

Preparation and In Vitro Evaluation of Self-Emulsifying Drug Delivery System for Oral Delivery of Glibenclamide

Akiladevi D^{*1}, Nappinnai M², Jerad Suresh A³, Amudha P⁴, Vetrichelvan T⁵

¹Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319, Tamilnadu, India

²Department of Pharmaceutics, Surya Group of Institutions, School of Pharmacy, Vikravandi, Villupuram-605 652, Tamilnadu, India

³Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-600 003, Tamilnadu, India

⁴Department of Pharmacology, CL Baid Mehtha College of Pharmacy, Thorapakkam, Chennai, Tamilnadu, India

⁵Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Tamilnadu, India

***Corresponding author:** Akiladevi D, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319, Tamilnadu, India, E-mail: akilaajcp@gmail.com

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Abstract

Objective: The present study was aimed to develop a novel o/w self-emulsifying drug delivery system (SEDDS) with novel synthetic oils, a variety of natural oils and surfactants/co surfactants to enhance the solubility and dissolution rate of poorly water soluble drug, glibenclamide thereby to improve its relative bioavailability. The novelty in this proposed research work is the simple process of production and easy to scale up with minimum infrastructure.

Methods: The design of an optimal SEDDS is performed by preformulation solubility and ternary phase-diagram studies. The prepared self-emulsifying formulations were performed for in vitro evaluation studies of droplet size, polydispersity index, drug loading, zeta potential, optical clarity, turbidity, cloud point, viscosity determination, self-emulsification time assessment, in vitro drug release and in vitro diffusion studies.

Results: The results showed that Peceol as oil, Labrasol as surfactant and Transcutol HP as cosurfactant prepared stable emulsions has a refractive index as same that of water clearly indicated that the microemulsions was highly transparent. The percentage drug release for optimal formulation of glibenclamide SEDDS after 30 minutes was found to be 99.7 % when compared to API (active pharmaceutical ingredient) and marketed tablet.

Conclusion: This study demonstrated that in vitro drug release and in vitro diffusion study were dependent on critical parameters such as particle size and drug loading in SEDDS formulations

Keywords: Self-Emulsifying Drug Delivery, Glibenclamide, In vitro evaluation, Particle size, Drug loading

Introduction

The major, popular route of administering drug from time immemorial is the oral route for both chronic and acute dosage regimen. Unfortunately, more than 50 % of drug compounds have unfavourable physicochemical property of which high lipophilicity is major one. Almost 40% of the new drug candidates exhibit low solubility in water which leads to poor bioavailability, high intra and inter -subject variability, lack of dose proportionality. Thus, for such compounds, among numerous factors limiting bioavailability is the absorption rate from gastrointestinal lumen. This is in turn is related to dissolution [1]. According to the BCS classification, two classes of drugs show poor aqueous solubility namely BCS II and BCS IV. BCS II drugs possess poor aqueous solubility but have good permeation properties. BCS class IV drugs are poorly water soluble and poorly permeable. The enhancement of oral bioavailability of poorly water soluble drugs remains one of the most challenging aspects of drug development. Although salt formation, solubilization and particle size reduction have commonly been used to increase dissolution rate, there are practical limitations of these techniques. The salt formation is not feasible for neutral compounds and the synthesis of appropriate salt forms of drugs that are weakly acidic or weakly basic may often not be practical. The solubilization of drugs in organic solvents or in aqueous media by the use of surfactants and co-solvents leads to liquid formulations that are usually undesirable from the viewpoints of patient acceptability and commercialization. Although particle size reduction is commonly used to increase dissolution rate, there is a practical limit to how much size reduction can be achieved by such commonly used methods as controlled crystallization, grinding, etc. The use of very fine powders in a dosage form may also be problematic because of handling difficulties and poor wettability [2]. To overcome these drawbacks, various formulation strategies have been adopted which including the use of cyclodextrins, nanoparticles, solid dispersions, and permeation enhancers [3]. SEDDS are considered one of the promising approaches for tackling formulation problems associated with drugs with poor aqueous solubility. SEDDS is

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an oral lipid dosage form composed of a mixture of natural or synthetic oils, solid or liquid surfactants, or with one or more solvents of hydrophilic capacity and co solvents/surfactants [4]. These formulations disperse freely when they come to contact with gastric fluids and form an o/w emulsion or micro emulsion utilizing mild agitation conditions provided by gastric motility. The lipophilic drug is delivered in liquid form, in small droplets of oil, leading to the elimination of the dissolution which is rate limited process in the absorption of poorly soluble drugs. The bioavailability of SEDDS is thereby improved and the drug content in plasma profile is reproducible in such systems.

Advantages of the system [5]

- The drug absorption will be more: SEDDS formulations can enhance the bioavailability by increasing the solubility of the drug and minimizes the gastric irritation. In SEDDS, the lipid phase interacts readily with water, forming a fine particulate o/w emulsion. The emulsion droplets will deliver the drug to the gastrointestinal mucosa in the dissolved state readily accessible for absorption.
- The drug can be protected from the gut environment: Many drugs are degraded in the physiological system because of acidic pH in the stomach, enzymatic degradation or hydrolytic degradation. Such drugs, when presented in the form of SEDDS, can be well protected against the degradation processes as a liquid crystalline phase in SEDDS might act as the barrier between the degrading environment and the drug [6].
- The sensitive drug compounds are protected: The self-micro emulsifying formulations spread readily in the GI tract and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. SEDDS typically produce an emulsion with a droplet size between 100 and 300 nm while SMEDDS form a transparent micro emulsion with a droplet size of less than 50 nm. When compared with emulsions which are sensitive and metastable dispersed forms, SEDDS and SMEDDS are

physically stable formulations that are easy to manufacture [7].

