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An approach to increase the bioavailability of glipizide by self-emulsification technique

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ABSTRACT

Glipizide is the first, potent second generation oral hypoglycemic drug of sulfonic urea class and is used in management of type II diabetes. It is one of the active ingredients used to treat diabetes. In the present work self-emulsifying drug delivery system of Glipizide was developed to treat diabetes. Self-emulsifying drug delivery system (SMEDDS) formulated to improve the oral bioavailability of lipophilic drugs. SMEDDS are isotropic mixtures of oils, surfactants and co-solvents. Pseudo-ternary phase diagrams were plotted to check the emulsification range and also to evaluate the effect of Glipizide on the emulsification behavior of the phases. The mixtures consisting of oil (oleic acid) with surfactant (Tween 80), co-surfactant (PEG 400) were found to be optimum formulations. The prepared formulations were evaluated for various parameters like robustness to dilution, drug content and *in-vitro* dissolution. The optimized SMEDDS formulation further evaluated for particle size distribution, zeta potential, TEM were carried out to confirm the stability of the formed SMEDDS. The release data was fitted to various mathematical models to evaluate the kinetics of drug release. The drug release follows mixed order kinetics and mechanism was found to be non-Fickian diffusion. From this study it can be concluded that the formulation was found to be showing significant improvement in terms of the drug release with complete release of drug within 18 minutes. Thus, Self-emulsifying formulation of Glipizide was successfully developed.

Keywords: Self micro emulsifying drug delivery system, Glipizide.

INTRODUCTION

Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost-effectiveness, least sterility constraints and flexibility in the design of dosage form. As a result, many of the generic drug companies are inclined more to produce bioequivalent oral drug products. However, the major challenge with the design of oral dosage forms lies with their poor bioavailability. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass

metabolism, pre-systemic metabolism and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability is attributed to poor solubility and low permeability [1].

Approximately 40% of new drug candidates have poor water solubility and the oral delivery of such drugs is frequently associated with low bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality. To overcome these problems, various formulation strategies are exploited including the use of surfactants, lipids, permeation enhancers, micronisation, salt formation, cyclodextrins, nanoparticles and solid dispersions. Recently, much attention has been paid to lipid-based formulations with particular emphasis on self-emulsifying drug delivery systems (SMEDDS) to improve the oral bioavailability of lipophilic drugs [2].

Self-emulsifying drug delivery systems (SMEDDS) are mixtures of oils and surfactants, ideally isotropic, and sometimes containing co-solvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation [3].

SMEDDS formulation is a simple binary system containing lipophilic phase and drug, or lipophilic phase, surfactant and drug. The formation of a SMEDDS requires the use of a co□surfactant to generate a micro emulsion. SMEDDS formulations are characterized by *in-vitro* lipid droplet ranging from 200 nm–5 mm and the dispersion has a turbid appearance. SMEDDS however have a smaller lipid droplet size (<200 nm) and the dispersion has an optically clear□to□translucent appearance. Both systems are associated with the generation of large surface area dispersions that provide optimum conditions for the increased absorption of poorly soluble drugs [4].

A recent trend to formulate semisolid self-nano emulsifying drug delivery systems (SNEDDS) and solid SMEDDS has been observed. Attempts have been reported for transformation of SMEDDS in solid dosage forms by addition of large amounts of solidifying excipients (adsorbents and polymers) [5].

MATERIALS AND METHODS

The materials used in the experiment are of laboratory grade such as Glipizide was provided by Micro labs Ltd. Bangalore, India, Poly ethylene glycol 200, Tween 80, Oleic acid, Sodium Hydroxide, collected from S.D. Fine Chem. Ltd, Mumbai, India.

FORMULATION OF SELF MICRO EMULSIFYING DRUG DELIVERY SYSTEM

The formulations were prepared by initially dissolving the accurately weighed amount of Glipizide in co-surfactant at 60˚C in an isothermal water bath. Oil was then added and mixture was cooled to ambient temperature. Then surfactant was added and the final mixture was mixed by stirring until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 hrs, and examined for signs of turbidity or phase separation prior to self-emulsification and particle size studies[6]. Final formulation was filled in hard gelatin capsule and stored in well closed container formula for the preparation of self-emulsifying drug delivery system of Glipizide as shown in (Table 1).

