Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

A Novel solid-self microemulsifying drug delivery system of Pioglitazone Hydrochloride: Formulation, Development and Characterization by *in vitro* and *in vivo* techniques.

Rupali K. Kadam, Tushar S. Raut*, Deokar G. S, S. J. Kshirsagar
Department of Quality Assurance Techniques, MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik- 422003, India.

Received July 10, 2016; received in revised form Sept. 10, 2016; accepted Sept. 01, 2016
***Corresponding author E-mail address:** tushar.raut85@gmail.com*

ABSTRACT
The objective of present study was to develop solid self-microemulsifying drug delivery system (SMEDDS) for improving the delivery of a BCS class II antidiabetic agent pioglitazone HCL and evaluated by in-vitro, and in-vivo techniques. Screening of excipients was done by determining the equilibrium solubility of pioglitazone HCL in different oils, surfactants and cosurfactants, pioglitazone HCL showed highest solubility in coconut oil (oil), Tween 80 (surfactant), PEG 400 (Co-surfactant) and phase diagram was constructed to identify the self-microemulsification region. Liquid SMEDDS was prepared and was converted to S-SMEDDS by spray-drying of the liquid SMEDDS in a laboratory spray dryer using aerosil 200 as solid carrier. Solid state characterization of the solid SMEDDS was performed by SEM, DSC and X-ray powder diffraction. The optimized system possessed a mean globule size of 201.2 nm, PDI 0.457 and Zeta potential -0.975 mV. The prepared S-SMEDDS was filled in hard gelatin capsule shell size '0' and *in-vitro* dissolution study were performed, from dissolution study it was concluded that S-SMEDDS form of showed complete and faster dissolution as compared to marketed formulation of pioglitazone HCL (piosys 15 tablet). *In-vivo* performance of S-SMEDDS was evaluated in Wistar rat using plasma glucose level was determined by oral glucose tolerance test. The test formulation (1:1 C) showed significant reduction in plasma glucose level, after oral administration. A one month stability studies were performed (40^0C & 75% RH) showed no change in physical appearance and dissolution rate of the drug.

KEYWORDS
Pioglitazone HCL SMEDDS, coconut oil, spray drying, oral glucose tolerance test.

1. INTRODUCTION

Oral route is the easiest and most convenient route of drug administration, being non invasive and cost effective. But major problem encountered in oral formulations (as estimated more than 50 % of oral formulations are found to be poorly aqueous soluble), is low bioavailability, giving rise to further problems like, high inter and intra subject variability, lack of dose uniformity and finally leading to therapeutic failure. The challenging task is to increase the bioavailability of drugs. Number of technological strategies are investigated for improving bioavailability like solid dispersions, cyclodextrins, micronization etc. But Self-microemulsifying Drug Delivery System (SMEDDS) have gained exposure for their ability to increase solubility and bioavailability of poorly aqueous soluble drugs with reduction in dose and also drugs are protected from hostile environment in gut. (Goyal, U. et al, 2011)

Self microemulsifying drug delivery system(SMEDDS) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants that have a unique ability of forming fine oil-in-water (o/w) micro emulsions upon mild agitation followed by dilution in aqueous media, such as GI fluids. SMEDDS spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. (Shukla, J.et al, 2010)

Pioglitazone HCl, a widely prescribed anti diabetic drug belongs to class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. Its oral absorption is dissolution rate limited and it requires enhancement in the solubility and dissolution rate for increasing its oral bioavailability. Chemically pioglitazone HCL is 5-(4-[2-(5-ethylpyridin-2- yl) ethoxy] benzyl) thiazolidine -2, 4-Dione. It is a thiazolidinedione derivative which is useful in the treatment of non-insulin dependent diabetes mellitus (NIDDM). Is a off-white crystalline powder, relatively insoluble in water. The pKa of drug is 5.19 and half-life**:** 3- 7 hrs.

Figure 1. Structure of pioglitazone HCL

1.1. Mechanism of action of pioglitazone HCl

Pioglitazone HCL is used for the treatment of diabetes mellitus type 2 either alone or in combination with sulfonylureas. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue, and the liver. As a result, pioglitazone reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose, insulin and glycated hemoglobin in the bloodstream. Although not clinically significant, pioglitazone decreases the level of triglycerides and increases that of high-density lipoproteins (HDL) without

changing low-density lipoproteins (LDL) and total cholesterol in patients with disorders of lipid metabolism.

The objective of the present work was to formulate a self microemulsifying drug delivery system (SMEDDS) for pioglitazone HCL because pioglitazone HCL have poor aqueous solubility, Since there is a decrease in solubility with increase in pH and the half life being 3-5 hrs, so is incomplete absorption and eliminated quickly from the conventional tablets. Pioglitazone HCL being a non-polar drug and cannot effectively break down the lattice structure of water and hence its aqueous solubility is low. The oral delivery of such drugs is frequently associated with low bioavailability which leads to high intra and inter subject variability and a lack of dose proportionality. So, study proposed to formulate a lipid-based system of pioglitazone hydrochloride to enhance its dissolution rate to achieve optimum oral bioavailability by using novel SMEDDS systems.

2. MATERIALS AND METHODS

2.1. Materials

Pioglitazone HCL was obtained as gift sample from Mylan Ltd., Sinnar. Coconut oil, Tween 80, PEG 400, aerosil 200 was purchased from Thomas Baker, Mumbai. Other reagents were of analytical-reagent grade. Marketed formulations of pioglitazone HCl tablet (Piosys 15) were purchased from the local drug store in Nashik city after checking their manufacturing license number, batch number, production and expiry date.

2.2. Preparation of pioglitazone HCL liquid SMEDDS

Liquid SMEDDS of pioglitazone HCL was successfully developed by determining solubility of pioglitazone in various oils, surfactants and co-surfactants and by constructing pseudo ternary diagram to identify micro emulsion region from that satisfactory composition was selected to prepare liquid SMEDDS.

Table 1 Composition of solid SMEDDS batches.

Sr. No.	Batch code	Oil (% w/w)	Smix (% w/w)
1	1:1 A	40.8	59.2
2	1:1 B	40.8	59.2
3	1:1 C	40.8	59.2
4	1:1 D	40.8	59.2
5	2:1 A	47.3	52.7
6	2:1 B	47.3	52.7

7	2:1 C	47.3	52.7
8	2:1 D	47.3	52.7

A series of SMEDDS formulations were prepared using Tween 80 and PEG 400 as the S/CoS combination and pioglitazone HCL. In all the formulations, the level of pioglitazone HCL was kept constant (i.e., 15 mg). Briefly, accurately weighed pioglitazone HCL was placed in a glass vial, and oil, surfactant, and cosurfactant were added. Then the components were mixed by gentle stirring and vortex mixing and were heated at 40 ºC on a magnetic stirrer, until pioglitazone HCL was perfectly dissolved. The mixture was stored at room temperature until further use. (Mahajan H. D. *et al*, 2011, Reddy S. M. et al, 2011)

