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Formulation and characterization of pioglitazone HCl self emulsifying drug delivery system

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ABSTRACT

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. The objective of the study was to screen various oils, surfactants and cosolvents and to formulate and evaluate self emulsifying drug delivery system (SEDDS) with selected vehicle. The highest solubility was observed in labrafac, tween 80 and propylene glycol. Then the feasibility of formulating Pioglitazone HCl SEDDS was evaluated and the effect of dilution on the dissolution rate and dissolution efficiency of Pioglitazone HCl was analyzed. A comparative release study was carried out in SGF and 1 % SLS. Pioglitazone dissolution was rapid from SEDDS and was higher when compared to pure drug. The rate and extent of release of Pioglitazone HCl from stable SEDDS (F1) was high in 1% SLS when compared to SGF. The FTIR spectra proved that there was no chemical interaction between excipients and drug.

Key words: Pioglitazone SEDDS, Labrafac, Oleic acid, Labrasol, Tween 80.

INTRODUCTION

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and having a short half-life are eliminated quickly from the blood circulation. For such drugs an incomplete release of the drug at prominent site for the absorption of drugs, will lead to lower bioavailability [1]. Several approaches have been developed to enhance the release rate by increasing the solubility. One such approach is the use of self emulsifying drug delivery systems (SEDDS).

SEDDS are defined as isotropic solutions of oil, surfactant, co-surfactant and drug which form o/w emulsions when introduced into aqueous phases under gentle agitation [2, 3]. SEDDS rapidly disperses in GI fluids to form droplets with gastrointestinal motility itself [4]. Therefore, it presents the drug in solution in small droplets of oil. Fine oil droplets should empty rapidly from the stomach and promote wide distribution of the drug throughout the gastrointestinal tract. For drugs subject to dissolution rate limited absorption, self-emulsified systems may offer an improvement in both the rate and extent of absorption [5]. Lipid based solid self emulsifying drug delivery systems require filling into soft or hard gelatin capsules. It would therefore be of interest to incorporate these vehicles into a powder to produce solid dosage forms. Recently, pellets containing a self emulsifying mixture are prepared by extrusion/spheronization. Solid-state microemulsion preconcentrate for the delivery of cyclosporine is prepared by coating the pre-microemulsion with an enteric coating material [6]. Similarly, solvent evaporation method is used to

prepare tocopheryl nicotinate tablets utilizing calcium silicates as the adsorbing agent [7]. Such methods often require elaborate processing and instrumentation.

Pioglitazone hydrochloride acts as an agonist at peroxisome proliferator-activated receptors (PPARs) in target tissues for insulin action, such as adipose tissue, skeletal muscle, and liver. It enhances tissue sensitivity to insulin by Activating the PPAR-g that regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization [8]. Pioglitazone hydrochloride has all the requisite characteristics suitable for developing SEDDS dosage form. 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 hydrochloride being a non-polar drug and cannot effectively break down the lattice structure of water and hence its aqueous solubility is low. Different techniques are used to enhance the aqueous solubility of drugs including salt formation [9], co crystal formation [10], addition of cosolvents [11], hydrotropes [12], surface active agents and SEDDS [13] and ionic liquids [14]. As Pioglitazone hydrochloride is delivered by oral route, there are number of investigations deal with improved solubilization techniques for Pioglitazone. [15, 16].

The objective of the present research work was to perform solubility studies in various oils and surfactants along with the cosolvents and to develop an optimum SEDDS for Pioglitazone HCl. The generated data was used to develop phase diagrams. The solubility of Pioglitazone hydrochloride in the mixed oils and surfactants was calculated using numerical methods and the accuracy of different methods are discussed by comparing the percentage deviations between calculated and experimental solubilities. SEDDS were characterized for size and zeta potential and *in vivo* studies were performed to assess the bioavailability.

MATERIALS AND METHODS

Pioglitazone hydrochloride was obtained as gift sample from Lupin Pharma Ltd., India. Labrafac PG (Propylene glycol dicaprylocaprate), Labrasol (Caprylocaproyl macrogol-8 glycerides EP, Caprylocaproyl polyoxyl-8 glycerides NF), and Transcutol HP (Diethylene glycol mono ethyl ether) were obtained as gift samples from Gattefosse, France. Oleic acid, Tween 80 and Propylene glycol were purchased from Merck Specialities Pvt. Ltd., Mumbai. PEG 400 was purchased from SD Fine chemicals, Mumbai. Other chemicals were of analytical grade.