Table 1: Formulations containing various concentrations of excipients

Formulations	Drug (Glipizide) In mg	Surfactant (Tween 80) in mg	Co-surfactant (PEG 200) In mg	Oil (Oleic acid) In mg
S1	5	600	300	**100**
S2	5	530	270	**200**
S3	5	470	230	**300**
S4	5	400	200	**400**

METHOD FOR IDENTIFICATION OF GLIPIZIDE

Infrared absorption spectrum

IR spectroscopy is one of the important analytical techniques for chemical identification. The drug polymer interaction was studied by FT-IR spectroscopy. The spectra were recorded for pure drug using FT-IR.

Determination of λmax of Glipizide

100mg of Glipizide was accurately weighed and dissolved in 100ml of 0.1N NaOH (SS-I), from this solution pipette out 10ml and added to another 100ml volumetric flask. The volume was made up with 0.1N NaOH to get a concentration of 100µg/ml (SS-II). From SS-II pipette out 0.5ml into 10ml volumetric flask and make up with 0.1N NaOH (SS-III). Ultraviolet scan was taken of SS-III between the wavelengths of 200-400 nm against 0.1N NaOH as blank which gave a highest peak at 275nm and the same was selected as λmax of Glipizide.

Preparation of standard curve of Glipizide

100mg of Glipizide was accurately weighed and dissolved in 100ml of 0.1N NaOH (SS-I), from this solution pipette out 10ml and added to another 100ml volumetric flask. The volume was made up with 0.1N NaOH to get a concentration of 100µg/ml (SS-II). From SS-II aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml, 1.4ml, 1.6ml, 1.8ml, 2ml were pipette into 10ml volumetric flasks and make up with 0.1N NaOH to get a concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/ml respectively. The absorbance of each concentration was measured at 275nm. The standard graph was drawn using the average values of four trails by plotting absorbance versus concentration of Glipizide.

Solubility study of drug in various excipients

Solubility of Glipizide was determined in different oil (oleic acid, olive oil, sunflower oil, castor oil, coriander oil), surfactants (Span 80, Tween 20, span 20,Tween 80) and co-surfactant (poly ethylene glycol 200, 400) by dissolving an excess amount of Glipizide in 3ml of oil, and other components using a stirrer at 37◦C ± 0.5 for 72 hrs. The equilibrated samples were then centrifuged at 1000rpm for 30 min to remove the undissolved drug. The solubility of Glipizide was determined by analyzing the filtrate spectro-photometrically at 275 nm [7].

Construction of Pseudo-ternary Phase Diagram

Pseudo-ternary phase diagrams of oil, surfactant/co-surfactant, and water were developed using the water titration method. The weight ratio of surfactant to co-surfactant (Km) was varied as 1:1, 2:1, 3:1, 4:1, and 5:1. For each pseudo ternary phase diagram at a specific surfactant/co-surfactant weight ratio, oil and surfactant/co-surfactant mixture were mixed thoroughly in different weight ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1). Water was added drop by drop to the mixture of oil and surfactant/co-surfactant under magnetic stirring at 37 °C until the mixture became translucent to get micro emulsion, and then the concentrations of the components were recorded[8].

EVALUATION PARAMETERS

Droplet Size Analysis

A 10mg quantity of the SMEDDS was placed inside the ring of the internally calibrated microscopic slide (Objective micrometer) and a drop of each non-solvent used above was added for a clearer view. The slide was covered with a cover slip and viewed under a binocular microscope at a magnification of 100. Different particles of the SMEDDS from a particular batch were counted manually since they were sizeable enough to be distinguished (n = 100) and the mean value was taken[9].

