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SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS): DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF PIOGLITAZONE HYDROCHLORIDE.

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ABSTRACT

The objective of present study is to develop self nano emulsifying drug delivery system for lipophillic drug, pioglitazone hydrocholoride (used to treat Type 2 diabetes). Capmul PG, kolliphour EL and propylene glycol were chosen as oil, surfactant and co-surfactant respectively as they show higher solubility for pioglytazone hydrochloride. Screening of surfactant, co-surfactant is done by percent transmittance and observed for turbidity or phase separation visually. To determine stable emulsifying region, pseudo ternary phase diagram was constructed. Optimization of formulation was performed by Box Bhenken design, chosen for further study. Characterization was done for optimized formulation. Percent drug content was found to be 90.2. Emulsifying study showed transparent appearance after 24 hr, dilutability test gave percentage transmittance was found to be 99.9. In-vitro dissolution studies were conducted in stimulated gastric fluid (S.G.F) pH 1.2 showed 95.94 percent drug release while pure drug exhibited only 58.67 % within 30 minute. cloud point determine to be 53.02, particle size found 111.4 nm which is under nano size range, performing poly dispersity index (PDI) 0.22, zeta potential found to be -15.3mv, by using particle size analyzer.TEM study shows uniform spherical nano emulsion droplets provide large surface area for drug absorption. Robustness to dilution determine by using thermodynamic study and centrifugation study shows homogenous and no phase separation. From the present study it is clear that SNEDDS can be formulated to improve the dissolution and oral bioavailability of poorly water soluble drug pioglitazone hydrochloride.

KEYWORDS: ***Pioglitazone hydrochloride, self nanoemulsifying drug delivery system, lipophillic, ternary phase diagram, bioavailability***

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INTRODUCTION

An ideal oral drug delivery system must protect the drug from the degradation in the gastrointestinal tract, and deliver the bioactive compounds to the specific area where it is better absorbed. Studies suggested that for development of oral route drugs, its stability in the GIT is very essential thus increasing drug solubility and further the bioavailability.[1] Past few decades, various lipid based formulations have been investigated to enhance the bioavailability. Oral bioavailability of the drugs through gastro intestinal tract depends on the lipophillicity and solubility profile of the drugs. Factors responsible for decreasing the oral bioavailability are drug stability in gastrointestinal tract, intestinal permeability, resistance to metabolism by cytochrome P450 enzymes present in gut enterocytes and liver hepatocytes and interaction with efflux transport system like P-glycoproten (P-gp) . Increasing the solubility of class II drugs amplify the concentration of dissolved drugs in the GI tract and thus boost the bioavailability.[2] Self emulsifying drug delivery system (SEDDS), have attracted interest particularly self nano emulsifying drug delivery system (SNEDDS). SEDDS and SNEDDS consist in micro or nano emulsions of oil containing the drug that spontaneously form in aqueous media on mild agitation. SNEDDS are isotropic concoction of lipid (generally triglycerides or diglycerides), surfactants (nonionic, HLB value above 10), co-surfactant and drug substances that rapidly form oil-in –water nano emulsion in gastrointestinal tract (GIT), the GI motility of the stomach and the intestine provide the necessary agitation for self emulsification. SNEDDS is thermodynamically stable formulation with high solubilization capacity for lipophillic drugs. Self emulsification process is specific to the nature of oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which self emulsification occurs. SNEDDS have globule size in the range of 20-200 nm[3,4] The increase in bioavailability by SNEDDS may be achieved by keeping the drug in solubilized form in the gut, avoiding dissolution step[5]. SNEDDS can be filled in hard gelatin /soft gelatin capsules nibbling its administration as unit dosage forms. [6,7]Pioglitazone hydrochloride (thiazolidinediones) Biopharmaceutics classification system (BCS) II is highly permeable and low soluble potent antidiabetic drug with short half life of 3-7 hours and is eliminated rapidly from the body. Pioglitazone hydrochloride has good bioavailability but low solubility in aqueous condition and slow dissolution limits the enhancement of pharmacokinetics and bioavailability of the drug[8-10]. Presence of food delays peak plasma concentration.Thus increasing the aqueous solubility and dissolution rate of pioglitazone hydrochloride by forming SNEDDS. The objective of present study was to develop and evaluate SNEDDS formulation of the drug.[11,12] The criteria for formulation selection were

a) Emulsion stability
b) Dispersibility /self emulsification ability
c) Incorporation of desired drug dose and
d) Desired nano-sized droplet size

MATERIAL AND METHODS

Materials

Pioglytazone hydrochloride was generous gift sample from USV Mumbai, India. capmul PG, capmul C8, captex 355, were donated by abitec corp, USA And were used as received. labrafac PG, labrafill 2125, labrafill 1944, Transcutol P were kindly donated by Gattefosse (Mumbai, Maharashtra, India). kolliphour EL gifted from BASF (Mumbai, Maharashtra India). oleic acid, PEG 200, tween 80, tween 20, Span 80, Propylene Glycol were brought from S.D fine chem.((Mumbai, Maharastra India).

Determination of solubility of drug in various solvents (oils, surfactants, and co-surfactants)

The solubility of Pioglitazone hydrochloride in various solvents was determined by dissolving excess amount of Pioglitazone hydrochloride in 1ml of each of the selected solvents in 5ml capacity in a Stoppard vials separately. Each glass vial was then mixed for 10 min using a vortex mixer. The mixture vials were then kept at 37±1.0 °C in a shaker bath for 72 hrs to get equilibrium. The equilibrated samples were removed from shaker and centrifuged at 18000 rpm for 30 min. The supernatant was taken and filtered through a 0.45µm membrane filter. The concentration of API was determined in each solvent by UV spectrophotometer by scanning from 200-400nm[13].

Screening of surfactant and co-surfactants

Screening of surfactants: (Emulsifying study)[14]

For this study, 150 mg of surfactant were added to 150 mg of oily phase and then this mixture was heated at 50ºC for 30 sec for homogenization.Of the components Then from each prepared mixture, 100 mg was withdrawn and diluted to 100ml in a volumetric flask. The ease of emulsification was judged by the number of flask inversions required to yield homogeneous emulsion. The emulsions were allowed to stand for 24 hrs and then % transmittance was evaluated at 638 nm by using UV spectrophotometer. They were also observed for turbidity or phase separation visually.