2.1 Saturation solubility study:

The solubility of Pioglitazone hydrochloride in various oils, surfactants and co-solvents were determined in one ml of selected vehicles present in capped vial containing excess of drug (50 mg). The mixture was heated at 40-50°C in water bath to facilitate the solubilization. Then the mixtures were transferred to orbital shaking incubator and maintained at 25°C for 24 hr. After reaching equilibrium, vials were centrifuged at 4000 rpm for 10 min and insoluble Pioglitazone hydrochloride was $^{\text{discarded}}$ by filtration using membrane filter (0.45 µm). Then the concentration of Pioglitazone hydrochloride was assessed by a sensitive, accurate and rapid high-performance liquid chromatography with UV-visible detection (HPLC-UV) method. Isocratic separation of Pioglitazone was carried out using a reversed-phase Phenomenex C18 (250 mm × 4.6 mm, 5µm) column with mobile phase consisting of methanol, 30 mM ammonium acetate buffer (pH adjusted to 3.5 with ortho-phosphoric acid) and acetonitrile in the ratio 50:30:10 (v/v) and quantified at 221 nm with a run time of 10.0 min. Linearity was constructed between 10 – 60 µg/ml concentration range.

2.2 Visual Observations and construction of ternary phase diagram

A series of formulations were prepared with the drug using varying concentrations of oil (20-40%), surfactant (20-60%), and co-solvent in the glass test tube and mixed by vortexing until a clear solution was obtained. The mixture was stored at room temperature until used. A visual test was performed to assess the self micro emulsification properties is formulation 50 µl was introduced into distilled water in glass beaker at 30°C and the contents were mixed gently with magnetic stirrer at 100 rpm. The tendency to emulsify spontaneously and also the final appearance was monitored. The phase diagrams were constructed identifying the micro emulsion region using Tri plot v1- 4 software.

2.3 Formulation design of Pioglitazone hydrochloride SEDDS

Various formulations of SEDDS were developed using different ratios of various oils, surfactants and co-solvent with constant amount of Pioglitazone hydrochloride (45mg). A series of formulations were prepared with varying

concentrations of Labrafac PG, oleic acid (20-40%), polysorbate 80 and propylene glycol (20-60%) as shown in table 1.0 and 2.0.

Table. 1. Formulations containing Labrafac PG

Formulation code / Ingredient(mg)	F1	F2	F3	F4	F5	F6
Pioglitazone hydrochloride	45	45	45	45	45	45
Labrafac PG	84	84	84	84	84	84
Propylene glycol	252	280	308	336	364	392
Tween 80	224	196	168	140	112	84

Table. 2. Formulations containing Oleic acid

Formulation code / Ingredient(mg)	F7	F8	F9	F10	F11	F12
Pioglitazone hydrochloride	45	45	45	45	45	45
Oleic acid	84	84	84	84	84	84
Propylene glycol	252	280	308	336	364	392
Tween 80	224	196	168	140	112	84

2.4. *In vitro* evaluation

2.4.1. Determination of Emulsification Time

The emulsification time, i.e., the time required for the pre-concentrate to form micro emulsion upon dilution, was monitored by visual observation. The emulsification time of SEDDS was determined by dispersing formulation in 500 ml of purified water at 37^{o}C in USP-dissolution apparatus type-II (paddle type) at 50 rpm.

2.4.2. Thermodynamic stability studies of Pioglitazone hydrochloride SEDDS

Each formulation of SEDDS containing Pioglitazone hydrochloride was taken in a test tube and was diluted with 10ml of distilled water at 37^{o}C.

Freeze Thawing

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at – 4°C for 24 hours followed by thawing at 40°C for 24 hours.

Centrifugation

Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.

2.4.3. Determination of droplet Size

The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption [17]. The mean size and polydispersity index of emulsion globules were determined by zeta seizer Nano ZS (Malvern Instruments, Malvern, UK) which utilizes photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) in measuring droplet size. Light scattering was monitored at 25°C at a 90° angle. The dispersed formulations were measured after dilution (1:10000) with distilled water to produce the required count rate (50-200) to enable accurate measurement.

2.4.4. Determination of Zeta Potential

The zeta potential of diluted SEDDS was determined using zeta sizer (Nano ZS, Malvern Instruments, Malvern, UK). Charge on emulsion droplets and their mean zeta potential values (±SD) were obtained. The SEDDS were diluted with a ratio of 1:10000 (v/v) with distilled water and mixed for 1min with cyclo mixer.