Robustness to Dilution

Robustness to dilution was studied by diluting the SMEDDS to 100-fold and 1000-fold with 0.1N NaOH. The diluted emulsions were stored for 24 hrs at 37.0±0.5°C and observed for any signs of phase separation and drug precipitation[10].

Zeta Potential Measurement

Electrical charges play an important role in determining the interaction between the particles of the dispersed phase and the resultant physical stability of the system, particularly for those in the colloidal size range. The potential between the tightly bound surface liquid layer (shear plane) of the particle and the bulk phase of the solution is

called as zeta potential. The measurement of the zeta potential tells about the stability. Zeta potential was observed with the help of Malvern Zetasizer[11].

Dissolution Studies

Dissolution profiles of the self-emulsified formulations were determined using USP paddle method at 37 °C and a rotating speed of 100 rpm in a 900 ml of 0.1N NaOH. The membrane selected was pretreated by soaking it in the dissolution medium for 24 hrs prior to commencement of each experiment. A 1 g quantity of the formulated SMEDDS from each batch was placed in a polycarbonate dialysis membrane containing 2ml of the dissolution medium, securely tied with a thermo-resistant thread and then placed in the appropriate chamber of the release apparatus containing the dissolution medium. Samples (5 ml) withdrawn after 5 min were filtered using a whaltman filter and subjected to spectrophotometric analysis. The sample volume was replaced each time with equal quantity of fresh medium[12].

Release Kinetics

The results of *in-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows:-

1. Zero- order Kinetic model – Cumulative % drug released versus Time.
2. First- order Kinetic model – Log cumulative % drug remaining versus Time.
3. Higuchi's model- Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppa's model- Log cumulative percent drug released versus log time.

Drug Content

Prepared SMEDDS containing Glipizide equivalent to 3 mg was added in 100 mL volumetric flask (VF) containing 0.1N NaOH and mixed it well with shaking or inverting the volumetric flask for two to three times. 1 ml of this solution was diluted with 10 ml fresh 0.1N NaOH and drug content was determined using UV-spectrophotometer at 228 nm[3].

Transmission Electron Microscopy

Transmission electron microscope is used as a visualizing aid for the observation of morphology of droplets. SMEDDS is diluted with water (1/100). A drop of the diluted emulsion is directly deposited on the holey film grid to observe the morphology of formulations. To improve the contrast, the samples were treated with a 1 wt % phosphotungstic acid solution for 2 hrs, deposited on copper grids, and allowed to dry for 48 hrs before TEM examination. The homogeneous and spherical droplets in emulsion were observed. The droplet on the emulsion appeared dark with the bright surroundings[13].

Stability Studies

Shelf life as a function of time and storage temperature was evaluated by visual inspection of the SMEDDS system at different time period. SMEDDS was diluted with purified distilled water and to check the temperature stability of samples, they were kept at two different temperature range (2˚C– 8˚C (refrigerator), room temperature) and observed for any evidences of phase separation, flocculation or precipitation.

RESULTS AND DISCUSSION

In the present work SMEDDS were prepared using Glipizide as a model drug for the treatment of diabetes. Formulations were prepared by using oil (oleic acid), surfactant (Tween 80), co-surfactant (PEG-200). The prepared formulations were evaluated for pre-formulation and post-formulation parameters like solubility, pseudo ternary phase diagram, *in-vitro* drug dissolution, particle size analysis, transmission electron microscopy and drug content.

CALIBRATION CURVE OF GLIPIZIDE

Calibration curve of Glipizide in 0.1 N NaOH

Table 2: Spectrophotometric data for the estimation of Glipizide in 0.1 N NaOH

Sl. No	Concentration(µg/ml)	Absorbance at 275nm
1	0	0
2	5	0.129
3	10	0.222
4	15	0.350
5	20	0.469
6	25	0.561
7	30	0.696
8	35	0.767