2.3. Spray drying of drug loaded SMEDDS –adsorbent suspension

Solid SMEDDS were prepared by spray drying method using aerosil 200 as solid carrier (adsorbent), pioglitazone HCL in coconut oil, tween 80 and PEG 400 as SMEDDS. In the SMEDDS sufficient alcohol was added. Aerosil 200 was suspended in sufficient alcohol the SMEDDS and alcohol solution was added to above suspension under continuous stirring at 200-300 rpm by using magnetic stirrer. The trial batches were formulated using varying concentrations of oil, Smix and adsorbent concentration was taken as 1:0.25 ratio (liquid SMEDDS: aerosil 200). (Hussain A. A. *et al*. 2014)

Table 2. Parameters for spray drying

Sr. No.	Batch	Inlet temperature (^{0}C)	Outlet temperature (^{0}C)	Feed rate (mL/min)	Aspirator (NM^3 /hr)
1	1:1 A	65.00	55.00	3.0	35.00
2	1:1 B	75.00	65.00	3.0	35.00
3	1:1 C	75.00	65.00	4.0	30.00
4	1:1 D	65.00	55.00	4.0	35.00
5	2:1 A	65.00	55.00	4.0	30.00
6	2:1 B	65.00	55.00	3.0	30.00
7	2:1 C	75.00	65.00	3.0	30.00
8	2:1 D	75.00	65.00	4.0	35.00

Atomisation air pressure (6.5 kg/cm^2) and Vacuum (-65 mm Wc) was same for all batches. In spray drying method drug loaded SMEDDS was adsorbed onto particles of aerosol 200 to make a immediate release solid SMEDDS formulations.

2.4. Evaluation of prepared solid SMEDDS

2.4.1. Percentage practical yield

Percent practical yield of solid SMEDDS is calculated using following formula:

$$\text{Percentage practical yield} = \frac{\text{Amount of solid SEDDS obtained (g)} \times 100}{\text{Theoretical amount (g)}} \quad \text{..... (A)}$$

2.4.2. Percentage drug content

Specific amount of S-SMEDDS theoretically equivalent to 15mg of drug weighed and dispersed in 100 ml of AR grade ethanol and sonicated for 10 min then concentration was determined by UV at 269 nm The percentage drug entrapment can be calculated by using following formula:

$$\text{Percentage drug entrapment} = \frac{\text{Practical drug content} \times 100}{\text{Theoretical drug content}} \quad \text{..... (B)}$$

2.4.3. Micromeritic properties of S-SMEDDS

1) Bulk density
2) Tapped density
3) Angle of repose
4) Carr's Compressibility Index
5) Hausner ratio

2.4.4. Determination of Emulsification time

In order to determine the emulsification time (the time needed to reach the emulsified and homogeneous mixture, upon dilution), solid SMEDDS theoretically equivalent to 15mg of drug was added to 100 mL of water at 37ºC with gentle agitation using magnetic stirrer at 100 rpm. The formulations were assessed visually according to the rate of emulsification and the final appearance of the emulsion. (Nawale R. B. *et al,* 2013, Madan J. R. *et al*, 2011) The formulation was visually assessed using the following grading system:

Grade A: clear or bluish appearance (within 1 min)
Grade B: Bluish white (within 1 min)
Grade C: Milky emulsion (within 2 min)
Grade D: Greyish white (longer than 2 min)

2.4.5. *Determination of Turbidity*

Nepheloturbidimetric evaluation was done to monitor the growth of emulsification. Solid SMEDDS theoretically equivalent to 15 mg of drug weighed and added to 0.1N HCl (100 mL) under continuous stirring (50 rpm) on magnetic stirrer at ambient temperature and the increase in turbidity was measured using Systronics nephelo-turbidometer. (Patil P. *et al,* 2004, Madan J. R. *et al*, 2011)

2.4.6. *Factorial design*

The aim of present work was to achieve optimized formulations determining the effects of some important factors and their interactions during the process preparation on SMEDDS physiochemical properties. Meanwhile the SMEDDS were being processed; the impact of different factors had been evaluated by making changes in their quantity. Finally, three of the most significant factors had been chosen as the independent variables. In the next step, for determining the low and high levels of each factor, some formulations were made. According to a 2^3 factorial design and considering these two variables, an experimental matrix was performed.

2.4.6.1. 2^3 Full factorial Experimental Design Layout.

Variables for Experimental Designs

Independent variable	Dependent variable
X1= Inlet temperature	Y1= Percent drug release
X2= Feed rate	Y2= Percent yield
X3= Aspirator	

Table 3. Coded Levels Translated in Actual Units

Coded levels	**Actual values**		
	X1 (^{o}C)	**X2 (mL)**	**X3 (%)**
-1	65	3	30
+1	75	4	35

2.4.7. Droplet size

This is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as the stability of the emulsion. Droplet size and distribution was determined by Malvern Zetasizer. About 1.0 gm sample was dissolved in double distilled water and agitated to get homogeneous dispersion. Mean globule diameter and distribution was determined (Bhagwat A. D. et al., 2012).

2.4.8. Zeta potential study

The micro particles were dispersed in distilled water. The S-SMEDDS were diluted with a ratio of 1:10000 (v/v) and mixed for 1min with cyclo mixer. This dispersion was filled in zeta cell and placed in the Zeta sizer and the zeta potentials were determined. (Madan J. R. *et al.*2014)

2.4.9. Surface morphology by scanning electron microscopy (SEM)

The SEM was carried out to characterize the surface morphology of solid SMEDDS and this was done by using Scanning electron microscope at 20 kV at ICON LAB Mumbai (Yi Y. *et al.*2008)

2.4.10. Differential scanning calorimetry (DSC)

For this study, the powder sample (1 to 5 mg) was packed in an aluminum pan and crimped. The crimped pan was placed in the sample cell along with an empty pan as a reference. Temperature was increased to 300 ^{0}C from 0 ^{0}C at a rate of 10 ^{0}C /min. (Yi Y. *et al.*2008).

2.4.11. X-ray powder diffractometry (XRD)

X-ray powder diffractometry method was used to investigate the effect of solubilisation of drug in SMEDDS and solidification by spray drying process on crystallinility of drug. The XRD patterns of drug powder and solid SEMDDS were recorded by using Panlytical Xpert Pro XRD at SAIF Punjab.

2.4.12. In-vitro drug release

The *in-vitro* drug release study was carried out as per IP 2014 and basket type dissolution apparatus (Electrolab, India) was used with some modifications. In 900 ml of 0.1 N HCL dissolution medium which was maintained at 37±0.5^{0}C and rotated at 75 rpm, solid SMEDDS theoretically equivalent to 15 mg of drug were filled in size "0" hard gelatin capsule. Appropriate aliquots were withdrawn at suitable time interval (5, 10, 15, 20, 25, 30, 35, 45 min.). After suitable dilutions aliquots were analyzed using UV spectrophotometer at 269 nm.

2.4.13. In vivo estimation of Blood glucose level

*2.4.13.*1 *Oral glucose tolerance test (OGTT)*

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee (IAEC), and all experiments were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Healthy Wistar rats of both sexes weighing 200 to 250 gm included in this study. Animals were randomly divided into four groups, six animals in each group.