- The oral dose is reduced therefore, the oral bioavailability is enhanced.

Glibenclamide (GBD) a second-generation sulfonylurea is orally used as a hypoglycemic agent to treat noninsulin-dependent (type II) diabetes mellitus. The aqueous solubility of GBD is low and highly pH-dependent in the physiological range because of its pKa value of 5.3. Low aqueous solubility gives rise to unsatisfactory dissolution profiles leading to potential problems of poor bioavailability and bioequivalence of the drug's dosage form GBD. The low oral dose (5-15 mg), suitable log p (octanol/water) of 4.8, and it being a BCS class II drug strongly provide a rationale to develop an SEDDS of GBD. In our study glibenclamide SEDDS was prepared and *in vitro* evaluation studies were performed to improve the solubility and dissolution rate.

Materials and Methods

Materials

Glibenclamide calcium was generously gifted by Goodman Pharmaceuticals, Pondicherry. Capryol PGMtranscutol HP, peceol, labrasol, labrafil M 1944 CS and labrafil M 2125 CS were gift samples from Gattefosse, Mumbai. Virgin sesame oil, virgin coconut oil and sunflower oil were obtained from Vama oil industries, Coimbatore, Olive oil from Shaah Enterprises, Chennai, Mustard oil from Green spice products, Coimbatore, Rice bran oil from Jupiter Manufacturing industry, Chennai, Corn oil from Arumuga group of industries, Tamilnadu were obtained for the research work. The dialysis membrane of molecular weight cut off 12000 daltons was purchased from Himedia Pvt. Ltd. All other chemicals were of analytical grade.

Methods

Fourier Transform Infrared (FT-IR) Spectroscopy of Glibenclamide [8]

The drug sample was mixed with anhydrous potassium bromide (KBr) in 1:4 ratios. Briefly about 100 mg of this mixture was made into fine powder using mortar and

pestle followed by compression to form transparent KBr pellet using Techno search hydraulic press set at 15-ton pressure. Each KBr pellet was scanned at 4 mm/s at a resolution of 2 cm over a wave number region from 4000 to 400 cm^{-1} in an FTIR spectrophotometer (8400S Shimadzu, Japan). Figure 1 shows FTIR spectrum of pure drug.

Solubility Studies

The solubility of glibenclamide was determined in distilled water, methanol, ethanol and dichloromethane. About 2 ml of each solvent was transferred into 5 ml glass vial and an excess quantity of drug (150 mg) was added to the vial. The solubility of the drug samples was also analyzed by adding excess amount (150 mg) of the drug to 2 ml of various oils, surfactants, and co-surfactants in screw capped glass vials followed by vortex mixing for 30 sec using vortex mixer (Sphinx, Japan). The mixtures were shaken for 48 h at 30°C in a thermostatically controlled shaking water bath, followed by equilibrium for 24 hr. The sample mixtures were then centrifuged at 3000 rpm [9] for 10 min and the supernatant liquid was filtered through a millipore membrane filter (0.45 μ). Samples were suitably diluted with methanol followed by sonication for 10 min and finally diluted with the same solvent. The final drug concentration was quantified by UV-visible spectrophotometer at 226.5 nm for glibenclamide. The experiment was repeated in triplicates. The results are represented as mean value (mg/ml) \pm SD.

Construction of Ternary Phase Diagram

The percentage limit of surfactant, co-surfactant and oil used was selected by considering their acceptable safe dose and decided on the basis of the requirements stated according to the lipid formulation classification system (LFCS) introduced by Pouton. A ternary phase diagram was constructed for the system containing oil-surfactant-co-surfactant by Chemix School software version 3.51. A series of self-emulsifying systems were prepared with varying weight percentage of oil, surfactant, and co-surfactant. Since the drug incorporated in the SEDDS may have some effect on self-emulsion boundary, every system in the series also consisted of 5% w/w for

glibenclamide. These mixtures were diluted dropwise with double distilled water with moderate agitation.

Preparation of SEDDS

A series of SEDDS formulations were prepared using labrasol and transcutool hp as the S/CoS combination and Peceol as the oil based on solubility studies as described in Table 1. In all the formulations, the level of glibenclamide was kept constant (5 mg). Briefly, accurately weighed glibenclamide was placed in a glass vial, and oil, surfactant, and cosurfactant were added. Then the components were mixed by gentle vortexing and sonicated for 10 minutes. Altogether nine formulations were prepared as shown by Table 2.

Evaluation of Prepared SEDDS

Self-Emulsification and Drug Precipitation Studies

The efficiency of self-emulsification of oral micro/nano-emulsion is assessed by dispersibility test using a standard USP dissolution apparatus II [10]. One ml of each formulation is added to 500 ml of water at $37 \pm 0.5^\circ\text{C}$. A standard stainless steel dissolution paddle rotating at 50 rpm tends to provide gentle agitation. The *in vitro* performance of the formulations is visually assessed from such dispersion, using a suitable grading system. The grading system has been reported to be based on the formation of a micro emulsion (o/w or w/o), micro emulsion gel, emulsion or emulgel. The drug/excipient precipitation was evaluated by visual inspection of the resultant emulsion after 24 h (Table 3). The *in vitro* performance of the formulations is visually assessed using the following grading system:

Grade I: Rapidly forming (within 1 min) nano emulsion, having a clear or bluish appearance. (Micro emulsion)

Grade II: Rapidly forming, slightly less clear emulsion, having a bluish white appearance. (Micro emulsion gel)

Grade III: Fine milky emulsion that formed within 2 min. (Emulsion)

Grade IV: A dull grayish white emulsion having slightly oily appearance that is slow to emulsify is formed (longer than 2 min) (Emulgel).