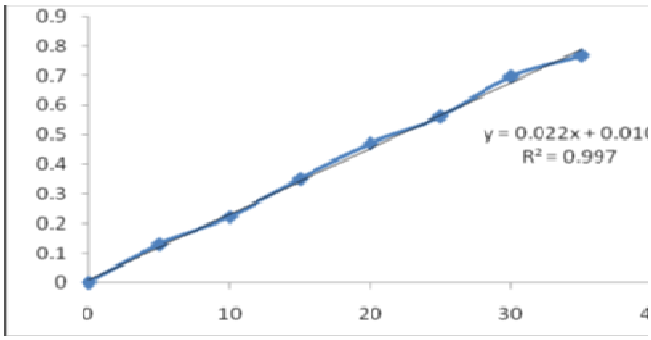

Figure 1: Calibration Curve of Glipizide in 0.1 N NaOH

As UV spectrophotometric method was selected for quality control purposes, the λmax was found to be 275 nm for Glipizide in 0.1 N NaOH. From the data obtained it can be said that the drug follows the Beer's law in the concentration rage of 2-16 µg/ml as shown in (Figure 1) and (Table 2).

FT-IR Studies

Table 3: Compatibility studies

IR Spectrum	Functional group	IR Range(Cm^{-1})
Glipizide+Oleicacid+Tween 80	C=N	**1650**
	C=O	**1692**
	N-H	**3325**
Glipizide	C=N	**1651**
	C=O	**1732-1651**
	N-H	**3323-3250**
Glipizide+Oleicacid+Tween 80+PEG 200	C=N	**1650**
	C=O	**1690**
	N-H	**3323-3250**

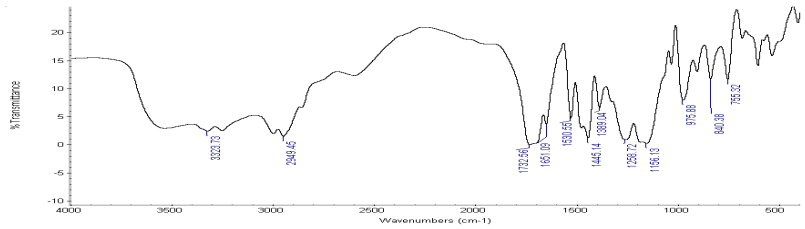

Figure 2: FT-IR Spectrum of pure Glipizide drug

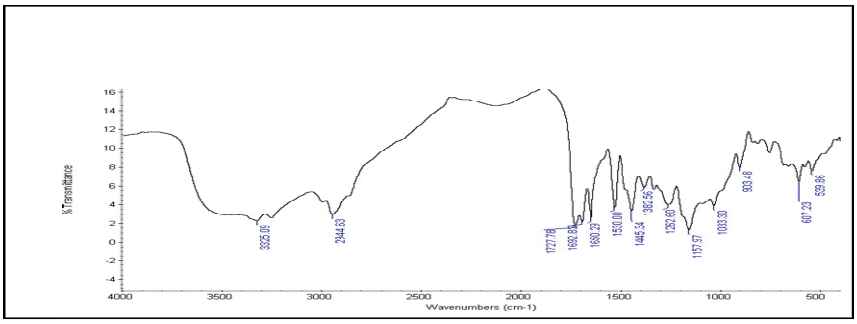

Figure4: FT-IR Spectrum of Formulation

The sample of Glipizide procured for study was identified by Fourier Transform infrared spectrum as shown in (Figure 2) and the excipients do not interact with drug in all formulations as shown in (Figures 3 & 4) and (Table 3).

SOLUBILITY STUDIES

Table 4: Solubility of glipizide in different oils/surfactants/co-surfactants

Vehicle	Solubility-of Glipizide(mg/ml),
Oleic acid	25.47
Castor oil	20
Olive oil	1.25
Sunflower oil	12.69
Coriander oil	10.29
Tween 80	32.01
Span 80	21.10
Span 20	6.01
Tween 20	19.62
PEG 200	17.62
PEG 400	13.51
Water	Not soluble