Group I: Vehicle control (normal saline).
Group II: Diabetic control (Glucose 2g/Kg).
Group III: Diabetic + Pioglitazone HCL [standard control (15 mg/Kg)]
Group IV: Diabetic + S-SMEDDS Formulation [test control (93 mg/Kg)]

Rats were dosed following an overnight fast for 16 hrs. 1 gm/kg glucose administered to each rat of glucose control group and blood samples were collected from rat tails and the glucose level was checked using blood glucose meter after 0, 30, 60, 120 min. Then glucose solution containing pure drug (1 gm of glucose+15 mg pioglitazone HCL) was administered to each of the rat orally of standard group The Plain drug suspension prepared by using 0.5% w/v CMC Na because pioglitazone HCL is virtually insoluble in water and glucose level were checked. The experiment was again repeated by administering samples containing glucose and optimized S-SMEDDS formulation in the same dose to test control. (Nipun T.S et al)

2.4.13.2. Determination of Urine Sugar and ketones

Capillary tube method of urine collection was used to collect the urine sample from the diabetic rats (Hayashi and Sakaguchi, 1975). The rat was held with one hand and the lower part of the abdomen, around the urinary bladder, was pressed with the thumb and the third finger of the other hand of the collector, to cause urinary excretion. The urine excreted was immediately collected directly into two capillary tubes held between the index and middle fingers. After collection, Urinary blood glucose and ketone level was determined using by reagent based strips. Only one drop of urine is kept on the test strips and the color change on the test strip was evaluated to determine the concentration of glucose by referring to the color index on test strip container [The Institutional Animal Care and Use Committee (IACUC) standard procedures. (Sabina E. P et al, 2014)

2.4.14. Stability studies

The stability studies were carried out as per the ICH guidelines. The solid SMEDDS formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies. Stability study of the best formulation was carried out for one month at 40ºC and 75% RH. Samples were charged in stability chambers (Remi, Mumbai, India) with humidity and temperature control. After one month, the formulation was analyzed for appearance, drug content and *in-vitro* drug release. (Hyma P. *et al,* 2014)

3. RESULT AND DISCUSSION

3.1. Evaluation of prepared solid SMEDDS

3.1.1. Percentage practical yield

The yield of all S-SMEDDS batches was found from 45%- 90.22%. Batch 2:1A showed highest yield 90.22%.

3.1.2. Percentage drug content

The content of drug in various S-SMEDDS formulation varies from 85.56 % to 98.10%. Batch 1:1C showed maximum drug content (98.10%). However, it was showed that as the surfactant increased in composition and oil decreased in composition of SMEDDS formulation, drug content was proportionally increased.

3.1.3. Micromeritic properties of S-SMEDDS

Table 4. Flow properties of solid SMEDDS batches

Batch	Bulk Density (gm/ml)	Tapped Density(gm/ml)	Angle of Repose(θ)	Hausner Ratio	Carr's Index (%)
1:1 A	0.248±0.02	0.283±0.02	24.2±0.3	1.10±0.02	9.0±0.28
1:1 B	0.325±0.02	0.371±0.03	23.02±0.17	1.14±0.03	12.5±0.30
1:1 C	0.265±0.04	0.296±0.02	20.10±0.2	1.11±0.01	10.47±0.3
1:1 D	0.299±0.05	0.319±0.03	21.6±0.3	1.06±0.03	9.37±0.25
2:1 A	0.248±0.03	0.269±0.03	19.2±0.10	1.08±0.05	7.8±0.2
2:1 B	0.288±0.02	0.252±0.05	20.1±0.15	1.14±0.02	9.7±0.15
2:1 C	0.294±0.03	0.324±0.02	22.8±0.3	1.10±0.02	9.2±0.15
2:1 D	0.277±0.02	0.317±0.01	21.03±o.3	1.14±0.01	12.9±0.3

All these results indicated that powder blend showed good flow properties.

3.1.4. Determination of Emulsification time

Batch 1:1C showed very less time (59 sec) for emulsification. From above it can be concluded that increase in amount of surfactant decreases emulsification time. Also increase in amount of adsorbent decreases emulsification time which may be due to available surface area in contact with liquid decreases.

Formulations 1:1B, 1:1C, 2:1B 2:1C were having slightly bluish appearance and come under grade A, while 1:1A, 1:1D, 2:1A and 2:1D formulation having bluish white appearance and come under grade B.

3.1.5. Determination of Turbidity

It was observed that there is increase in turbidity with increase in amount of adsorbent. It was least for the formulation 1:1 C (154 NTU) where it is possible that the droplet size is least which decreased the turbidity.

3.1.6. Factorial design

3.1.6.1. Percentage Drug Release (% Drug release)

A. Analysis of Variance for Experimental matrix (ANOVA)

Table 5. Analysis of Variance (ANOVA) of % release

Component	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF

Intercept	9.60	1	0.070	9.37	9.82	
A-Temperature	0.27	1	0.070	0.042	0.49	1.00
C- Aspirator	0.26	1	0.070	0.032	0.48	1.00
AC	-0.25	1	0.070	-0.47	-0.026	1.00
BC	-0.11	1	0.070	-0.34	0.11	1.00

Table 6. Experimental design of the optimization step for formulations

Run	Factor 1 Inlet Temperature (ºC)	Factor 2 Feed rate (ml/min)	Factor 3 Aspirator (%)	Response 1 Release %	Response 2 Yield %
1	65.00	3.0	35.00	96.84	73.31
2	75.00	3.0	35.00	98.38	45.0
3	75.00	4.0	30.00	98.85	65.7
4	65.00	4.0	35.00	96.66	58.27
5	65.00	4.0	30.00	83.64	90.22
6	65.00	3.0	30.00	72.35	75.26
7	75.00	3.0	30.00	95.5	77.08
8	75.00	4.0	35.00	96.4	65.69

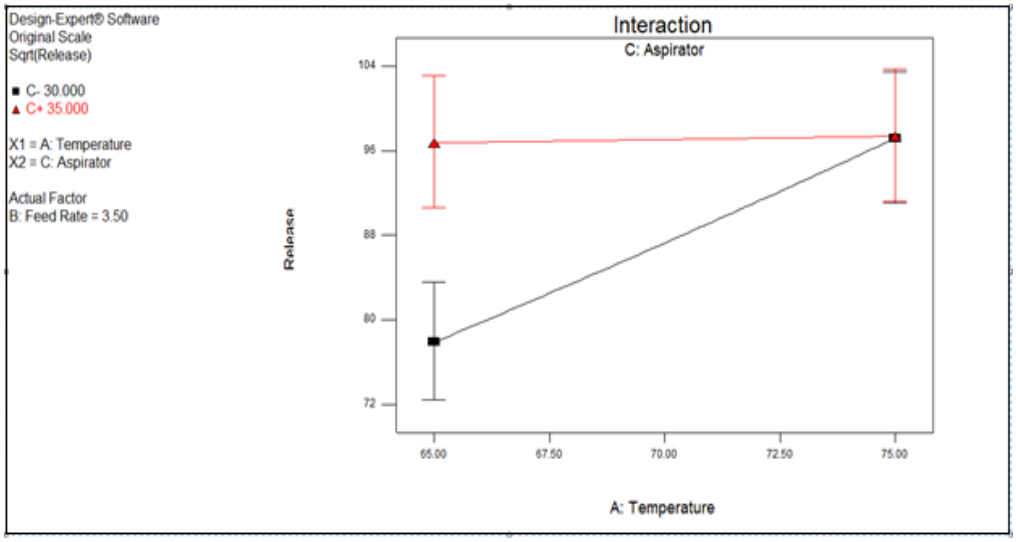

Figure 2. Interaction plot of % Drug release

In interaction graph, inlet temperature and aspirator is evaluated by keeping release as a response, in which temp increases the release pattern linearly while Aspirator doesn't show much significant impact on release.