Screening of co-surfactants: (Emulsifying study)

For screening of selected co-surfactants, the oil: surfactant: co-surfactant was taken as 300mg: 200mg: 100mg i.e. in the ratios of 3:2:1. Out of the total 600mg of the mixture 100 mg was withdrawn and then added drop wise in a100 ml volumetric flask containing distilled water drop wise, then it was inverted 50-60 times and kept overnight. After which the percent transmittance was determined by scanning in the range from 800-200 nm (wavelength 650 nm) using UV-visible spectrophotometer. After the completion of screening the next step was to optimize the combination showing good % transmittance.[15]

Construction Of pseudo-ternary phase diagram

Phase diagrams involve the plotting the three components surfactant

co-surfactant (S_{mix}), oil and water each of them representing an apex of triangle. Ternary mixtures with varying compositions of the components were formed. For any ternary mixture formed the total of surfactants,

co-surfactants and oil concentrations always added to 100%. The required amount of the three components was weighed accurately. The mixture was then gently heated at 45–50ºC and vortex to form homogenous mixture. To this mixture distilled water was added drop by drop until a transparent solution was formed. The surfactant and co-surfactant was varied in mass ratios 1:1, 1:2, 2:1. The different concentration ratios of oil and mixture of surfactant and co-surfactant were taken as 0.5:9.5, 0.5:9, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8, 3:7, 4:6 and 5:5. Ternary mixtures were formed in these ratios and then quantity of water forming transparent solution was plotted with other components in the pseudo-ternary phase diagram.[16-18]

Preparation of pioglitazone hydrochloride containing SNEDDS using Box Bhenken design.

Different types of surface response methodology (RSM) include 3-lavel factorial design, Box Bhenken design and D-optimal design. Based on the principal of design of experiments (DOE), the methodology encompasses the use of various types of excremental designs, generation of polynomial equations and mapping of the response over the excremental domain to determine the optimum formulation. RSM is used for optimization of formulation. As it is more efficient and cost effective than conventional methods. The required amount of the three components and pioglitazone hydrochloride (15mg) were weighed accurately and vortex for 1 minute until drug was perfectly dissolve followed maintained the pH of formulation. The mixture was then gently heated at 45–50ºC and vortex to form homogenous mixture.[19-20]

Table 1
Composition of SNEDDS using Box Behnken design

Factor	Name	Units	Minimum	Maximum
A	Oil	%	4.63	14.03
B	Surfactant	%	29.71	63.30
C	Co-surfactant	%	28.85	63.61

Formulation

Preparation of pioglitazone hydrochloride Loaded SNEDDS

Unless otherwise stated, the optimized SNEDDS formulation was prepared by accurately weighed 15mg of drug placed in glass vial and mixed with each of oil from 4.63 to 14.03%, surfactant from 29.71 to 63.30% and co-surfactant from 28.85 to 63.61% .After adding all the components, the mixture was vortexed for few min and then homogenized at 45^{o}C for 5 minutes. After homogenization, the mixture is sonicated and in bath sonicator occasionally vortexed until drug was perfectly dissolved.

Evaluation of different SNEDDS formulation

Percentage drug content

The drug content evaluation for the selected formulations was done by dissolving weighed amount of drug to the particular formulation. SNEDDS formulation was centrifuged diluted at 18000 rpm for 60 minutes and diluted with methanol. These drug loaded formulations were subjected to assay by analyzing it in UV spectrometer at respective λ max. The percentage drug content is then calculated by the formula,

$$\% \text{ Drug content} = \frac{Amount\ of\ drug\ in\ supernatant}{Initial\ amount\ of\ drug}$$

Thermodynamic stability studies

SNEDDS was subjected to thermodynamic stability studies in order to access any phase separation and stability of the prepared formulation [20-21]

Heating cooling cycle

The SNEDD formulations six Heating and cooling cycle was done in refrigerator, the temperature ranging between 4^{0}c and 45^{0}c for 48 hours. The formulations which were stable at these temperatures were subjected to centrifugation test.

Centrifugation study

The formulation was centrifuged at 18000 rpm for 30min. The resultant formulation was then checked for any instability problem, such as phase separation, creaming, or cracking. The formulations which do not show any phase separation were taken for the freeze thaw stress test.

Freeze thaw cycle

Four freeze thaw cycle were carried out between a temperature -4^{0} C and +40^{0} C, where the formulation were stored for not less than 48 hours at each temperature. Those formulations, which passed these thermodynamic stress tests, were selected for further study.

Partition coefficient of pioglitazone hydrochloride

Partition coefficient was calculated using the equation.It can be determined by the formula

$$K_{o/w} = C1/C2$$

Where, C1 = conc. of solute in organic phase.
C2 = conc. of solute in aqueous phase.
$K_{o/w}$ = Partition coefficient
Log P = log ($K_{o/w}$)

Dilutability test process

The dilutability of the SNEDDS was assessed toknow whether these systems would be diluted with threw aqueous phase of the system without separation or not. For this purpose, selected and drug- loaded microemulsions were diluted with 900ml distilled water and there transparency was assessed visually initial and after 24 hr. Percentage transmittance reflects the clarity and visual appearance which increases the acceptability of formulation.

Cloud point measurement

Cloud point was determined as the temperature at which there was a sudden appearance of cloudiness [22]

In-vitro dissolution

In vitro dissolution profile for pure drug (pioglytazone hydrochloride) and percentage drug release from optimized SNEDDS formulation performed in USP type-II dissolution apparatus (basket type) using 900 ml of stimulated gastric fluid (SGF) without containing enzyme (pH 1.2) for 30 minute at temperature maintained 37±0.5^{0}C. The drug content was determined spectrophotometrically at the predetermined λ_{max} (269nm). The volume withdrawn was replaced with fresh medium to maintain sink condition. All the experiments were done in triplicate.

Determination of globule size and zeta potential

Total 50mg of the optimized SNEDDS formulation was diluted with water to 100ml in flask, and gently mixed by hand. The droplet size distribution and zeta potential of the resultant emulsion was determined by laser diffraction analysis using a particle size analyzer (Malvern zetasizer, U.K). All studies were repeated in triplicates. ($P<0.05$) Zeta potential measure electric charge at the surface of particles indicates the physical stability of colloidal system it measure by zeta sizer. Each sample suitably diluted with double distilled filtered water and placed in disposable zeta cell. The zeta potential values were assessed by determining the particle electrophoretic mobility. The electrophoretic mobility was converted to the zeta potential via the helmholtz-smoluchowski equation, all measurement perform triplicate. The result expressed as mean ±sd. Polydispersity index (PDI) measurement is the parameter which shows the uniformity in globule size distribution. The minimum is the PDI the maximum is the uniformity in the globule size distribution. PDI less than 0.5 indicate homogenous distribution, while a greater than 0.5 indicates a higher heterogeneous dispersion.