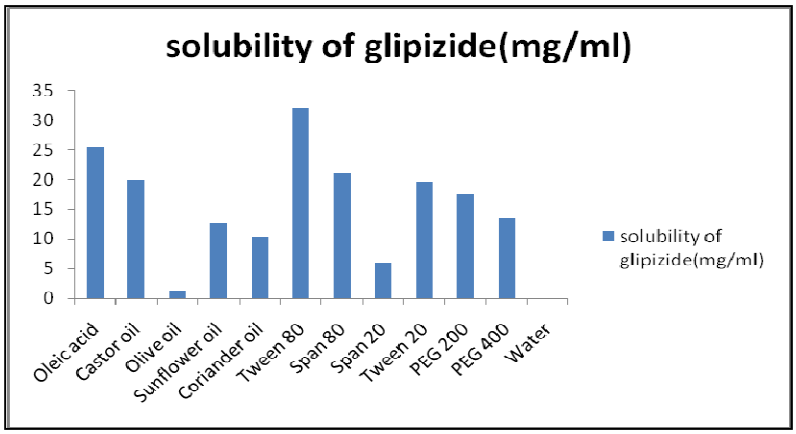

Figure 5: Solubility of Glipizide in different oils/surfactants/co-surfactant

The self-emulsifying formulation consisted of oil, surfactants, co-surfactants and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase, hence it should have good solvent properties to allow presentation of drug in solution. The solubility of Glipizide in different vehicles is presented in (Table 4). Based on this study oil (oleic acid), surfactant (Tween 80), co-surfactant (PEG-200) selected for the formulation of SMEDDS of Glipizide as shown in (Figure 5).

Ternary phase diagram

Phase diagrams are constructed in the presence of Glipizide to obtain the optimum concentrations of oil, surfactant, and co-surfactant. SMEDDS form fine oil-water emulsion with only gentle agitation, upon its introduction into aqueous media. Since the free energy required to form an emulsion is very low, the formation of emulsion spontaneous. Surfactants form a layer around the emulsion droplets and reduce the interfacial energy as well as providing a mechanical barrier to coalescence. The visual test measures the apparent spontaneity of emulsion formation. The series of SMEDDS were prepared and their self-emulsifying properties were observed visually.

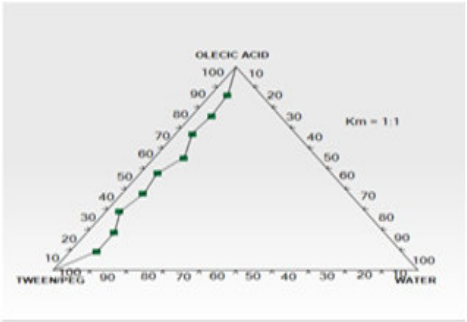

Figure 6: Ternary plot (Km=1:1)

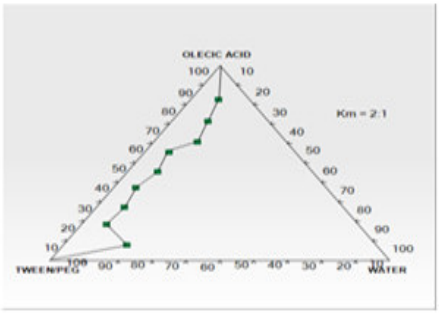

Figure 7: Ternary plot

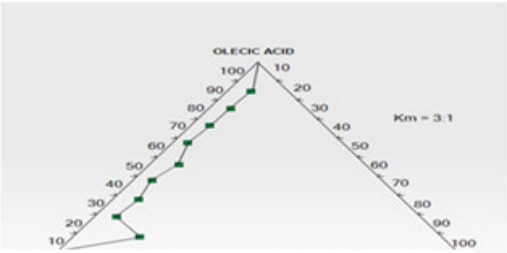

Figure 8: Ternary plot (Km=3:1)

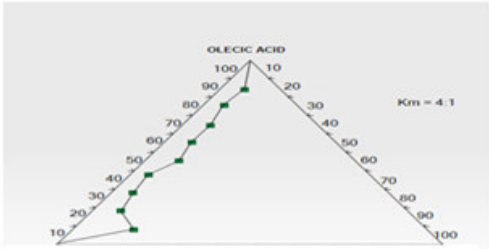

Figure 9: Ternary plot (Km=4:1)