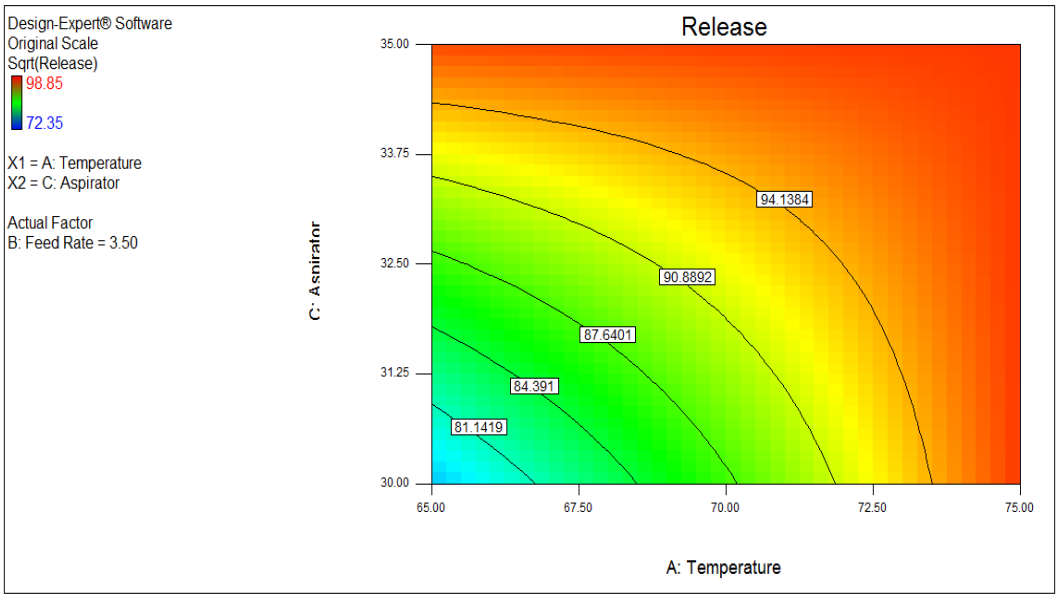

Figure 3. Contour plot of % Drug release

Counter plot was plotted to evaluate impact of Temperature and Aspirator on release of drug, and it was concluded that Increase in temperature and increase in Aspirator will lead to show gradual increase in release.

3.1.6.2. % Yield

A. Analysis of Variance for Experimental Matrix (ANOVA) of % yield

Table 7. Analysis of Variance for Experimental Matrix (ANOVA)

Component	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	8.26	1	0.068	8.07	8.45	1.00
A- Temperature	-0.33	1	0.068	-0.52	-0.15	1.00
C-Aspirator	-0.51	1	0.068	-0.70	-0.32	1.00
ABC	0.48	1	0.068	0.29	0.67	1.00

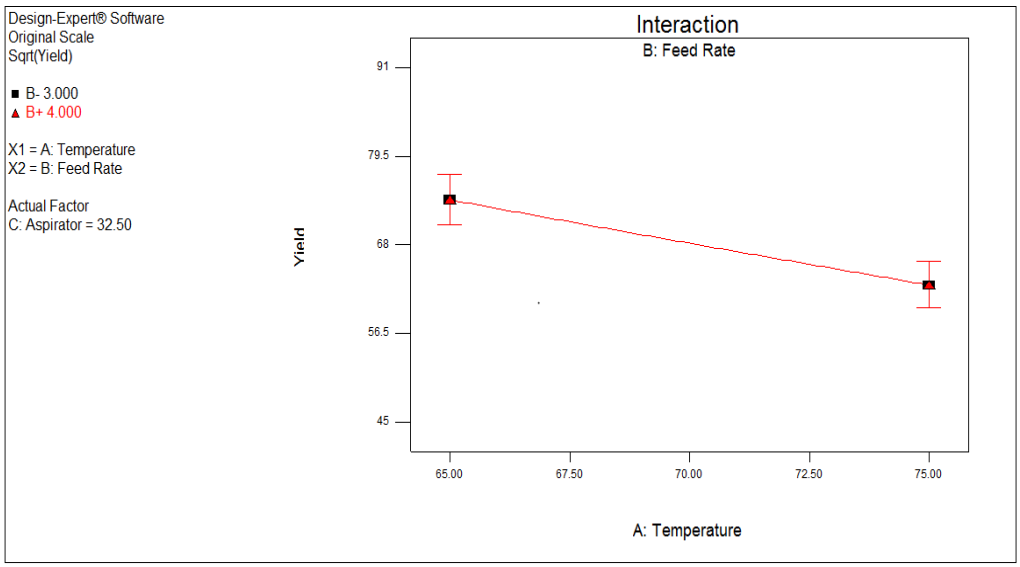

Figure 4. Interaction plot of % yield

All interaction occurs when the response are different depending on settings of two factors. Plot appears with one parallel line. This shows as temperature increase the Yield was decrease, additionally feed rate and temperature does have significant impact on Yield of S-SMEDDS.

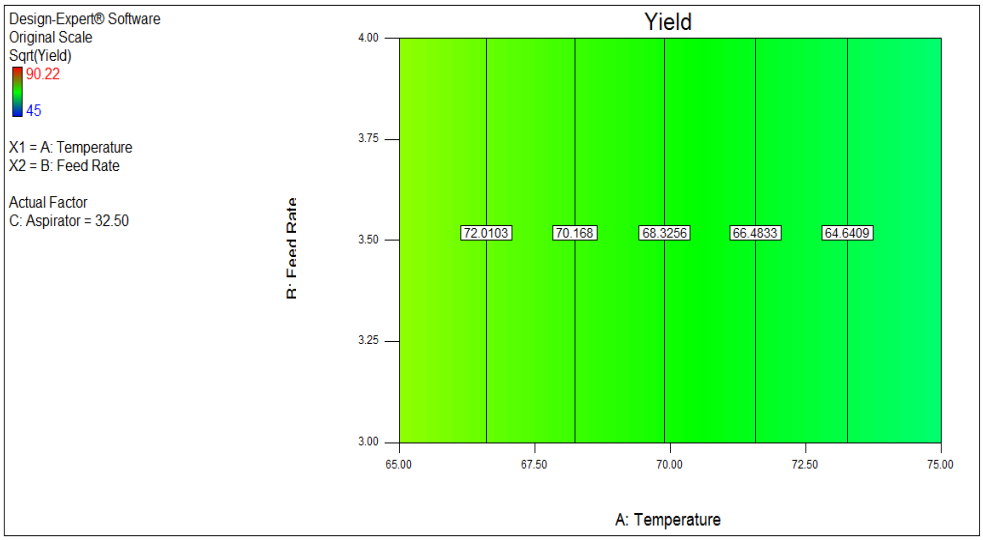

Figure 5. Contour plot of % Drug release

From the graph it was observed that Yield was increased at lower temperature and higher Feed rate.