Transmission electron micrograph (TEM)

Morphology of oil droplets in the emulsion formation was visualized with transmission electron micrograph (TEM) (Hitachi, H-7500, Tokyo, Japan), operated at 80kv and at 50,000 times magnification. The TEM analysis also performed to visualize any precipitation of the drug upon addition of the aqueous phase. A drop of nanoemulsion after suitable dilution was allowed to deposit directly on the microscope gold coated grid and observed after drying.

Differential scanning colorimetry (DSC)

Differential scanning colorimetry (DSC) measurements were carried out using metter-toledo star system, Japan, equipped with liquid nitrogen sub ambient accessory. Calibration was carried using indium and zinc as reference materials. Samples were sealed in aluminum pans and lids. DSC thermograms were recorded at heating rate of 10°c/min from 40-350°c.

RESULTS AND DISCUSSION

Miscibility studies and excipient selection

Key step for SNEDDS formulation is to find a suitable oil and surfactant mixture that can dissolve the drug within the therapeutic concentration. Long chain triglycerides and medium chain triglycerides, oil with different degree of saturation have been used in the design of SNEDDS. The result of solubility of pioglytazone hydrochloride in various excipient (oils, surfactants, co-surfactants) shown in figures 1,2,3. The components in formulation of SNEDDS were selected having maximum solubility of drug along with good miscibility with each other and to produce stable formulation.

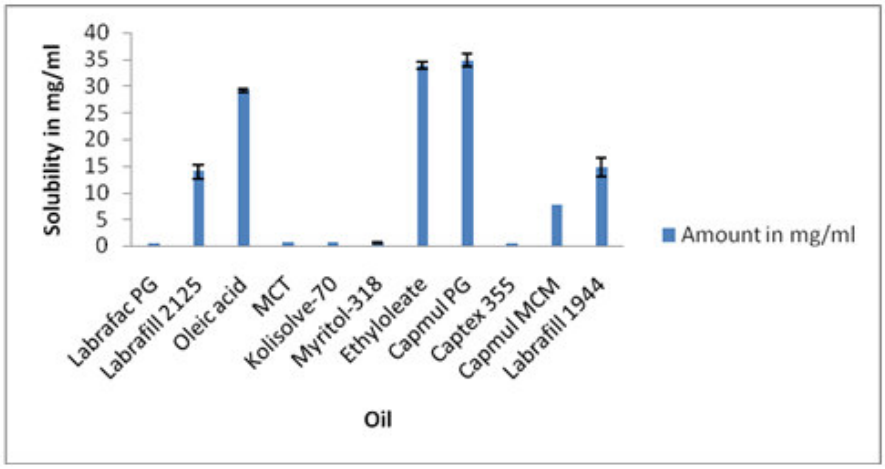


Figure 1
Solubility study of pioglytazone hydrochloride in different oils

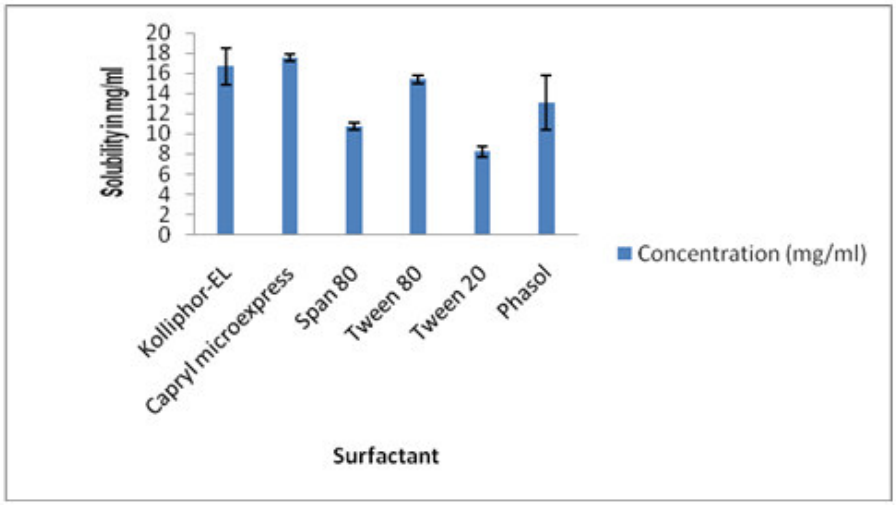


Figure 2
Solubility study of pioglytazone hydrochloride in different surfactants

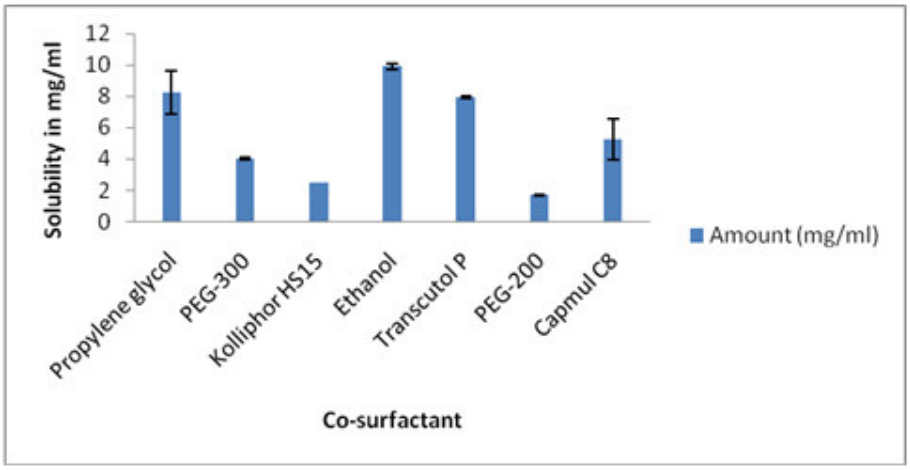


Figure 3
Solubility study of pioglytazone hydrochloride in different co-surfactants

On the basis of solubility study (Figure 1,2,3) following excipient was selected for further study
Oil: Ethyl oleate, capmul PG, labrafil 1944, oleic acid
Surfactant: kolliphor EL, Tween 80, capryl microexpress,
Co-surfactant: Propylene glycol, PEG-300, ethanol, Transcutol P, capmul C8