Grade V: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface with phase separation is observed.

Grade VI: The drug is precipitated.

Grade I and Grade II formulation will remain as nano-emulsion when dispersed in GIT. The formulation falling in Grade III could be recommended for SEDDS formulation. The primary means of self-emulsification is a visual evaluation. The efficiency of self-emulsification could be estimated by determining the rate of emulsification, droplet size distribution, and turbidity measurements.

Phase Separation Study

The self-emulsifying formulation was diluted with distilled water up to 5 times and the temperature was maintained at 25°C . The mixture was then mixed for 2 min, stored for about 2 h and visually observed for any phase separation.

Determination of Emulsification Time

The emulsification time (the time for a preconcentrate to form a homogeneous mixture upon dilution) was monitored by visually observing the disappearance of SEDDS and the final appearance of the emulsion in triplicate. A dissolution apparatus USP II (Electrolab) was employed with 500 ml water and with a paddle speed of 50 rpm at 37°C . The SEDDS (1 ml) was added drop wise to the medium by dropping the pipette and time required for the disappearance of SEDDS was recorded [11].

Spectroscopic Characterization of Optical Clarity

SEDDS formulations disperse in aqueous phase forming the emulsion or micro emulsions and can be detected by the final appearance and droplet size. In practice, the key difference between the emulsion and micro emulsions concerns with their appearance. Emulsions are cloudy while micro emulsions are clear or translucent and the reason for their transparency appearance is due to very small droplet size. The optical clarity may be checked visually. But in order to measure it quantitatively, a UV-visible spectrophotometer was used to measure the amount of light of a

given wavelength absorbed by the solution. The cloudier solutions will absorb more of the incident light, resulting in higher absorbance values and lower absorbance is obtained with optically clear solutions.

The optical clarity of aqueous dispersions of SEDDS formulations was measured spectroscopically. About 1 ml of SEDDS formulations were diluted to 50 times with double distilled water. The absorbance values of each formulation were measured by a UV-visible spectrophotometer (Shimadzu) at 400 nm [12].

Turbidity Measurement

The measurement of turbidity is to analyze whether the dispersion reaches equilibrium rapidly and in a reproducible time. The growth of emulsification is done by nepheloturbidimetric evaluation. The turbidity measurements in nephelometric turbidity unit (NTU) were performed on the resultant emulsion stored in a screw capped sample vials using a turbid meter (Elico D-10-model 331). 0.5 ml of the SEDDS formulation was introduced into 250 ml of distilled water in 500 ml conical flask under an action of magnetic stirrer rotating at constant speed. The emulsification was done at room temperature [13].

Viscosity Determination

The viscosity studies are necessary for SEDDS to characterize the system physically and to control its stability. If the system has low viscosity then, it is o/w type of the system and if a high viscosity then it is w/o type of the system. SEDDS preconcentrate (10 ml) was taken and its viscosity was measured by using Brookfield viscometer (Brookfield engineering Laboratories, USA) using spindle C 16-1 at 25 ± 0.5 °C with a shear rate of 50 rpm.

Cloud Point Measurement

Cloud point temperatures (T_c) was determined by visual observation. 0.5 ml of preconcentrate (Oil-20 mg and Smix-40 mg) was diluted to 50 ml with distilling water in a glass beaker. The sample was heated at the rate of about 0.5°C/min. A close observation was made at the appearance of the dispersion with

the increase in temperature. The temperature at which the dispersion became turbid was taken as T_c . After the temperature exceeds the cloud point, the sample was cooled below T_c , and then it was heated again to check the reproducibility of the measurements. It mainly insists about the stability of micro emulsion at body temperature [14].

Determination of Refractive Index

The refractive index, n , of a medium is defined as the ratio of the speed, c , of a wave such as light or sound in a reference medium to the phase speed, v_p , of the wave in the medium represented by $n=c/v_p$. It was determined using an Abbes type refractometer. The clarity of micro emulsion could be estimated by measuring the refractive index of the formulations [1517]. The SEDDS formulations were diluted 100 times with water. The refractive index of the system was measured by an Abbe refractometer by placing 1 drop of solution on the slide and it compares with distilled water. The refractive index (RI) was determined to prove the isotropicity of the developed SEDDS formulations close to that of the water (1.333). The closure of the formulations RI value to water indicated the transparency property of the formulations.

Droplet Size and Polydispersity Index (PDI) Analysis

The droplet size of the micro/nano emulsions is determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to a brownian motion of the particles) using a Zetasizer which can measure sizes between 10 and 5000 nm. Polydispersity was determined according to the equation:

$$\text{Polydispersity} = \frac{D(0.9) - D(0.1)}{D(0.5)}$$

Where $D(0.9)$ corresponds to particle size immediately above 90% of the sample, $D(0.5)$ corresponds to particle size immediately above 5% of the sample, and $D(0.1)$ corresponds to particle size immediately above 10% of the sample. PDI is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the PDI value the more homogenous are the particles. The mean droplet size and polydispersity index of formulations were determined by using

Malvern Nano Zeta sizer-90. The resultant SEDDS 0.5 ml, was diluted to 100 ml with double distilled water. The samples were loaded into a cuvette placed in a thermostatic chamber and light scattering was monitored at 25°C at a 90° angle [16] after external standardization with spherical polystyrene beads.