3.1.6.3. Graph of Desirability Function

According to the final results, this program suggests some formulations and also predicted their responses containing a probability factor named 'Desirability' that ranged between 0-1 that the

most presumable answer would be the nearest to 1 and from graph optimized formulations are showing Desirability 1.

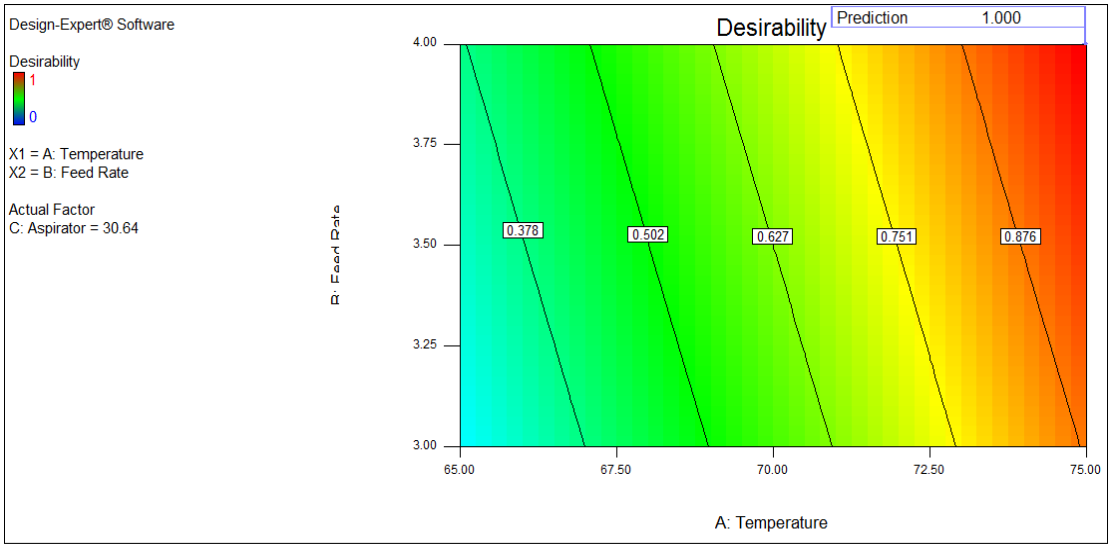

Figure 6. Graph for Desirability Function

From the graph it was observed that desirability value increases as Temperature increases and also increase in Feed rate. Desirability was observed 1at highest inlet temperature (75°C) and at high feed rate (4mL/min).

Table 8. No. of Solutions Obtained DE 7.0

Number	Temperature	Feed rate	Aspirator	Release	Yield	Desirability
1	75.00	3.10	30.48	98.89	63.61	1.00

The optimized solution obtained from the model was formulated and the results are performed in the triplicates for determination of % Drug release, % yield. The solution was found to complies all specifications hence considered optimized.

Table 9. Optimized parameters for spray drying

Sr. No.	Parameters	Specification
1	Inlet temp.	75 ^{0}C
2	Outlet temp.	55 ^{0}C
3	Aspirator speed	30 NM^3 /hr
4	Atomisation air pressure	6.5 kg/cm^2
5	Feed rate	4 ml/min
6	Vacuum	-65 mmWc

2.4.6.4. Evaluation of optimized batch

Table 10. Evaluation of Physical Parameters of Optimized Formulation

Sr. No.	Parameters	Result
1	% yield	65.70±0.01%
2	% drug release	98.93±0.05%
3	% drug content	98.10±0.011
4	Bulk density	0.265(gm/ml)
5	Tapped density	0.296 (gm/ml)
6	Angle of repose	20.10±0.2 (θ)
7	Hausner's ratio	1.11
8	Compressibility index	10.47%
9	Emulsification time	57.6±0.52 sec
10	Turbidity	153.33 ± 2.0NTU
11	Refractive Index	1.45

3.1.8. Droplet size

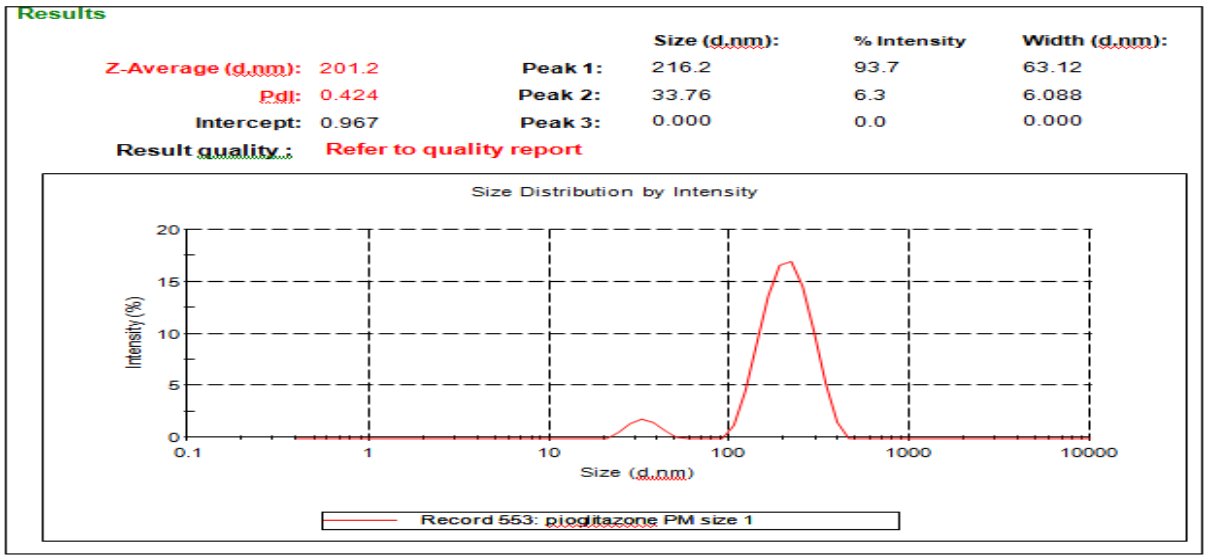

Figure 7. Droplet size of optimized S-SMEDDS formulation

Mean droplet size of reconstituted S-SMEDDS was found to be 201.2 nm with polydispersity index 0.457**.** S-SMEDDS showed polydispersity index less than 1, indicating uniform distribution of droplets throughout formulation.