Screening of surfactant and co-surfactant
Screening of surfactants
Surfactant chosen must be able to lower the interfacial tension during the formation of the nanoemulsion. Surfactants are amphillic in nature and generally dissolve or solubilize relatively high amount of hydrophobic drug compounds [23] For an effective absorption the precipitation of the drug compound within the GI lumen, it should be prevented and the drug should be kept solubilized for a prolonged period of time at the site of absorption.[24] Surfactants has pivotal role in stabilizing nanoemulsion, its nature and amount determining droplet size and stability. Following formulation was selected for further screening of co-surfactant

A1 Labrafil 1944 and kolliphor EL
C1 Ethyl oleate and kolliphor EL
D1 Capmul PG and kolliphor EL
D3 Capmul PG and Tween 80

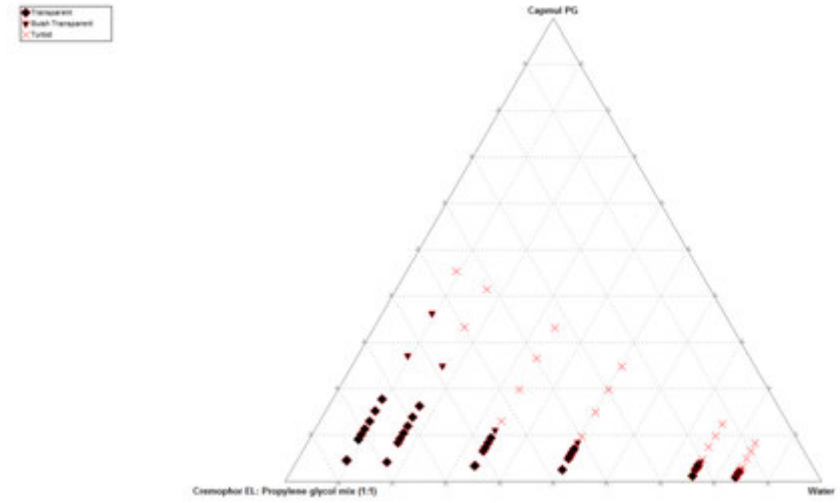
Screening of co-surfactant
Surfactant and co-surfactant got preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence, then improved the stability of the formulation[17.] Co-surfactant could increase interfacial fluidity by penetrating into the surfactant film [18] Addition of co-surfactant to the surfactant containing formulation improved the dispersibility and drug absorption from the formulation. Form the screening of surfactant and co-surfactant two combinations were selected for the ternary phase diagram. The two combinations are

A21 Capmul PG, kolliphor EL and propylene glycol +Transcutol (1:1)
A11 Capmul PG, kolliphor EL and propylene glycol

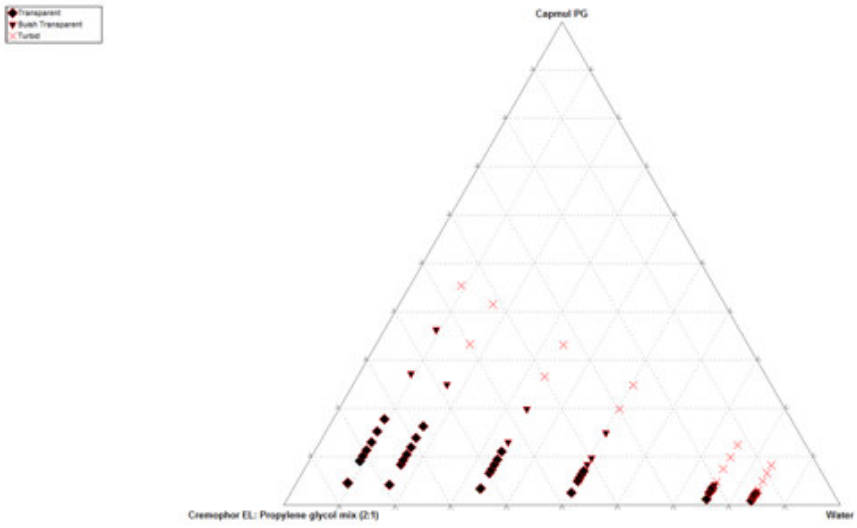
Pseudo-ternary phase diagram

The key factor influencing the phase properties is the surfactant and co-surfactant ratio.

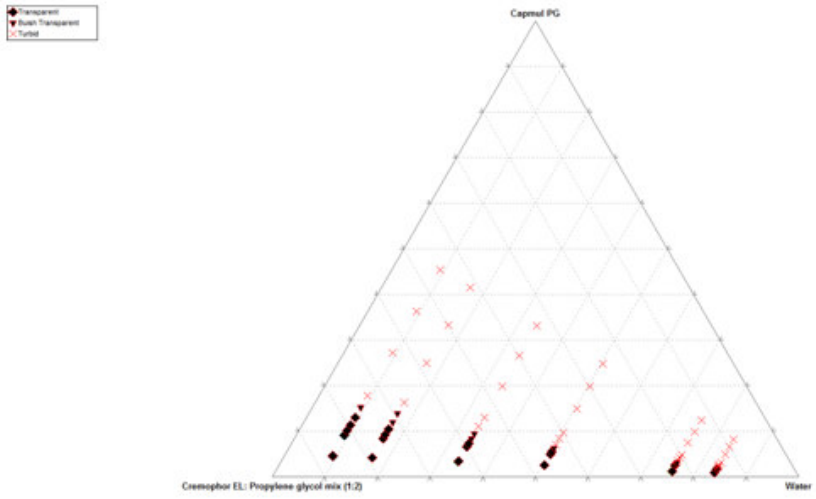
Attwood et al; 1992 reported that size and location of nanoemulsion was changed on changing the mass ratio. Surfactants have a pivotal role in solubilizing nanoemulsion, its nature and amount determining droplet size and stability. The area enclosed within the solid line represents the region of self-emulsification. With in this area the SNEDDS form fine oil in water emulsion with only gentle agitation. This is possible as surfactant strongly localized to the surface of the emulsion droplet reduces interfacial free energy and provide a mechanical barrier to coalescence resulting in a thermo mechanically spontaneous dispersion[17]. Furthermore, co-surfactant increases interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules.[18] This eventually led to decrease in the energy required for the formation of nanoemulsion (Shafiq et al., 2007) Result of pseudo ternary phase diagram (Figure 4) showed that by using sufficient concentration of surfactants reduces the interfacial tension between the molecules of both the phases oil and aqueous. Increasing oil ratio results cloudiness (turbid) in the formulationKolliphour EL (non ionic surfactant) formed larger self emulsifying regions by using capmul PG (oil) and propylene glycol (co-surfactant) (figure 4.d). The better emulsification performance of kolliphour EL can be ascribed to its higher HLB value being required in forming a good emulsion.