Each determination was done in triplicate. The nanometric size range of the particle is retained even after 100 times dilution with water which proves the systems compatibility with excess water.

Zeta Potential Measurement

The zeta potential of prepared SEDDS formulations was determined using a Zeta sizer ZS 90 (Malvern Instruments UK) by using laser Doppler micro-electrophoresis. An electric field is applied to a solution which will cause the particles to move to the electrodes with a velocity related to their zeta potential calculated using Helmholtz–Smoluchowski equation. A suitable amount of the sample (50–100 µl) was diluted with 5 ml of distilled water and after sonicating in a bath sonicator to achieve a homogeneous state. Measurements were carried out at 25°C using disposable polystyrene cuvette with a zeta dip cell. All the measurements were performed in triplicate and the data presented is mean ± SD [1717].

Zeta potential determination using following equation

$$\zeta = \frac{V}{E} \cdot \frac{\eta}{\epsilon \cdot \epsilon_0}$$

ζ zeta potential, E electrical intensity, v particle velocity, η viscosity, ε·ε0 dielectric constant

Drug Loading Efficiency [18]

The drug efficiency was done to investigate the effect of drugs on a self-emulsifying performance of SEDDS. Approximately 10 mg of glibenclamide was added to 1 ml of boundary formulations of SEDDS and checked for a formation of the clear solution. The prototype formulations of glibenclamide were prepared by varying peceol in 1:1 ratios of the mixture of labrasol and transcitol HP as per the formula composition mentioned in Table 2. In

the first trial, the oil was used at 15% with an interval of 5% and increased up to 25%. The ratio of surfactant to co-surfactant was maintained at 1:1. Then drug of one dose equivalent of 5 mg of glibenclamide was added and stirred for 15 min. The mixture was heated to 30-40°C till the drug was solubilized. The drug loading capacity of each mixture was determined by adding the excess of glibenclamide to each prototype mixture till the clear solution was obtained. The solution was filtered using a membrane filter (0.45 µm, 13 mm, Whatman filter).

The drug content of the SEDDS formulation was determined by diluting the solution in methanol and the volume was made up to 10 ml with methanol (1 mg/ml). From the above stock solution, 0.2 ml (200 µg/ml) was withdrawn and diluted up to 10 ml with methanol (20 µg/ml). From the above solution 0.2 ml (20 µg/ml) diluted up to 10 ml with methanol (2 µg/ml) Samples were prepared in triplicate and absorbance was measured at 226.5 nm using UV-visible Spectrophotometer [19] (Shimadzu UV-1700) using methanol as a reference solution. The drug content was determined from the standard calibration curve of glibenclamide in methanol using the regression equation $y=0.118x+0.002$ with the correlation coefficient of 0.999. The procedure mentioned above with the SEDDS formulation equivalent to 5 mg of glibenclamide and the absorbance was measured at 226.5 nm using UV-Visible spectrophotometer (Shimadzu UV-1700).

Drug loading efficiency was calculated by equation

Drug loading efficiency = Amount of drug in known amount of formulation x 100 / Initial drug load

In Vitro Dissolution Studies of Glibenclamide

The SEDDS formulation of GF7* were optimized by trial error and method and validated by preparing four formulations with similar composition as that of GF7*. The *in vitro* evaluation studies were performed and the resulting formulation was considered as best and optimized formulation as the results obtained were with minimum particle size and maximum drug loading for stable formulation of SEDDS which was showed in Table 2 and

Table 4. The *in vitro* release rate of four validated formulation were similar to that optimized formulation of GF7* as shown *in vitro* release graph depicted in Figure 3. The *in vitro* drug release profiles of optimized SEDDS formulations of glibenclamide GF7*, API glibenclamide and marketed glibenclamide tablet (Daonil 5 mg, Aventis Pharma Ltd) were carried out using USP type II dissolution test apparatus (Electrolab) in 900 ml of phosphate buffer (pH 7.4) as dissolution media [20]. The temperature was maintained at $37 \pm 0.5^\circ\text{C}$ and the speed of the paddle was set at 50 rpm. About 70 mg of each optimized SEDDS formulation (GF7*) were filled into soft gelatin capsules (size '3') and used for dissolution studies. The capsules were held to the bottom of the vessel using copper sinkers. At predetermined time intervals of 5, 10, 15, 20, and 30 min, an aliquot (2 ml) of a sample was collected and filtered through the membrane filter (0.45 μm , Whatman). The withdrawn samples were diluted suitably with phosphate buffer (pH 7.4) and the drug content was analyzed by standard calibration curve method using the linear regression equation $y=0.018x+0.001$ of glibenclamide in phosphate buffer pH 7.4 with the correlation coefficient of 0.999 by UV-visible spectrophotometer (Shimadzu UV-1700) at 226.5 nm. An equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain the sink condition. Each test was performed in triplicate ($n = 3$), and calculated mean values of cumulative drug release were used while plotting the release curves as illustrated in Figure 3. The dissolution profile of the API glibenclamide and marketed tablet (Daonil 5 mg) were assessed by the same method.