2.4.5. *In vitro* release studies:

The release of Pioglitazone hydrochloride from the SEDDS formulation was determined according to USP dissolution apparatus type-II. To permit the quantitative drug release from SEDDS formulation, 900ml of SGF (0.1N HCl) without enzymes were added [18]. The SEDDS formulation filled in hard gelatin capsule was placed in the dissolution medium and was agitated at 50rpm at 37 ± 0.5 ^{o} C. At predetermined time intervals, 5ml of the samples were withdrawn at 5, 10, 15, 20, 30, 45, 60 min and samples were filtered through 0.45m Membrane filters

the drug concentration was determined in at 221nm. The volume removed was replaced each time with fresh dissolution medium to maintain sink conditions. Cumulated released amounts were plotted as a function of time.

2.4.6. Effect of dilution on globule size:
In order to simulate *in vivo* dilution behavior, effect of dilution volume by distilled water and other aqueous media on droplet size was measured. Triplicate samples of selected pre-concentrates were diluted in different ratios like 1:50, 1:100 and 1:1000 and then the size was measured.

2.4.7. Drug-excipients compatibility study
This was carried out by FT-IR analysis of pure drug, oils and surfactants (Labrafac PG, Oleic acid and tween80) and their formulations to study the possible interaction between drug and polymers.

RESULTS AND DISCUSSION

3.1. Solubility of Pioglitazone in Various Oils and Surfactants
The self-emulsifying formulations consist of dissolved drug in a mixture of oils, surfactants and co-solvents. The mixture should be a clear, monophonic liquid at ambient temperature and should have good solvent properties to allow solubilization of drug in solution. The solubility of Pioglitazone in various surfactants and oils are shown in Fig. 3.0. From the results it was observed that, among all the oils, Labrafac PG and oleic acid shown to have higher solubility. Among all surfactants, Tween 80 showed better solubility and from three co-solvents propylene glycol showed better solubility for Pioglitazone hydrochloride. The standard graph for solubility study was performed in Methanol: Buffer: Acetonitrile (50:40:10). The wave length selected was 221nm with a run time of 10 min. The R^2 obtained was 0.998 (Fig. 1.0) at linearity range was 10-60µg/ml. The retention time for Pioglitazone Hydrochloride was 7.07 min (Fig. 2.0).