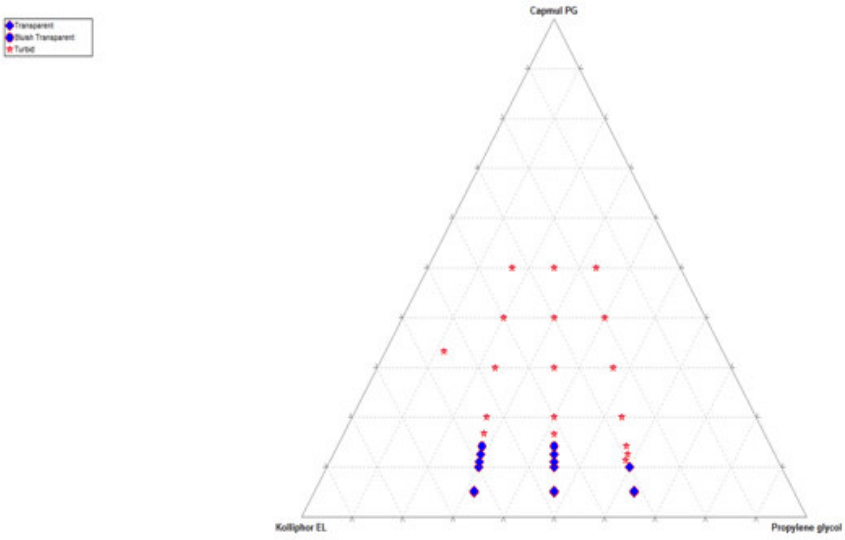
(A)



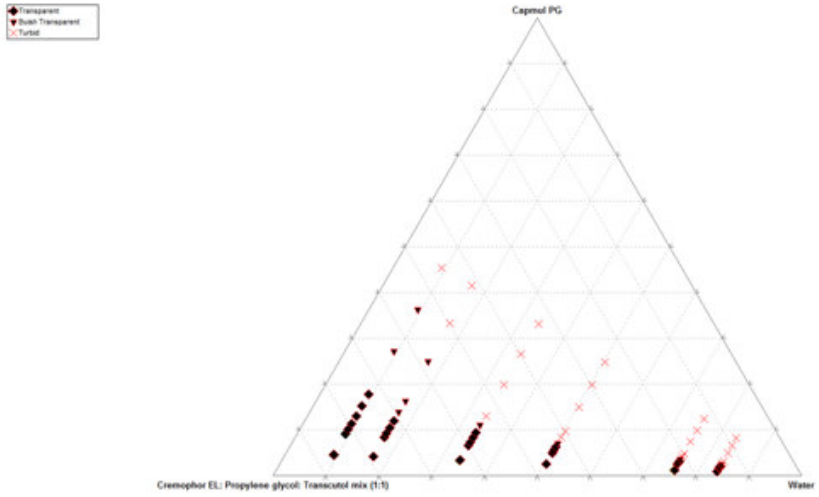
(B)



(C)



(D)



(E)

Figure 4
Pseudo ternary phase diagrams of system containing capmul PG kolliphour EL:propylene glycol mix and water a)1:1, b) 2:1 c) 1:2,d) capmul PG kolliphour EL propylene glycol e) capmul PG, kolliphour EL:propylene glycol Transcutol mix water 1:1

Optimization of formulation by Box Bhenken desine

In this study Box Behnken statically design was used to optimized and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the in vitro performance. Seventeen experiments were required for the response surface methodology on this design. Constraints on the formulas were placed so that the oil phase ranged from 4.63 to 16.75% and surfactant ranged from 27.93 to 64.07% and co surfactant ranged from 26.98 to 63.61%. The different variable with their constraints, excremental runs and the results of characterization were given in table. Based on experimental design, the factor combinations resulted in different SNEDDS and drug release (Figure 5).

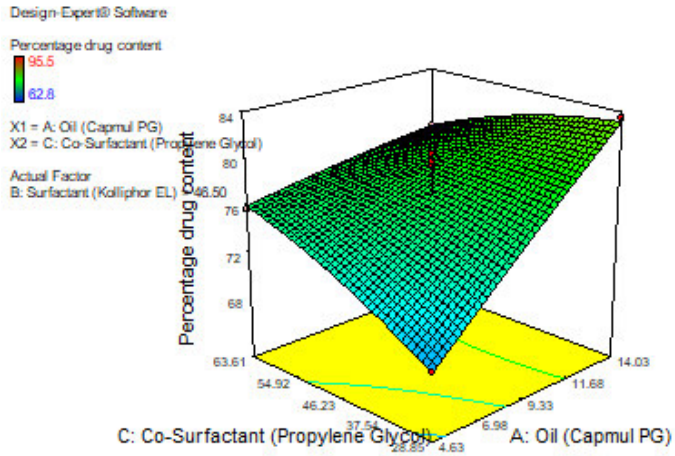


Figure 5(a)

3D Surface Response Graph between Oil (X_1), Surfactant (X_2) with % Entrapment (Y_1)

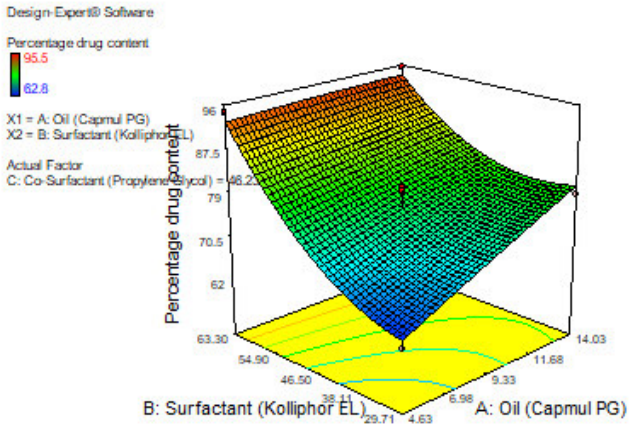


Figure 5(b)

3D Surface Response Graph between Oil (X_1), Co-Surfactant (X_3) with % Entrapment (Y_1)