***In Vitro* Diffusion Release Study of Glibenclamide**

The *in vitro* diffusion was performed using the dialysis membrane diffusion technique by a dialysis membrane method. The dialysis membrane of molecular weight cut off 12000 daltons was soaked in distilled water for four hours and then rinsed thoroughly with distilled water. One end of pretreated cellulose dialysis bag (7 cm tubing) was sealed firmly with clamp and 0.5 ml of optimized self-emulsifying formulation was introduced in it along with 0.5 ml of dialyzing medium

(phosphate buffer pH 7.4). The other end of the bag was also secured with clamp and was allowed to rotate freely. The bags were incubated in beakers containing 500 ml phosphate buffer 7.4 for glibenclamide. 5 ml of samples were withdrawn individually at 0.5, 1, and 2 hours for glibenclamide, which was simultaneously replaced with equal volumes of fresh medium at the same time. The drug content was determined spectrophotometrically from the standard calibration curve of glibenclamide in phosphate buffer (pH 7.4) as shown in Figure 4 using the linear regression equation $y=0.018x+0.001$ with the correlation coefficient (r^2) of 0.9995 at 227.5 nm UV-visible spectrophotometer (Shimadzu UV-1700). The diffusion of the drug from optimized formulation was compared with the API and marketed tablet.

Discussion

Solubility Study

Peceol a novel a semi synthetic medium chain derivative was chosen as oil phase for glibenclamide since it was found to have maximum solubility when compared to the other oils and in which the solubility was found to be minimum in sunflower oil as indicated in results of Table 1. Peceol is an efficient solubilizer for glibenclamide which was proven by Patil Prashant P et al. [21] in his research work. From Table 1 it was proved that labrasol was chosen as surfactant because it showed maximum solubility followed by labrafil 1944 CS, capryol PGMC and labrafil 2125 for formulation of glibenclamide SEDDS. The medium length alkyl chain surfactant labrasol was selected as a surfactant for both the drugs since it has maximum solubilizing capacity and it was represented for drugs having poor intestinal absorption [22]. Transcutol HP showed maximum solubility for glibenclamide and it was selected as the cosurfactant for the formulation of SEDDS as indicated in Table 1. Transcutol HP is a strong solubilizer with low toxicity has a long history of safe use as a solvent in many products including pharmaceuticals, cosmetics and food applications [23].

Construction of Phase diagram

In the present study peceol (oil) with labrasol and Transcutol HP as the S/CoS mixture was selected from the solubility studies. From the ternary plot as shown in Figure 2, Peceol gave a wider micro emulsion region at 1:1 of Smix ratio for glibenclamide. The co-surfactant was incorporated along with the surfactant in equal proportion at Smix ratio of 1:1 a higher nano-emulsion region was observed in glibenclamide SEDDS. This may be due to the addition of co-surfactant leading to further decrease in the interfacial tension, which will lead to increase in the fluidity of the interfacial film, thus increasing the entropy [24]. The percentage of oil, surfactant and cosurfactant selected for both the drugs were selected from the phase diagram and only those formulations which used the minimum and maximum concentration of Smix were taken for the formulation of SEDDS. Moreover, the self-emulsification ability of SEDDS depends on the formulation parameter variables such as surfactant/cosurfactant and oil ratio, a polarity of the emulsion, globule size and charge on the droplets. The stability and efficiency of the drug is increased by self-emulsification property.

Self-Emulsification, Drug Precipitation, Phase Separation And assessment of Emulsification Time Studies

The self-emulsification was visually assessed to measure the apparent spontaneity of nanoemulsion formation. SEDDS when diluted in water were found to be non-turbid and bluish transparent in appearance indicating spontaneous emulsification. All the resulting nanoemulsions were transparent with some opalescence in appearance and did not show any sign of phase separation. All the results of the nanoemulsion formulations were transparent and their absorbance's were below 1 which showed good optical clarity as illustrated in Table 4. The selection of surfactants and cosurfactants are determined by emulsification ability which depends on the physicochemical properties such as globule size, Zeta potential, turbidity measurement and PDI of the resulting nanoemulsion. All the formulations of both the drugs showed rapid emulsification time within a minute which proves the performance of the formulations for enhancing the dissolution profile. Thus, it can

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be concluded that the absorption of the drug can be increased *in vivo* if the formulations have low emulsification time. Labrasol a hydrophilic surfactant having HLB value of 12 rendered very good nanoemulsions that required a short emulsification period.

Turbidity Measurements

The rapid equilibrium reached by the dispersion and reproducibility of the process is determined by turbidity measurements. The results obtained are within the limits for both the drugs. The emulsification efficiency is confirmed by a decrease in turbidity values which results in the corresponding decrease in droplet size. In the formulation GF5 of glibenclamide SEDDS the turbidity value was high of 142 NTU due to the larger droplet size of the emulsion formed of 402.3 nm which was shown in Table 2 and Table 4.

Refractive Index and Viscosity Measurement

There was no significant difference in the refractive index values of the formulations tested. The refractive index values close to that of the water (1.333) prove the isotropicity of the formulations as indicated in Table 4.

Droplet Size

The globule size observed for all the formulation was less than 620 nm. The drug loading did not show significant difference in the polydispersity values. The droplet size distribution is one of the most important characteristics of nano-emulsion for stability evaluation and is a critical step in the pathway of enhancing drug bioavailability. The smaller nano-emulsion particle size leads to larger interfacial surface area, thus promoting rapid absorption and improved bioavailability. The particle size and drug loading are critical formulation parameters used to help maximize the pharmacokinetics of small molecules [25]. In this present work, the particle size of SEDDS after dilution was selected as criteria for *in vitro* evaluation. If the particle size of SEDDS is less, the release of drug will be more resulting with better bioavailability in the formulation of SEDDS. The particle size of around 20 nm gives total transparent system upon dilution, which acts as a solution.