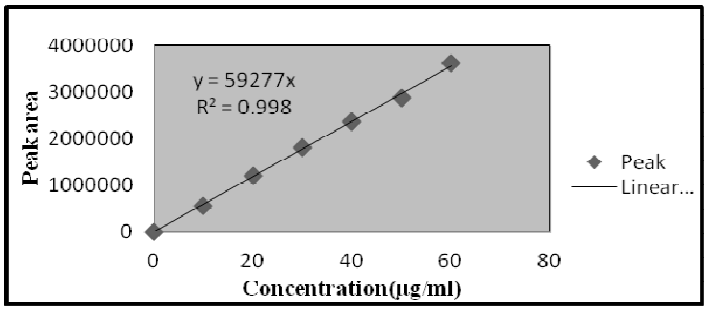

Figure. 1. Standard graph of Pioglitazone Hydrochloride in HPLC

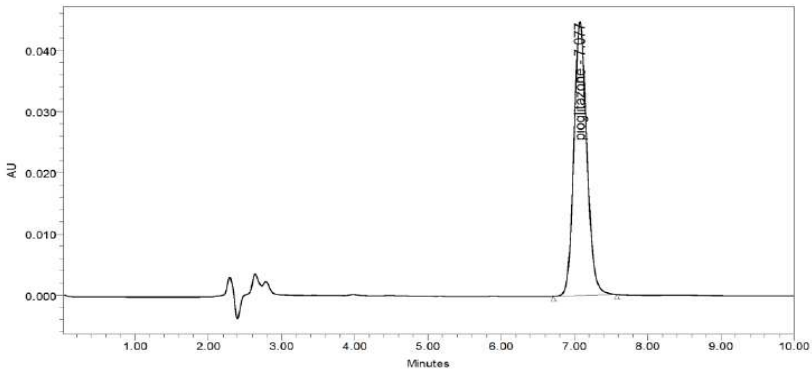

Figure. 2. HPLC spectrum of Pioglitazone hydrochloride

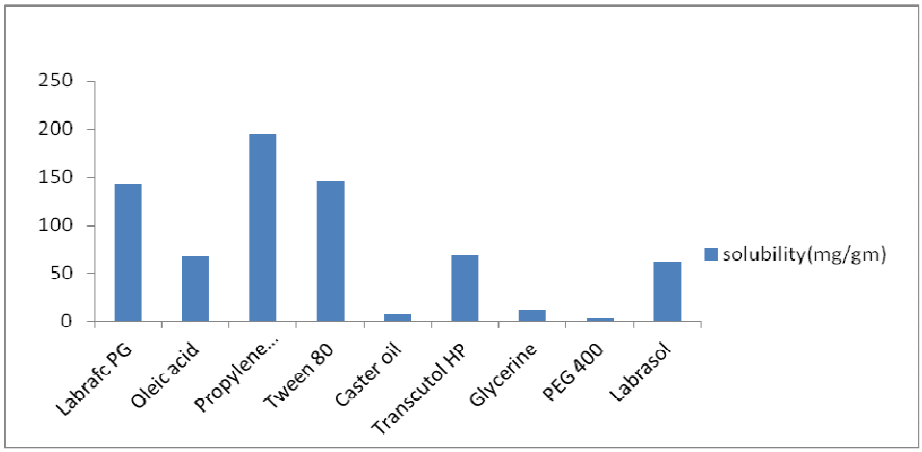

Figure. 3.0. Solubility of Pioglitazone hydrochloride in various oils, surfactants and co- solvents

3.9.2 Visual observations and construction of ternary phase diagram:

From the above chosen oils, surfactant and co-solvents were taken in different ratios (Table 3.0 and 4.0) for the construction of ternary phase diagrams to know the emulsion and micro-emulsion domains such that at particular concentration of oil, surfactant and co-solvent ratios, a stable self-emulsifying formulation is formed.

The Self emulsification process is affected on the concentration of Tween 80 and propylene glycol and their ratio. Micro emulsion region was appeared at surfactant concentration (20-65%) of W/W, co-solvent concentration at (20-60%). The phase diagram representing that series I and series II both showed a narrow micro emulsifying domain (Fig. 4.0 and 5.0). So the concentration of oil, surfactant and co-solvent was selected in these domains for the study. From the phase diagrams, it was observed that self emulsifying region increased with increasing concentrations of surfactant or combination of surfactant and co-surfactant. Efficiency of self-emulsification was good when the surfactant concentration was increased.

Table. 3. Composition of combinations containing Labrafac PG,Tween 80 and Propylene glycol (oil,surfactant,co-solvent)

Formulation code	Labrafac PG (%)	Tween 80 (%)	Propylene Glycol (%)	Visual Observation	Inference
1A1	50	25	25	turbid	Not stable
1A2	50	20	30	turbid	Not stable
1A3	50	10	40	turbid	Not stable
1B1	30	35	35	turbid	Not stable
1B2	30	20	50	turbid	Not stable
1B3	30	15	65	turbid	Not stable
1C1	15	40	45	**Transparent**	Stable
1C2	15	20	65	**Transparent**	Stable
1C3	15	10	75	turbid	Not stable
2AI	50	25	25	turbid	Not stable
2A2	50	30	20	turbid	Not stable
2A3	50	40	10	turbid	Not stable
2B1	30	35	35	turbid	Not stable
2B2	30	50	20	turbid	Not stable
2B3	30	65	15	turbid	Not stable
2C1	15	45	40	**Transparent**	stable
2C2	15	65	20	**Transparent**	stable
2C3	15	75	10	Turbid	Not stable

Table. 4. Composition of combinations containing Oleic acid,Tween 80 and Propylene glycol (oil,surfactant,co-solvent)

Formulation code	Oleic acid	Tween 80	Propylene glycol	Visual Observation	Inference
3A1	50	25	25	turbid	Not stable
3A2	50	20	30	turbid	Not stable
3A3	50	10	40	turbid	Not stable
3B1	30	35	35	turbid	Not stable
3B2	30	20	50	turbid	Not stable
3B3	30	15	65	turbid	Not stable
3C1	15	40	45	Transparent	Stable
3C2	15	20	65	Transparent	Stable
3C3	15	10	75	turbid	Not stable
4AI	50	25	25	turbid	Not stable
4A2	50	30	20	turbid	Not stable
4A3	50	40	10	turbid	Not stable
4B1	30	35	35	turbid	Not stable
4B2	30	50	20	turbid	Not stable
4B3	30	65	15	turbid	Not stable
4C1	15	45	40	Transparent	Stable
4C2	15	65	20	Transparent	Stable
4C3	15	75	10	turbid	not stable