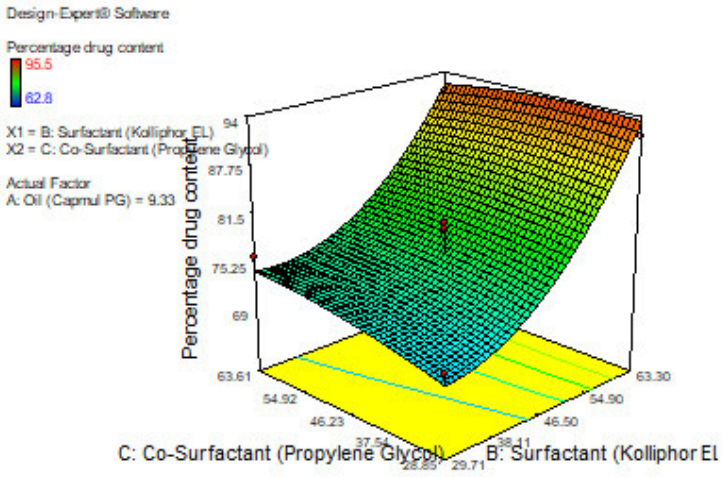


Figure 5(c)

3D Surface Response Graph between Surfactant (X_2), Co-Surfactant (X_3) with % Entrapment (Y_1)

ANOVA for response surface quadratic model

Table 2
ANOVA analysis using Response Surface Quadratic Model

ANOVA	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	1269.316676	9	141.0351863	14.39879681	0.0010	significant
A-Oil (Capmul PG)	154.00125	1	154.00125	15.72254957	0.0054	
B-Surfactant (Kolliphor EL)	871.53125	1	871.53125	88.97780557	< 0.0001	
C-Co-Surfactant (Propylene Glycol)	5.78	1	5.78	0.590101291	0.4675	
AB	58.5225	1	58.5225	5.974775576	0.0445	
AC	37.21	1	37.21	3.798904681	0.0923	
BC	7.84	1	7.84	0.800414209	0.4007	
A^2	0.199184211	1	0.199184211	0.020335443	0.8906	
B^2	133.5791842	1	133.5791842	13.63758635	0.0077	
C^2	2.321289474	1	2.321289474	0.236988913	0.6413	
Residual	68.5645	7	9.794928571			
Std. Dev.	3.129685059		R-Squared	0.948751428		
Mean	79.75882353		Pred R-Squared	0.683891023		
C.V. %	3.923935837					
PRESS	422.91625					

The model (table:2) F-value of 14.39 implies the model is significant. There is only a 0.10% chance that a "model F value" this could occur due to noise. Value of prob>f less than 0.0500 indicate model term is significant. In this case a^2, b^2 ,c^2 are significant model terms. Value greater than 0.1000 indicate the model terms are not significant. The "pred R-squared" of 0.6839 is not close to the "adj R-squard" of 0.9487 as one might normally expect. The ratio 0.6839 indicates as adequate signal. This model can be used to navigate the design space. Optimized Concentration of Dependent Variables: Data with desirability one (table:3) were run again and calculated for minimum standard deviation between theoretical and experimental response value. Minimum standard deviation was observed in combinatorial sets (marked in bolds), thus considered as optimized parameters of the process.

Table 3
Number of Solutions with desirability=1

Formulation code	Oil (Capmul PG)%W/W	Surfactant (Kolliphor EL) %W/W	Co-Surfactant (Propylene Glycol) %W/W	Predicted Percentage drug content	Desirability	
A1	5.53	61.41	45.7	90.26158892	1	Selected
A2	8.12	42.86	34.38	72.58791737	1	
A3	7.14	47.56	29.6	73.60315524	1	
A4	5.17	58.26	29.67	82.981086	1	
A5	10.31	56.13	38.79	85.97693444	1	
A6	11.18	32.35	48.39	75.78216234	1	
A7	7.55	53.28	43.6	81.36244225	1	Selected
A8	10.8	63.27	51.22	93.18344526	1	Selected
A9	12.43	63.11	49.46	93.10461518	1	
A10	6.81	41.87	38.5	70.73214119	1	

Formulation code (table:3) A1, A7 and A8 selected for evaluation study.

Evaluation of optimized SNEDDS formulations
Percentage drug content
The percentage drug content showed uniform distribution of drug in the prepared SNEDDS formulations. Percentage drug content found highest in SNEDDS formulation A1, 90.26, A8, 93.18 and A7, 81.36. These formulations selected for further evaluation.

Thermodynamic stability studies
The objective of this thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SNEDDS formulations. The SNEDDS formulation A1 was stable during heating and cooling cycle's evaluation with no phase separation. On centrifugation study both formulations were stable after centrifugation. No sign of phase separation was seen in both formulations. They are transferred for the freeze thaw stress test. The formulation passed freeze- thaw cycles test, it showed good stability with no phase separation, creaming, or cracking.

Partition coefficient of pioglitazone hydrochloride
The partition coefficient of drug is 2.78. So the drug is lipophillic in nature.

Emulsification study

The formulations were categorized as clear (transparent or transparent with bluish tinge), non clear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours) From the basis of design, drug content and percentage emulsification study we find three formulations that were A1, A7 and A8 for further evaluation.

Dilutability test process

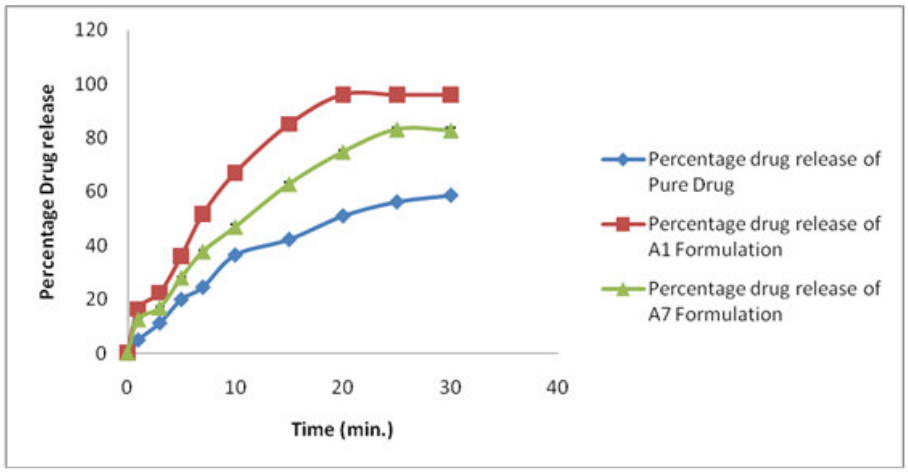
Percentage transmittance reflects the clarity and visual appearance which increases the acceptability of formulation. Percentage transmittance of formulation A1 was 99.9±1.55 has transparent appearance after 24 hrs[25].