Zeta Potential

The emulsion stability is directly related to the magnitude of the surface charge and the zeta potential is stability indicative parameter in colloidal systems means the system will resist aggregation. The reason for this behavior could be attributed to the strong repulsive Coulomb force between charged particles, which counterbalances the Vander Waals attraction force. Generally, an increase in electrostatic repulsive forces between micro emulsion droplets prevents the coalescence of micro emulsion droplets. High absolute (positive and negative) zeta potential values (above +30 or -30 mV) should preferably be achieved in most of the emulsions prepared in order to ensure the creation of a high-energy barrier against coalescence of the dispersed droplets [26]. The zeta potential of the optimized formulation of glibenclamide was found to be -24.8 mV which were nearer the limits with good separation. The zeta potential values were found to carry negative charges due to the presence of free fatty acids. Significant increase in the value of zeta potential was observed after drug loading, higher absolute values of zeta potential generally, indicated an increase of electrostatic repulsive forces between emulsion droplets preventing the coalescence droplets and increases in the stability. Among all the vehicles tested Labrasol (surfactant) and Transcutol HP (co-surfactant) proved to be the most promising vehicles for SEDDS formulation.

Polydispersity Index (PDI)

The PDI for all the formulations were less than 0.5, formulation with combination of Smix showed lower PDI values as illustrated with the results given in Table 4, thus indicating the uniform size distribution improving the performance of the spontaneous emulsification. Labrasol is a high dispersible surfactant produces small droplet size and good PDI from all surfactants.

Cloud Point

The cloud point is an essential factor in the SEDDS consisting of non-ionic surfactants, and it is responsible for the successful

formation of a stable microemulsion. When the temperature is higher than the cloud point, an irreversible phase separation will occur and the cloudiness of the preparation would have a bad effect on drug absorption, because of the dehydration of the polyethylene oxide moiety. Hence, the cloud point for SMEDDS should be above 37°C, which will avoid phase separation occurring in the gastrointestinal tract. The cloud point for all the formulation as shown in Table 4 tested was above 37°C. Therefore, it would suggest a stable micro emulsion can be formed at physiological temperature *in-vivo*.

Drug Loading

The maximum drug loading for glibenclamide ranged from 8.2 mg to 9.23 mg for all nine formulations. Drug loading is a critical parameter which and toxicity of the drug. Higher drug loading is preferable because less non-active excipie affects the therapeutic efficacy, pharmacokinetics nts are used to produce the same quantity of API in the SEDDS formulation. At a higher drug loading, lower quantity of oils and surfactants (non-active ingredients) need to be manufactured to deliver an equivalent dose of API. The benefit of maximum drug loading is that the quantity of surfactants incorporated in the SEDDS formulation can be reduced which in turn large quantity of the surfactants causes the irritation on GIT. It can also reduce the manufacturing and processing time, raw material usage. So, particle size was selected as criteria for the optimization. The percentage drug loading was selected as another critical parameter for *in vitro* evaluation because the higher the drug loading in SEDDS formulation reduces final dose of the drug and improves patient compliance with minimum GIT irritation and side effects.

In Vitro Dissolution Study

The dissolution study of API, marketed formulation and optimized formulation GF7* were performed in 7.4 pH phosphate buffer for glibenclamide. The optimized glibenclamide SEDDS formulation of GF7* showed a drug release of above 90% within 5 min was illustrated in Table 5. It could be suggested that the SEDDS formulation resulted in spontaneous formation of a micro emulsion

with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain glibenclamide and marketed tablet as shown in Figure 3. Thus, this greater availability of dissolved glibenclamide from the SEDDS formulation could lead to higher absorption and higher oral bioavailability. It was also showed that increase in surfactant concentration and decrease in oil concentration in formulation increase in drug release.

***In Vitro* Diffusion Release Study**

Diffusion study was carried out to study the release behavior of formulation from liquid crystalline phase around the droplet using dialysis technique. *In vitro* diffusion profile of glibenclamide from optimized SEDDS in phosphate buffer (pH 7.4) is given in Table 6. It was observed that at the end of 2 h, the optimized formulation GF7* SEDDS showed about 99.8% and 96.23% for the marketed tablet (Daonil 5 mg) at the end of 2 h.

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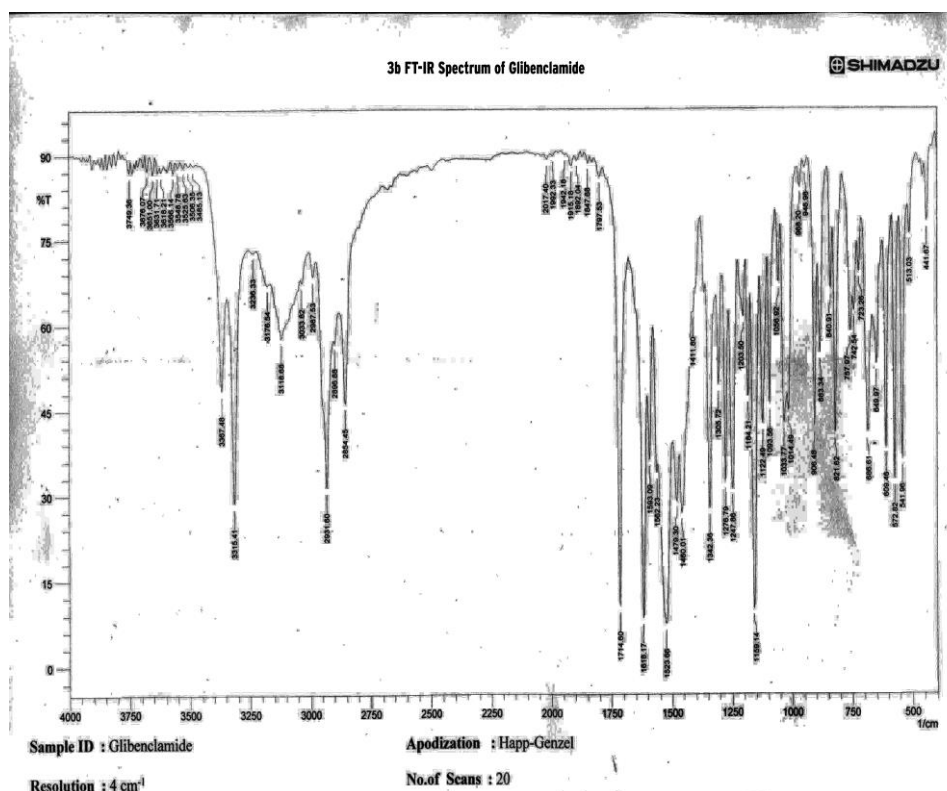