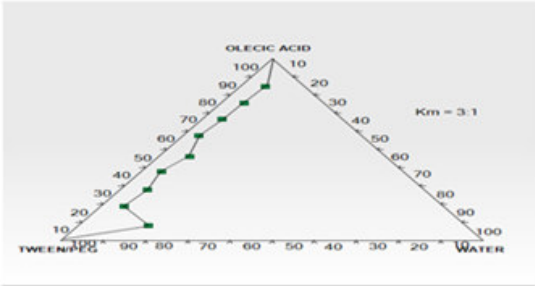

Figure 10: Ternary plot (Km=5:1)

Pseudo ternary phase diagrams were constructed to identify the self-emulsify regions and optimized concentration of oil, surfactant, and co-surfactant was used for formulation S1-S4. From this study surfactant: co-surfactant (Km) ratio 2:1 has been selected as the optimized concentration as shown in (Fig. 7).

In-vitro drug dissolution study

Table 5: *In-vitro* drug release profiles of formulations

Time (min)	% Drug release from the formulation Formulation code			
	S1	**S2**	**S3**	**S4**
0	0±0.00	0±0.00	0±0.00	0±0.00
3	21.44±0.50	22.86±0.23	21.54±0.19	26.02±0.11
6	38.46±0.25	45.55±0.10	36.67±0.28	48.34±0.27
9	44.61±0.31	52.96±0.35	40.55±0.52	58.12±0.13
12	64.57±0.13	71.44±0.56	67.86±0.26	76.09±0.35
15	73.61±0.22	90.19±0.33	80.70±0.12	91.81±0.45
18	86.22±0.17	94.52±0.14	88.50±0.05	96.01±0.15

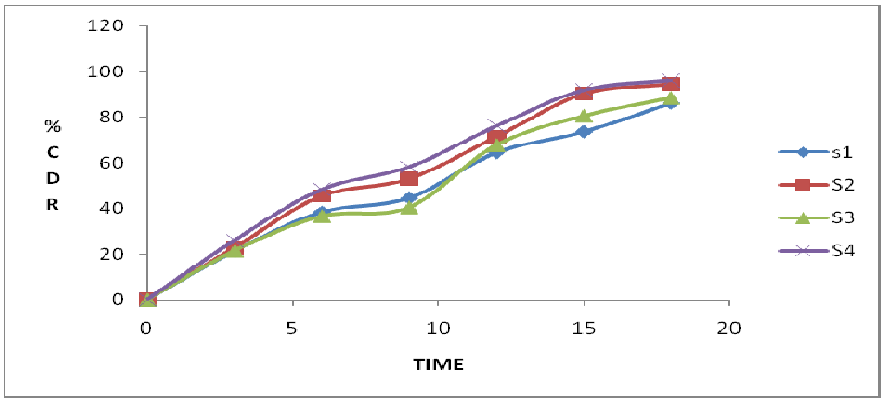

Figure11: Cumulative percentage release Vs time profile of formulations

The release of Glipizide from the formulations containing the smallest amount of drug (3 mg) is completed 96.01% within 18 min as shown in (Table 5)and(Figure 11).

Determination of % drug content

Table 6: Percentage drug content of the formulations

Formulations	Absorbance of pure drug solution	Absorbance of Formulation	% drug content
S1	0.7041	0.6389	90.79
S2	0.7041	0.6551	93.02
S3	0.7041	0.6497	92.27
S4	0.7041	0.6682	94.82

Formulation S4 contained maximum drug and hence showed highest percent drug content of 94.82% as shown in(Table 6).