Cloud point measurement

Cloud point was determined as the temperature at which there was a sudden appearance of cloudiness[21]. Optimized formulation A1 cloud point was 53.03±0.933

In-vitro dissolution

In vitro dissolution profile for pure drug and percentage drug release of A1 and A7 SNEDDS formulation (Figure 6) performed in USP type II apparatus in stimulated gastric fluid (SGF) containing without enzyme pH 1.2 . Drug release profile from SNEDDS formulation showed higher drug release i.e. percentage of drug release in 30 minutes, from SNEDDS A1 formulation is 95.94 ±0.18 and A7 SNEDDS formulation shows 82.74 ±1.14 than compared with the pure drug which shows only 58.67 ±0.57 ensuring that the SNEDDS formulation preserved the improvement of in vitro dissolution higher than pure drug. This may be, when the SNEDDS formulations were introduced into the aqueous media upon mild agitation, these systems could form fine oil-in-water (o/w) nanoemulsion and most amounts of drug pioglitazone distributing on the interface of oil/water which may lead to release into water rapidly. Otherwise, the release of pioglitazone in oil phase may be limited by diffusion through the oil droplet and by interfacial barrier at the droplet surface. Haiyan li et al., 2014.



The result shows highest percentage of drug release from formulation A1.

Figure 6

Percentage drug release pure pioglitazone hydrochloride, formulation A1 & A7

Determination of globule size and zeta potential

With globule size in the range of 20-200nm due to anhydrous nature, SNEDDS can be filled in hard capsules enabling its administration as unit dosage form. Small droplets provide large surface area for drug absorption. The droplet size of an emulsion is a vital factor in self emulsification characteristics because it determines the rate and extent of drug release as well as absorption. It has been reported that droplet size have an effect on drug absorption, smaller is the size of droplets larger is the interfacial surface area available for the drug absorption.

Table 4

Formulation A1 particle size, zeta potential, polydispersity index (PDI)

S.no.	Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mv)
1	A1	111.4	0.22	-15.3

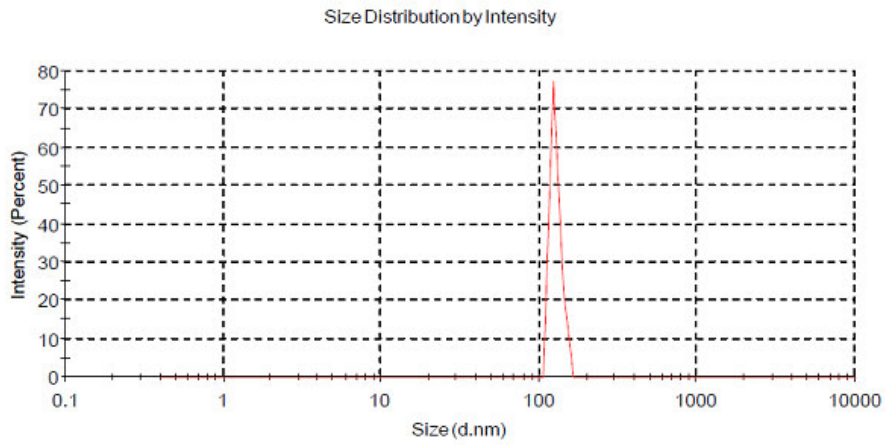


Figure 7
Graph of particle size of A1 formulation

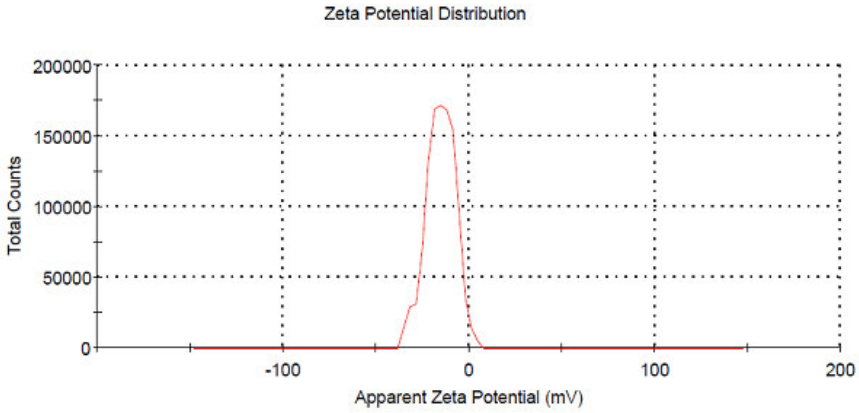
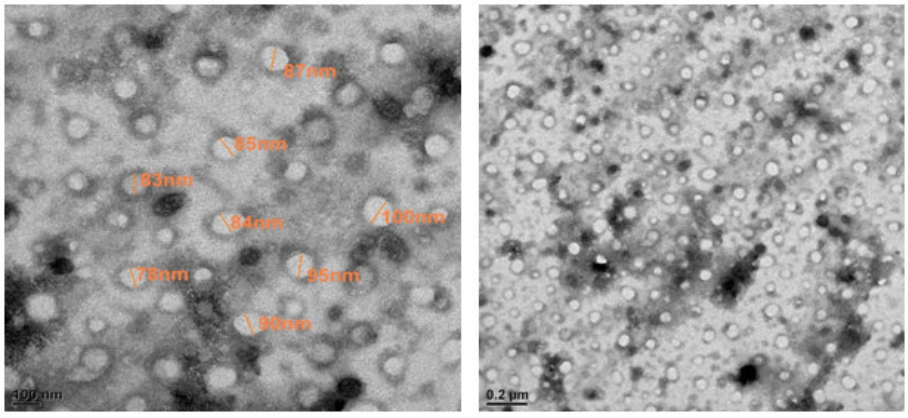


Figure 8
Graph of Zeta Potential of A1 Formulation

Droplet size, polydispersity index (PDI) and ζ-potential Shown in Table.4 .The optimized formulation A1 has particle size 111.4nm hence the system is in nano size range. PDI is 0.22 (figure 7) Polydispersity index (PDI) measurement is the parameter which shows the uniformity in globule size distribution. The minimum is the PDI the maximum is the uniformity in the globule size distribution. PDI less than 0.5 indicates homogenous distribution, while a greater than 0.5 indicates a higher heterogeneous dispersion ζ-potential Figure 8 was-15.3.The charge range from -30 to +30mv is reported typically as high zeta potential. The negative sign of the zeta potential indicated the formulations were negatively charged, and the magnitude of charge showed the stability of the formulation.