Figure 1: FT-IR spectrum of glibenclamide

Ternary Phase diagram of SEDDS

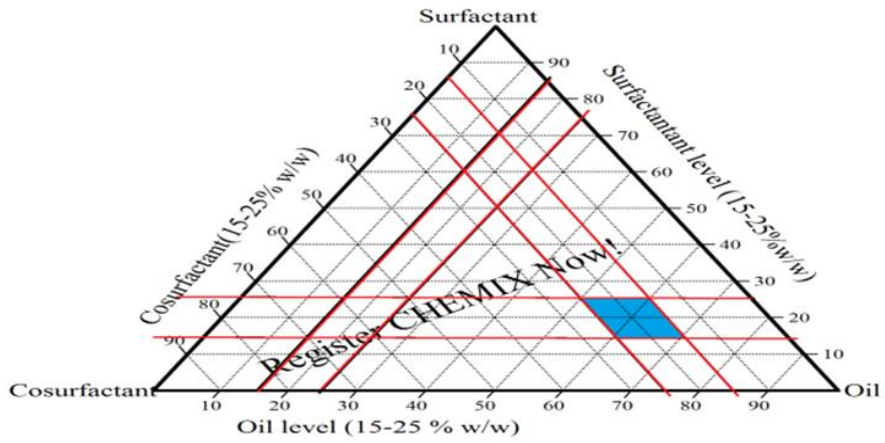


Figure 2: Ternary phase diagram of glibenclamide SEDDS

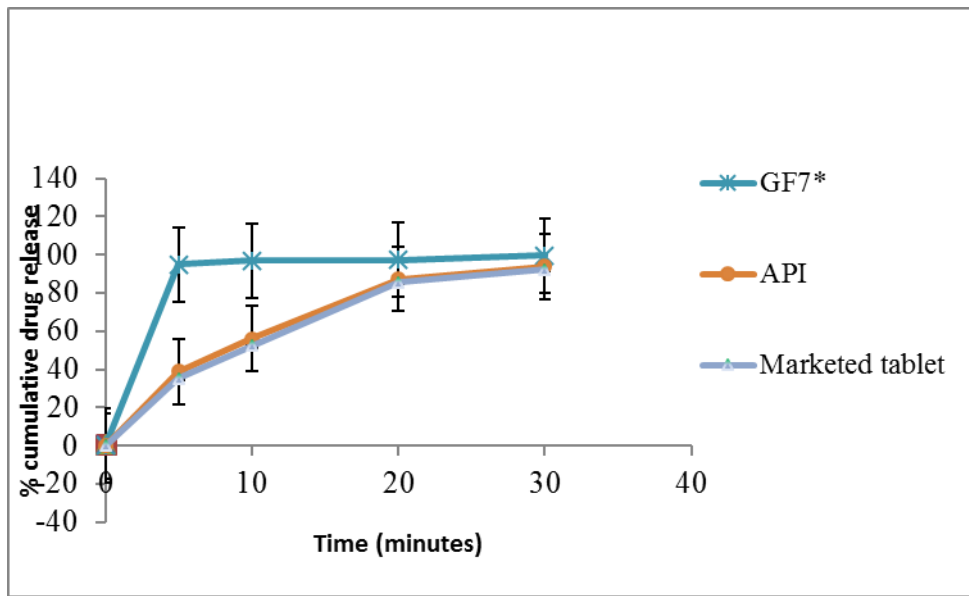


Figure 3: Dissolution comparison graph of API, marketed formulation and optimized formulation of glibenclamide SEDDS

