential serves as an indicator of the potential stability of a colloidal

system. When all particles exhibit high negative and positive zeta potentials, they will remain dispersed and separate from each other, ensuring dispersion stability. Conversely, low zeta potential leads to particle attraction and dispersion instability. Aerosil® 200 Pharma carrier comprises exceptionally fine particles with a substantial specific surface area, resulting in a high oil adsorption capacity. Additionally, silica derivatives capable of forming a threedimensional network with hydrogen connections between silanol groups are increasingly employed for adsorbing lipid formulations (22). It's important to note that while DSC studies do not provide a precise understanding of the interactions, they do suggest potential interactions between the drug and the adsorbent, which can be further explored through changes in the carrier's glass transition temperature (23). The release from L-SMEDDS) was slightly superior to solid SMEDDS. Several factors are likely responsible for these outcomes. Firstly, the initial phase of drug release is delayed due to the tablet disintegration process. Additionally, certain excipients, such as Aerosil® 200 Pharma, exhibit strong interactions with the adsorbed SMEDDS. Lastly, a minor increase in droplet size during reconstitution may be the third factor. This results in a reduced surface area, which is likely to decrease the rate and extent of glimepiride release (5,7). Nanosized globules, the inclusion of surfactants that function as p glycoprotein inhibitors, absorption via the Peyer's patch, and greater penetration of the SMEDDS formulation may be the causes. It was also mentioned that lipid-based systems frequently experience lymphatic uptake, which enhances absorption and bioavailability even more. Because glimepiride belongs to the BCS class II group and hence has strong permeability, the permeation difference between suspension and SMEDDS was not particularly significant (10,24). These findings of antidiabetic activity align with previous research on solid nano dispersion (1) and micro emulsion (25) of glimepiride, which aimed to improve its bioavailability.

CONCLUSION

The current investigation effectively showcased the creation and formulation of liquid and solid SMEDDS for glimepiride, intended for oral administration. The refined formulation encompasses Capryol® 90 as the oil phase, Kolliphor® EL serving as the surfactant, and Transcutol® P functioning as the co-surfactant. These components harmoniously emulsify in the aqueous environment, facilitated by gentle gastrointestinal motility, resulting in the formation of finely-sized globules. The use of Aerosil® 200 Pharma as a carrier for adsorption showed better physical properties. The particle size and zeta potential showed nanosize and considerable stability for SMEDDS. The analysis of the solid-state indicated that glimepiride was present in a molecular dispersion or amorphous state. Both the liquid and solid SMEDDS showed enhanced *in-vitro* drug release when compared to plain glimepiride. The *ex-vivo* permeability studies proved better permeation of glimepiride SMEDDS in comparison with pain glimepiride. *In-vivo* antidiabetic activity in albino mice proved significant improvement in hypoglycemic activity when compared to plain glimepiride. These findings prove the improved bioavailability of glimepiride through SMEDDS and oral solid dosage forms.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
All experimental procedures involving animal studies were conducted in accordance with the guidelines and regulations established by the Institutional Animal Ethics Committee (IAEC), Vivekanand Education Society's College of Pharmacy, Affiliated to the University of Mumbai, Mumbai 400 074, Maharashtra, INDIA. The IAEC approved the protocols for pharmacokinetic experiments involving animals, excluding non-human primates, under Reference No. VESCOP/06/2016 dated October 14, 2016.

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