Transmission electron microscopy

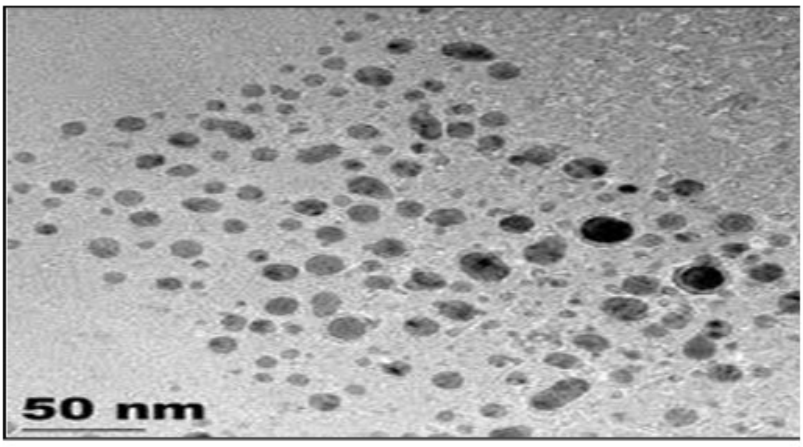

Figure 12: Transmission electron microscopy of formulation S4

The photographic image of micro particles showing surface morphology under TEM as shown in (Figure 12). From the TEM study the average particle size was found to be within 50 nm and the shape was found to be spherical.

Robustness to dilution

Table 7: Robustness to dilution

Formulation	Phase Separation	Precipitation
S1	No phase separation	No precipitation
S2	No phase separation	No precipitation
S3	No phase separation	No precipitation
S4	No phase separation	No precipitation

Robustness to dilution was studied by diluting the SMEDDS 100 and 1000 folds with 0.1N NaOH. The diluted emulsions showed no visible signs of phase separation or drug precipitation after storage for 24 hrs at 37.0°+0.5°C as shown in (Table 7).

Zeta potential

Table 8: Particle size and zeta-potential of formulations

Formulation	Particle Size(nm)	Zeta potential(mV)
S2	50	-28.12±10
S4	58	-35.45±2.8

Formulations are stabilized by a greater zeta potential (negative) by preventing droplet coalescence upon random collisions of particles leading to repulsive forces which can stabilize the formulation. The zeta potential of formulation S4 (-35.45 ± 2.8mV) was high when compared to other formulations as shown in (Table 8).

Mathematical modeling and drug release kinetics of formulation

Table 9: Drug release kinetics of formulation

Formulation Code	KINETIC MODELS				
	Zeroorder	Firstorder	Higuchi	Korsmeyer - Peppas	
	R^2	R^2	R^2	n	R^2
S1	0.949	0.9452	0.978	0.479	0.9871
S2	0.932	0.9304	0.9796	0.479	0.9777
S3	0.953	0.9416	0.9488	.4979	0.9713
S4	0.890	0.9389	0.9877	.4349	0.9823

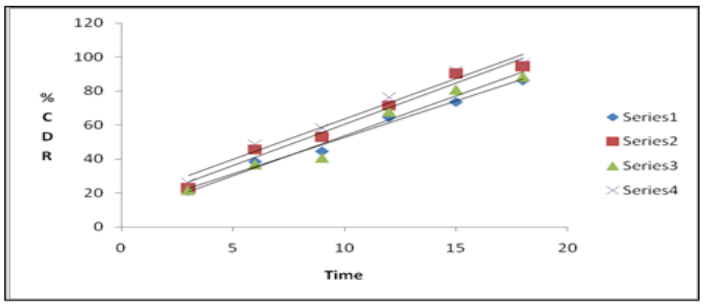

Figure 13: Plot of % Cumulative drug released Vs Time of formulations (Zero order kinetics)

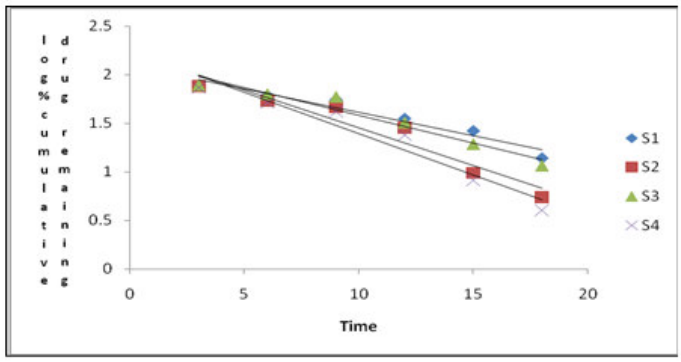

Figure 14: Plot of Log % Cumulative drug remaining of formulations Vs Time of formulation (First order kinetics)