TEM images of A1 drug loaded SNEDDS formulation

TEM analysis Figure 9 revealed that emulsion droplet was almost spherical in shape. The droplet size of the diluted SNEDDS formulations was evaluated by TEM as described elsewhere.



From TEM images of formulation showed uniform spherical nanoemulsion droplet.

Figure 9
TEM images of A1 drug loaded SNEDDS formulation

DSC of drug, physical mixture and optimized formulation

Pioglitazone hydrochloride Figure 10 showed an endothermic peak at 201.6 ℃ with onset at 195.7℃ that corresponds to the melting point of pioglitazone hydrochloride.

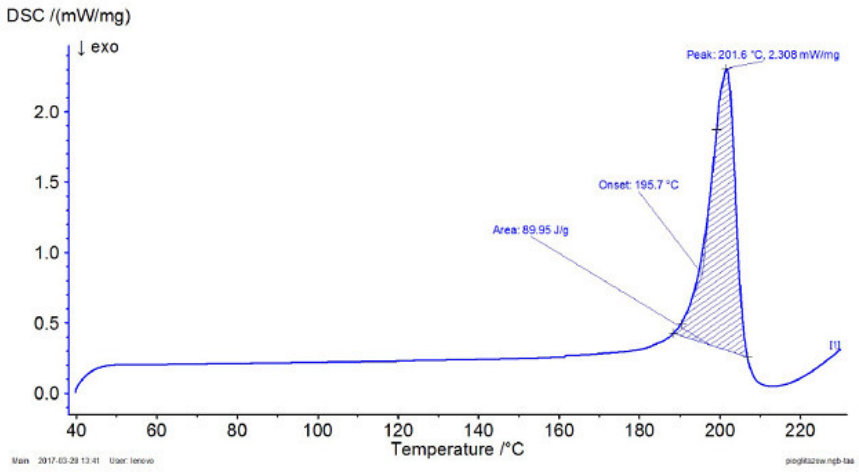


Figure 10
DSC thermogram of pioglitazone hydrochloride

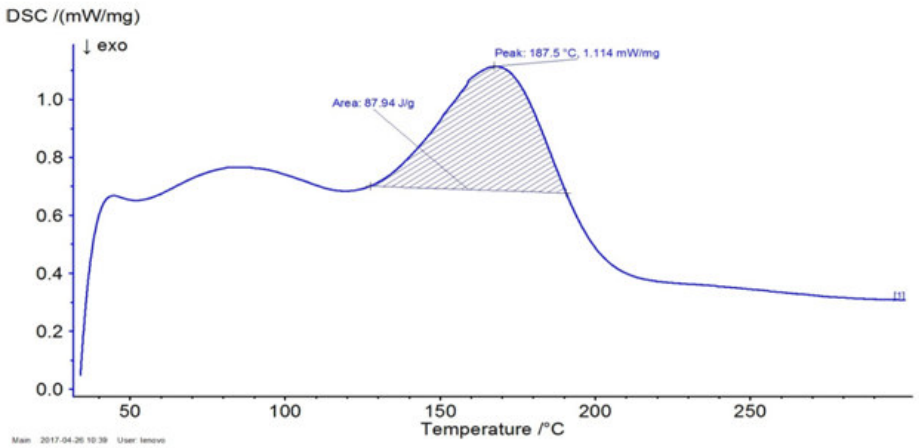


Figure10 a)
DSC thermogram of physical mixture

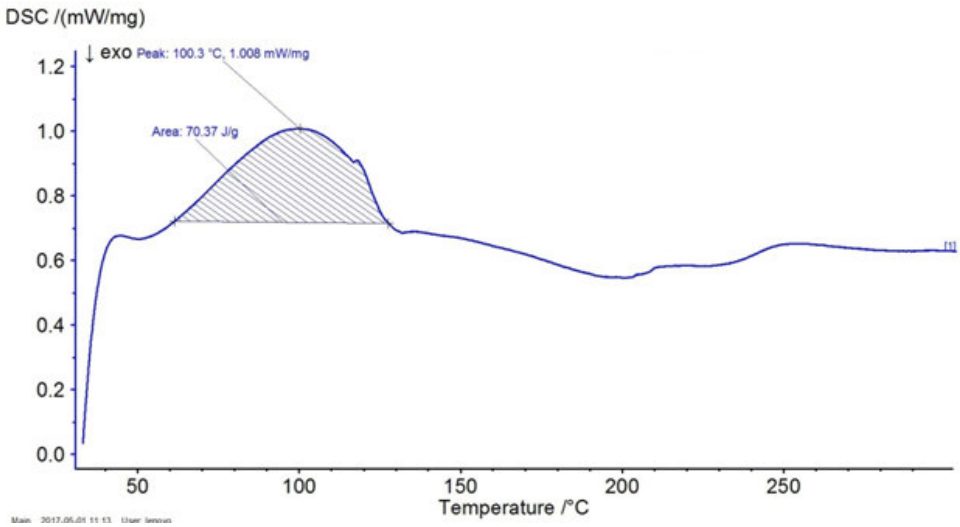


Figure 10 b)
DSC thermogram Of SNEDDS formulation A1

No endothermic peaks were found in the optimal formulations indicating that Pioglitazone hydrochloride must be molecularly dissolved in an amorphous state in the formulations.

CONCLUSION

The present study has demonstrated the potential utility of SNEDDS of pioglytazone with improved dispersibility, stability and bioavailability. The optimized SNEDDS formulation consist of capmul PG (5.53% w/w) kolliphour EL (61.4%w/w) and propylene glycol (45.7%w/w) showed improvement in dissolution and diffusion across the intestinal membrane compared to plain drug of pioglytazone. The synergistic effect of oil and surfactant enhances the absorption of the drug. Small droplet size produces large surface area for absorption and Improved permeation of the drug by SNEDDS is because presence of surfactant which reduces the interfacial tension. Thus SNEDDS is a promising approach for formulating poorly water soluble drugs and increasing bioavailability.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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