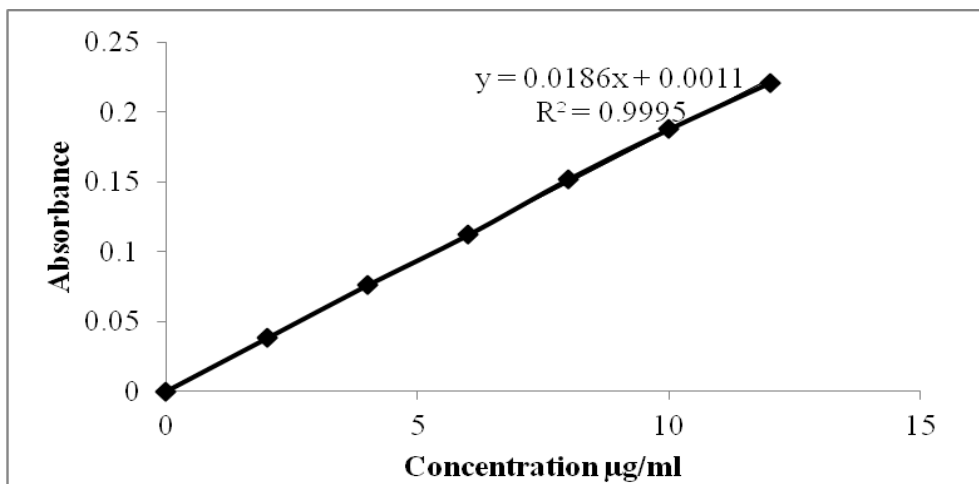


Figure 4: Calibration curve of glibenclamide in phosphate buffer pH 7.4

Table 1: Solubility of glibenclamide in various excipients

S.No.	Excipients	Glibenclamide Solubility (mg/ml)
1.	Virgin sesame oil	3.5 ± 0.012
2.	Virgin coconut oil	6.30 ± 0.007
3.	Sunflower oil	2.28 ± 0.001
4.	Corn oil	4.89 ± 0.009
5.	Mustard oil	2.34 ± 0.002
6.	Rice bran oil	4.25 ± 0.001
7.	Olive oil	5.65 ± 0.003
8.	Peceol	7.83 ± 0.015
9.	Labrasol	9.52 ± 0.016
10.	Labrafil 1944CS	6.24 ± 0.004
11.	Labrafil 2125	1.26 ± 0.011
12.	Capryol PGMC	2.26 ± 0.052
13.	Transcutol HP	18.12 ± 0.018
Solvents		
14.	Distilled water	0.0001 ± 0.008
15..	Methanol	0.0092 ± 0.002
18.	Ethanol	0.0085 ± 0.012
21.	Dichloromethane	0.0305 ± 0.004

Table 2: Composition of glibenclamide SEDDS formulations

Formulation Code	Oil (mg)	Smix (mg)	Particle size (nm)	Drug Loading (%)
GF1	15	40	222.2	92.3 ± 3.26
GF2	15	30	284.2	88.8 ± 2.38
GF3	25	30	415.2	82 ± 1.9
GF4	25	40	233.1	84 ± 4
GF5	20	40	402.3	91.2 ± 5.4
GF6	25	50	229.7	84.6 ± 3.7
GF7*	20	40	421.6	89 ± 4.16
GF8	20	30	464.1	85.6 ± 2.64
GF9	20	50	616.3	92.3 ± 4.12

Table 3: Self- emulsification and drug precipitation of glibenclamide SEDDS

Formulation Code (FC)	Visibility grade	Phase separation	Precipitation
GF1	IV	+	++
GF2	III	+	++
GF3	IV	+	++
GF4	V	+	++
GF5	II	X	XX
GF6	III	+	++
GF7*	I	X	XX
GF8	V	+	++
GF9	III	+	++

X-No phase separation, XX-No precipitation, +-phase separation and ++-precipitation.

Table 4: Refractive index, Turbidity, Optical clarity, Polydispersity index (PDI), Viscosity, Cloud point measurement and Emulsification time of SEDDS formulations of glibenclamide

FC	Refractive Index \pm SD (n=3)	Turbidity (NTU)	Absorbance	PDI \pm SD (n=3)	Viscosity (cps) \pm SD (n=3)	Cloud point measurement (°C) \pm SD (n=3)	Emulsification time (sec)
GF1	1.3333 \pm 0.0006	98	0.583	0.204 \pm 0.017	211 \pm 2.22	68 \pm 2.16	23
GF2	1.3332 \pm 0.0003	93	0.578	0.315 \pm 0.21	215 \pm 2.36	69 \pm 3.61	32
GF3	1.3356 \pm 0.0005	127	0.598	0.2 \pm 0.01	324 \pm 3.24	72 \pm 2.23	24
GF4	1.3331 \pm 0.0002	134	0.488	0.284 \pm 0.01	356 \pm 1.72	77 \pm 2.46	25
GF5	1.3334 \pm 0.0002	142	0.571	0.438 \pm 0.01	318 \pm 2.34	71 \pm 2.76	27
GF6	1.3335 \pm 0.0003	132	0.472	0.224 \pm 0.02	365 \pm 3.24	79 \pm 4.20	20
GF7*	1.3330 \pm 0.0002	92	0.494	0.244 \pm 0.04	200 \pm 1.95	62 \pm 2.25	21

GF8	1.3358 ± 0.0004	125	0.687	0.423 ± 0.06	348 ± 1.89	73 ± 2.36	30
GF9	1.3349 ± 0.0004	132	0.708	0.328 ± 0.015	298 ± 2.97	78 ± 4.28	22

Table 5: Cumulative percent release of glibenclamide SEDDS from various formulations

Time in min	GF7* SEDDS	API	Marketed Tablet
0	0	0	0
5	95 ± 3.51	38.7 ± 1.98	35.31 ± 2.22
10	96.8 ± 1.97	55.9 ± 2.24	52.4 ± 3.71
20	97.2 ± 3.14	87.3 ± 3.21	85.5 ± 3.26
30	99.7 ± 2.74	93.8 ± 2.67	92.3 ± 3.47

Table 6: Percent cumulative drug absorbed through dialysis membrane of optimized glibenclamide SEDDS formulations

Time in hours	GF7* SEDDS	Marketed Tablet
0	0	0
0.5	97.2 ± 0.95	82.92 ± 1.97
1	99.3 ± 1.74	90.32 ± 2.19
2	99.8 ± 2.12	96.23 ± 1.41

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