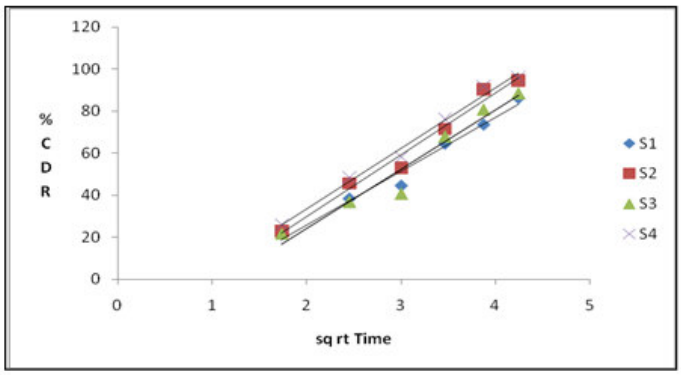

Figure 15: Plot of % Cumulative drug release Vs SQRT of Time of formulations (Higuchi's model)

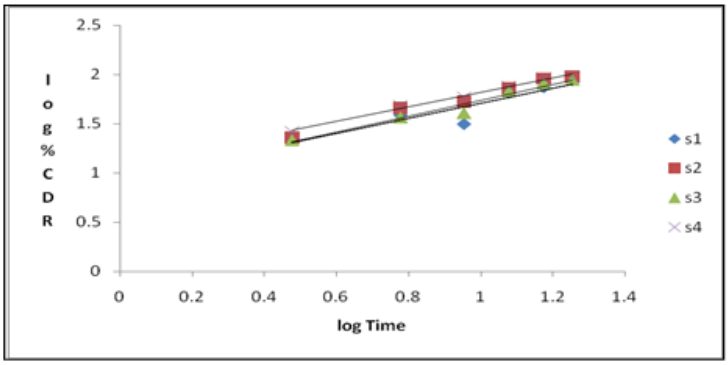

Figure 16: Plot of Log % Cumulative drug release Vs Log time of formulations (Korsmeyer- Peppas model)

The release data was fitted to various mathematical models such as Higuchi, Korsmeyer-Peppas, Zero order and first order to evaluate the kinetics of drug release as shown in (Table 9). The drug release follows mixed order kinetics and mechanism was found to be non-Fickian diffusion as shown in (Figs. 13, 14, 15 & 16). From the obtained results it was summarized that the formulation S4 released drug to a greater extent when compared to other formulations. From the stability studies it was concluded that SMEDDS formulation was stable thermally as well as under stressful conditions. From the above said it can be concluded that the self-emulsifying drug delivery system of Glipizide showed a better bioavailability.

CONCLUSION

Self-emulsifying drug delivery systems are a promising approach for the formulation of Glipizide. The oral delivery of hydrophobic drugs can be made possible by SEDDS, which have been shown to substantially improve oral bioavailability with future development of this technology. SEDDSs will continue to enable novel applications in drug delivery and solve problems associated with the delivery of poorly soluble drugs. Glipizide can be used to develop the SEDDS. *In-vitro* drug dissolution studies revealed that release of Glipizide from SEDDS was faster than the conventional formulation. The SEDDS showed good release profiles as drug delivery systems. Results of this study indicated that *in-vitro* drug release varied with the formulation and particle size. From the drug content study it is found that all the formulation contain above 90% of drug. Formulation S4 has released a maximum of 96.01% drug from the formulation. From the TEM study the average particle size was found to be within 50 nm and the shape was found to be spherical. The short term stability studies carried out were confirmative of the drug stability in the SEDDS during the present study. From the above said it can be concluded that the self-emulsifying drug delivery system of Glipizide showed a better bioavailability.

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