3.1.9. Zeta potential study

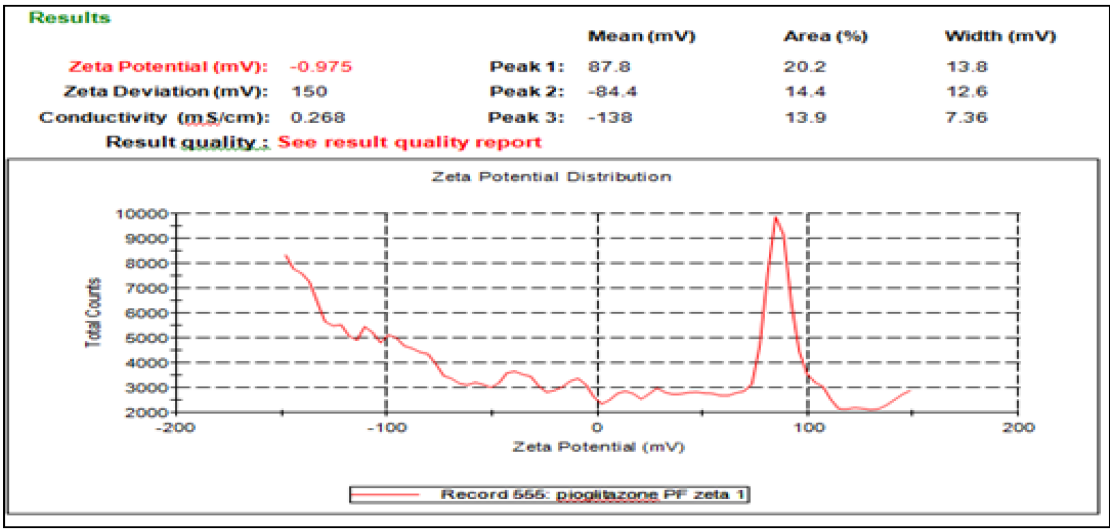

Figure 8. Zeta potential of optimized S-SMEDDS formulation

zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. Zeta potential of the system negative (−) mV, which indicates the droplets of microemulsion have negative charge. The zeta potential of optimized 1:1 C formulation was found to be −0.975and confirms the formulation of 1:1 C S-SMEDDS was stable.

3.1.10. Surface morphology by scanning electron microscopy (SEM)

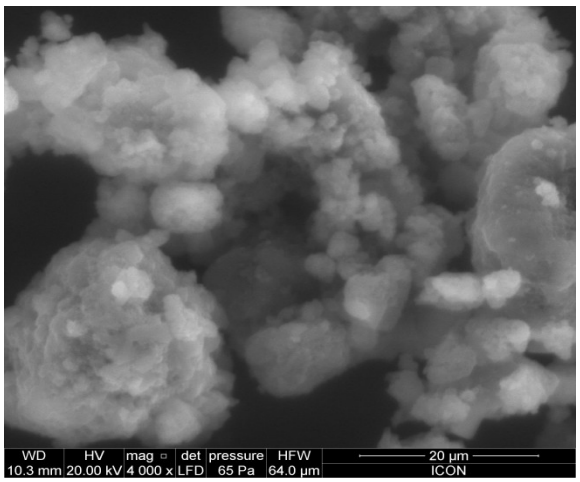

Figure 9. SEM of optimized S-SMEDDS formulation

SEM study showed that S-SMEDDS appeared as smooth surfaced S-SMEDDS particles, indicating that the liquid SMEDDS is adsorbed or coated inside the pores of Aerosil 200 with a lesser amount of aggregation.

3.1.11. Differential scanning calorimetry (DSC)

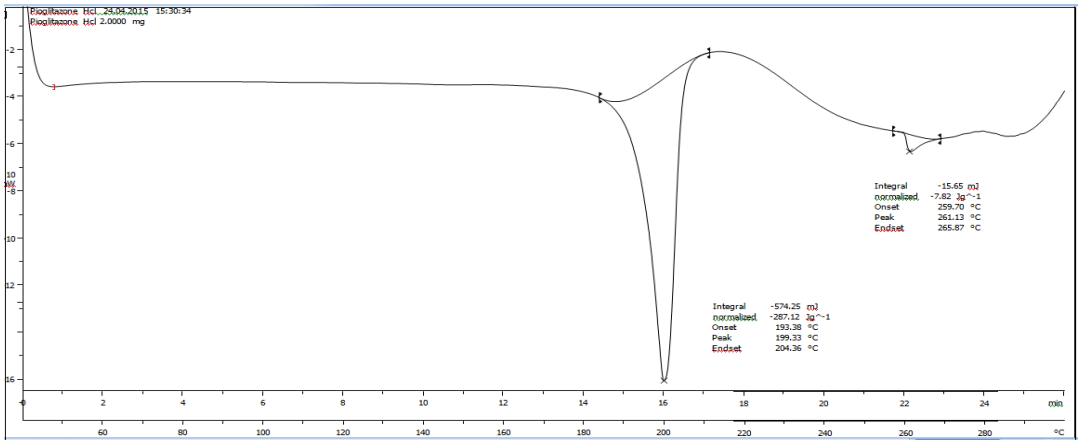

Figure 10. DSC thermogram of Pioglitazone HCL

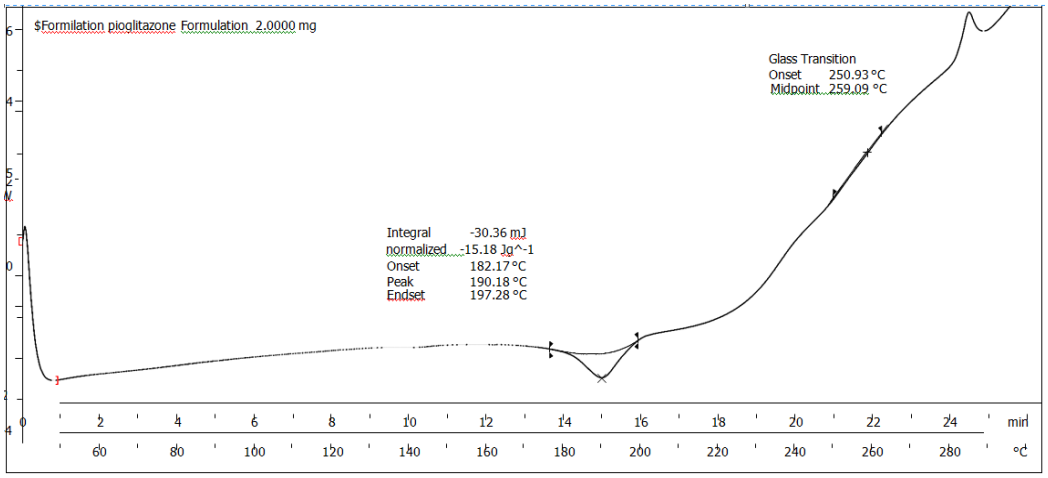

Figure 11. DSC thermogram of S-SMEDDS

DSC curves of pioglitazone HCL shows sharp endothermic peak at near about 199.33°C. The S-SMEDDS exhibit retained small endothermic peak at 190.18°C for pioglitazone HCL and it may be due to solubilization of pioglitazone HCL in S-SMEDDS.