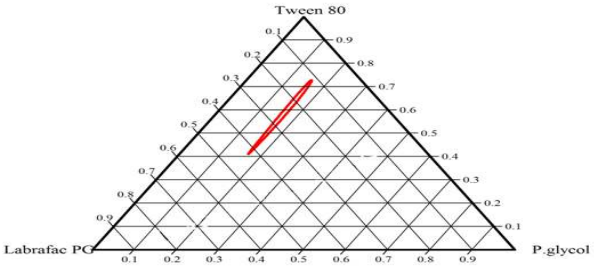

Figure. 4. Ternary phase diagram of series 1A, 1B, 1C and 2A, 2B, 2C consisting of Labrafac® PG, Tween 80 and propylene glycol.

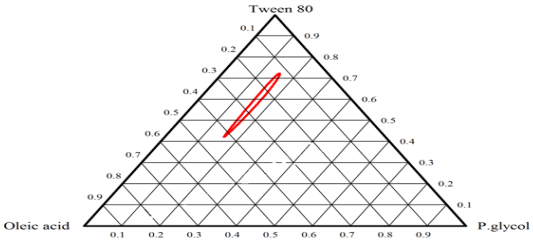

Figure. 5. Ternary phase diagram of series 3A, 3B, 3C and 4A, 4B, 4C consisting of Oleic acid, Tween 80 and Propylene glycol (red circle consisting of region where stable micro emulsion exist)

3.9.3. *In vitro* evaluation

3.9.3.1 Determination of emulsification time

The rate of emulsification is an important index for the assessment of the efficiency of emulsification. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. The SEDDS should disperse completely and quickly when subjected to aqueous media under mild agitation. The Evaluation of emulsification time for the prepared formulations was conducted in 0.1N HCl.

Table. 5. Emulsification times of various SEDDS formulations

Formulation code	Emulsification time (sec) (in 0.1 N HCl)	Formulation code	Emulsification time(sec) (in 0.1 N HCl)
F1	85	F7	98
F2	80	F8	90
F3	115	F9	95
F4	132	F10	126
F5	144	F11	125
F6	178	F12	129

Table 5 shows that, as the concentration of surfactant increases, the spontaneity of emulsification process increased. This may be due to capacity of Tween 80 in reducing the interfacial tension and that the co-solvent further lower the interfacial tension between O/W interface and also influenced interfacial film curvature, may show impact on spontaneous emulsification process.

3.9.4 Thermodynamic stability studies of Pioglitazone Hydrochloride SEDDS

The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SEDDS formulations. Pioglitazone hydrochloride SEDDS were diluted with aqueous medium and centrifuged at 15,000 rpm for 15 minutes and formulation were observed visually for phase separation. Formulations were subjected to freeze cycle (-4°c for 24hrs followed by +40°c for 2 days) and results are summarized in Table 6.

Table. 6. Thermodynamic stability studies of Pioglitazone hydrochloride SEDDS

Formulation code	Centrifugation	Freeze thaw method	
		-4°C for 2 days	+40°C for 2 days
F1	No phase separation	No change	No change
F2	No phase separation	No change	No change
F3	No phase separation	No change	No change
F7	No phase separation	No change	No change
F8	No phase separation	No change	No change
F9	No phase separation	No change	No change

The six formulations shown in Table 6.0 undergo neither phase separation nor precipitation of drug in micro emulsions after 24 hr. It representing that these formulations are resulting in stable micro emulsion upon dilution. But F4, F5, F10, F11 were showing phase separation upon centrifugation, F6 and F12 forming turbidity upon storage at room temperature with in 24 hr. If they form precipitation on storage, the drug release will be negatively affected. Hence the formulations which were stable for precipitation after study were selected for further investigation.

3.9.5 Droplet size analysis

SEDDS have the size range of 100-300 nm. The dispersed formulations were measured for droplet size after dilution (1:10000) to produce the required count rate (50-200) to enable the accurate measurement. The 6 formulations showed size below 300 nm (Table 7.0). The formulation F1 showed least size 76.91nm followed by F7 & F2. The polydispersity index was in the range of 0.1-0.4 indicating the uniformity. The formulations F1 and F7 showed no effect on dilution as represented in Table 8.0.

Table. 7. Mean droplet size, Poly dispersity index of various developed formulations of SEDDS.