3.1.12. X-ray powder diffractometry (XRD)

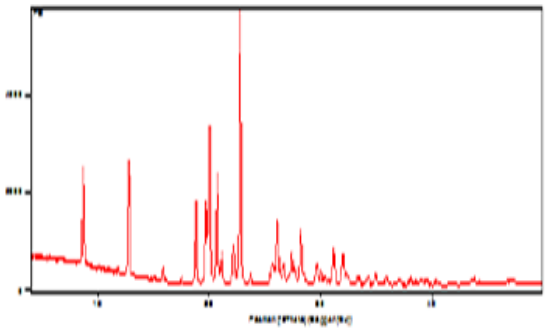

Figure 12. XRD pattern of pioglitazone HCL

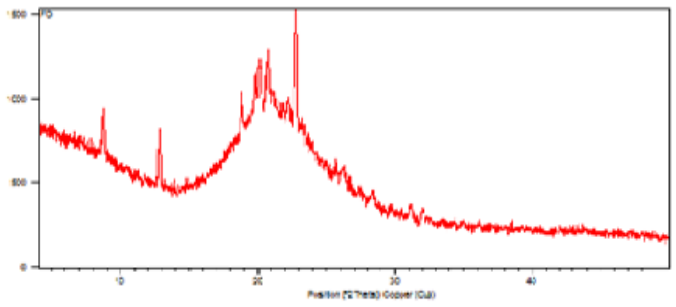

Figure 13. XRD pattern of S-SMEDDS

The X-ray powder diffraction pattern of pure drug shows crystallinities. S-SMEDDS revealed that the intensity of the peak for the pure drug was sharp, when it was incorporated into the self emulsifying system, then the peak intensities was decreased. It indicates that the crystalline nature of drug was changed to amorphous or must be present in molecularly dissolved state after formulation into S-SMEDDS.

3.1.13. In-vitro drug release

It was observed from the above results of *in-vitro* drug release study of all batches for 45 min, highest drug was released at 25 minute of dissolution in all batches. Also it was found that as the amount of adsorbent increases, drug release decreases. Increase in amount of Smix increases drug release to some extent. Maximum drug release was found in batch 1:1C. It may be due to amount of oil must be optimum for self emulsification. Also the proportion of oil with Smix sufficient to form droplets of smaller size so that maximum surface area available to get in contact with dissolution medium and hence maximum release.

Also *in vitro* dissolution study of marketed tablet of pioglitazone HCL (Piosys 15) was studied with S-SMEDDS in HGF & 0.1 N HCL. So, from study it was found that drug release from S-SMEDDS is higher than marketed formulation and drug release rate depend on droplet size of emulsion. It could be suggested that the S-SMEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of marketed tablet. Thus, this greater availability of dissolved pioglitazone HCL from the S-SMEDDS formulation could lead to higher absorption and higher oral bioavailability.

Table 11. *In-vitro* Dissolution Data and % of the Optimized Formulation

Sr. No.	Time (min)	% release (Optimized formulation)	% release (Marketed tablet)
1.	5	51.16	79.11
2.	10	83.85	86.92
3.	15	89.85	94.23
4.	20	93.71	98.05
5.	25	98.93	94.88
6.	30	96.89	90.96
7.	35	95.65	88.85
8.	45	96.26	86.42

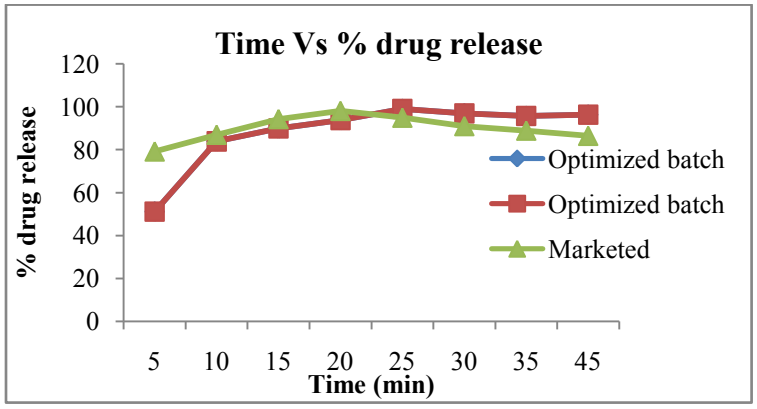

Figure 14. Cumulative drug release profile of marketed tablet and solid SMEDDS

Table 7. Dissolution Kinetic Models for Optimized Formulation

Formulation code	R^2				
	Zero order	**First order**	**Higuchi**	**Hixon Crowell**	**Korsmeyer Peppas**
1:1C	0.784	0.747	0.864	0.748	0.907

Release kinetic model was found to be zero order. Mechanism of drug release was found to be Korsemayer Peppas with highest R^2 value. n value was found to be 0.459 this proves that the formulated solid SMEDDS shows immediate release with diffusion mechanism.

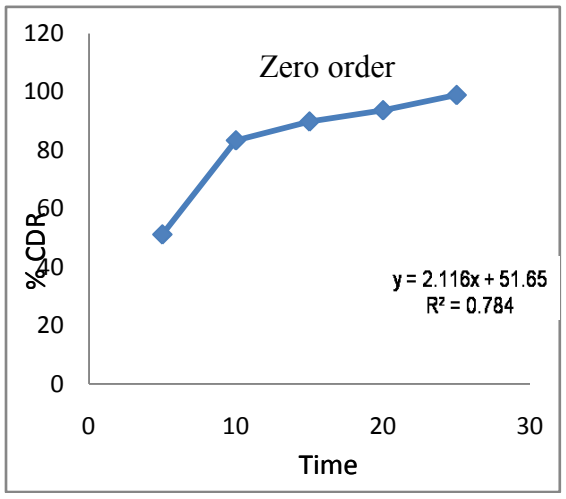

Figure 15. Graph of Zero order release for optimized batch

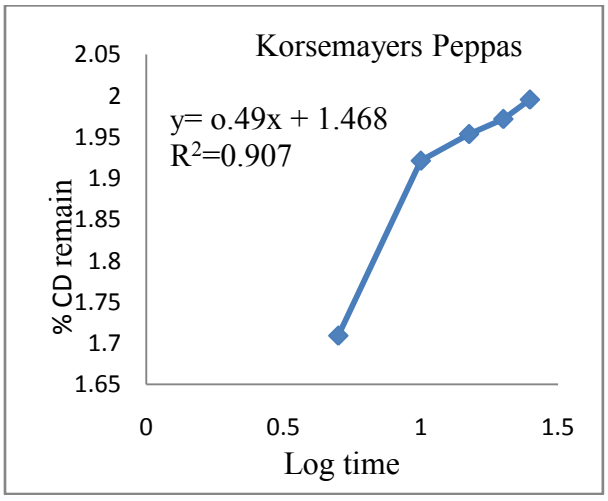

Figure 16. Graph of Korsemayer Peppas release for optimized batch

3.1.14. In vivo estimation of Blood glucose level

3.1.14.1. Oral glucose tolerance test (OGTT)

Results are expressed as statistical analysis using one way ANOVA, followed by student Newman Keul's Multiple Test; P<0.05 implied significance. Administration of glucose leads to significantly elevation of fasting blood glucose level. The supplementation of solid SMEDDS formulation (standard control) at 30 min improved the glucose tolerance in the fasted rats. Serum glucose level was lowered significantly (P < 0.05) at 60 and 90 min and varied significantly (P< 0.01) lowered at 120 minutes. Standard control (pure pioglitazone HCL) also showed significant hypoglycemic effect after 60 and 90 min of treatment. Test control showed significant reduction in blood glucose level than standard control.

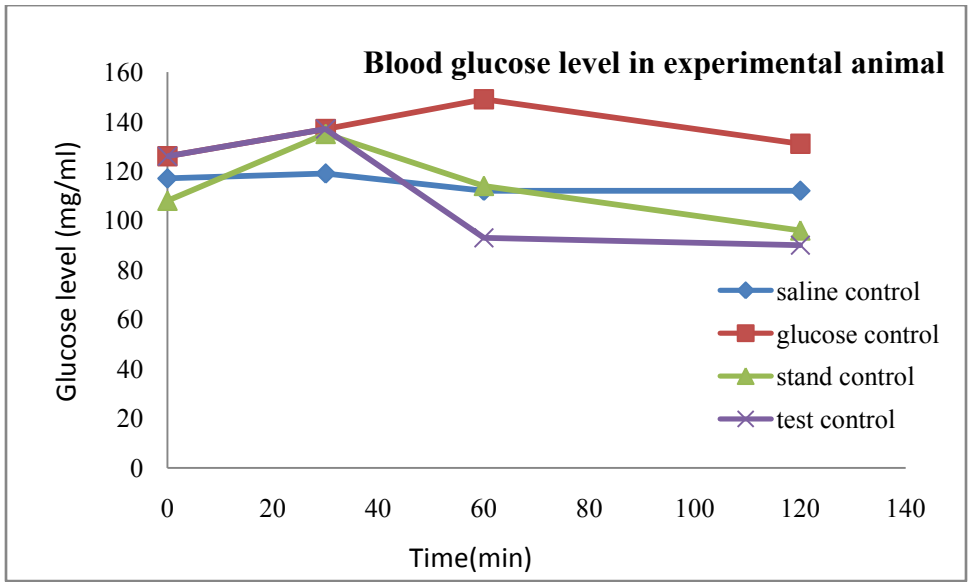

Figure 17. Fasting blood glucose level (mg/dl) in rats

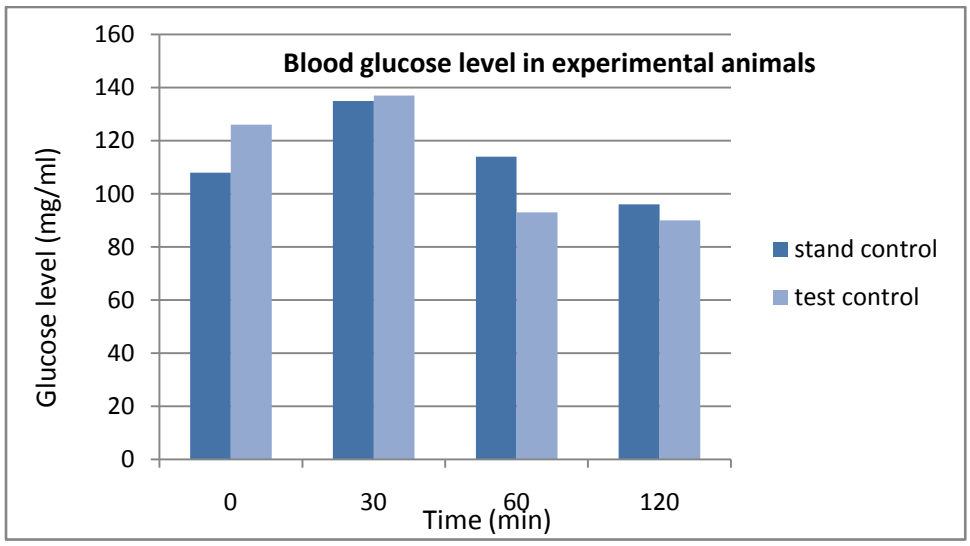

Figure 18. Effect of solid SMEDDS on blood glucose level

3.1.14.2 Determination of Urine Sugar and ketones

Reagent based strips showed no colour change. So, all groups showed absence of urine glucose and ketones.

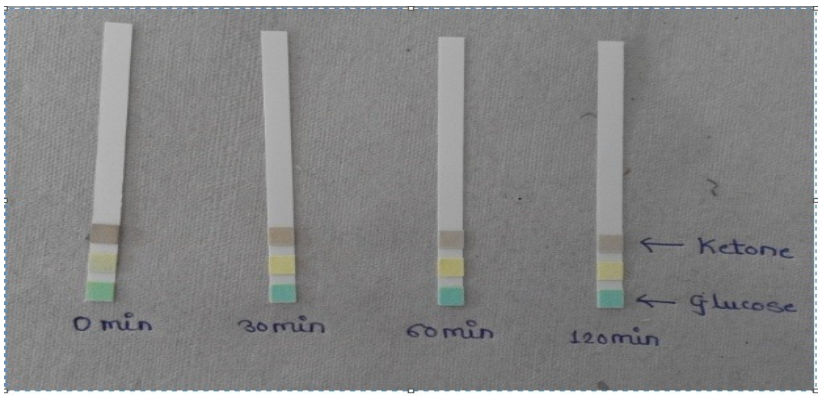

Figure 19. Estimation of urine glucose and ketones

3.1.15. Stability studies

Generally, solid SMEDDS formulations are put into hard gelatin capsules as the final dosage form. The entire system has a very limited shelf life owing to its powder characteristics. Thus, the developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form. The results of the evaluation test conducted on stability samples. There was no significant change in the drug content, drug release (98.93%). It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There were also no significant changes in the appearance, or microemulsifying property. Thus, these studies confirmed the stability of the developed formulation.

Table 12. Effect of Temperature and Humidity on Optimized Batch

Formulation	Appearance	% drug content	% drug release
Optimized batch	Fine white powder	97.90±0.01	98.85±0.01

From the above tabulated results it can be concluded that there was no significant changes in the optimized batch of drug release & drug content.

4. CONCLUSION

Study concluded that S-SMEDDS of pioglitazone HCL could be developed using coconut oil, Tween 80 as surfactant, and PEG 400 as co surfactant in 1:1 ratio. The optimized formulation evaluated for drug content, DSC, SEM. This showed a minimum droplet size, zeta potential, good emulsification property and highest drug content also higher in-vitro drug release and better control of plasma glucose level in rats. From dissolution study it was concluded that S-SMEDDS formulation showed complete and faster drug release as compared to marketed tablet i.e., Piosys

15 tablet. Hence adsorption process using Aerosil 200 as solid carrier may efficiently formulate S-SMEDDS which enhance dissolution rate and concomitantly bioavailability. However stability studies indicates no significant degredation in developed S-SMEDDS and its compatibility with hard gelatin capsules. So study indicates that the potential use of S-SMEDDS for the oral delivery of pioglitazone HCL can be an alternative to improve its systemic availability.

5. ACKNOWLEDGEMENT

The author is thankful to MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik for providing facilities to carry out the research work.

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