Formulation code	Mean Dispersion Size(nm)	Poly dispersity index
F1	76.9 ± 2.3 nm	0.195
F2	105.8 ± 2.8nm	0.199
F3	111.1 ± 3.1 nm	0.206
F7	102.6 ± 3.6 nm	0.393
F8	124.2 ± 2.8 nm	0.486
F9	129.9 ± 2.5 nm	0.421

Table. 8. Effect of drug loading on size

Formulation code	1:100	1:1000
F1	81.4 ± 2.6 nm	89.2 ± 2.1nm
F7	124.6 ± 3.1 nm	127.4 ±3.8 nm

3.9.6 Zeta potential measurement

The emulsion stability is directly related to the magnitude of the surface charge. The Zeta potential of SEDDS were determined using Zeta sizer 3000 HAS (Malvern Instruments, Malvern, UK).

Table. 9. Zeta potential measurement of different formulations

Formulation code	Zeta potential (mv) (mean±SD)
F1	-29.3±0.34
F2	-15.9±0.21
F3	-9.08±0.39
F7	-21.3±0.51
F8	-17.86±0.32
F9	-2.24±0.24

The zeta potential results of the diluted SEDDS formulations are expressed in mean ± SD and shown in Table 9.0. Diluted SEDDS formulations F1, F7 have a significantly higher absolute zeta potential than other 4 formulations. This is may be due to small droplet with more surface area which has more charge density. This indicates that upon emulsification these formulations generate a more stable emulsion than the other SEDDS.

3.9.7 Physical stability of preconcentrate

F1 and F7 SEDDS were stable at room temperature for more than 12 weeks in SGF as shown in Table 10.0 with no significant effect on droplet size with aging (Fig 1.4, 1.5, 1.6 and 1.7).

Table. 10. Effect of age on particle size of different formulations

SEDDS	1st Week	4th Week	8th Week	12th Week
F1	82.45	79.4	86.14	89.47
F7	108.21	105.25	112.56	117.97

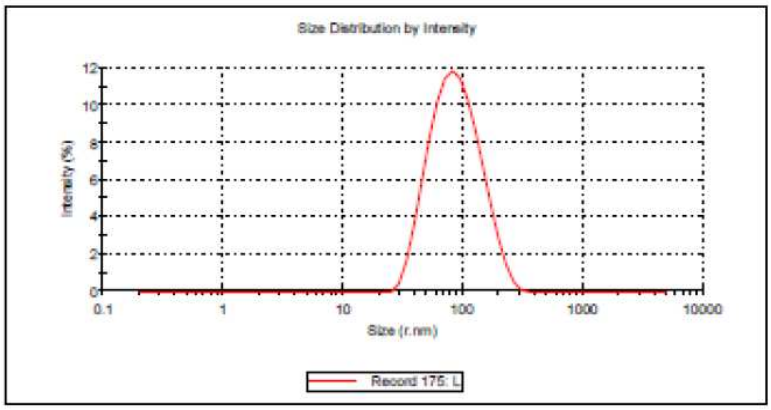

Figure 6.0. Size distribution study of F1 formulation by intensity (%)

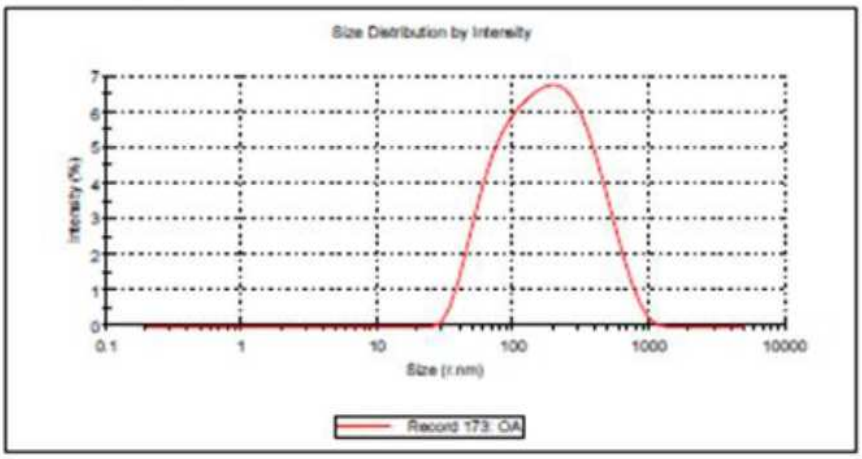

Figure. 7. Size distribution study of F7 formulation by intensity(%)

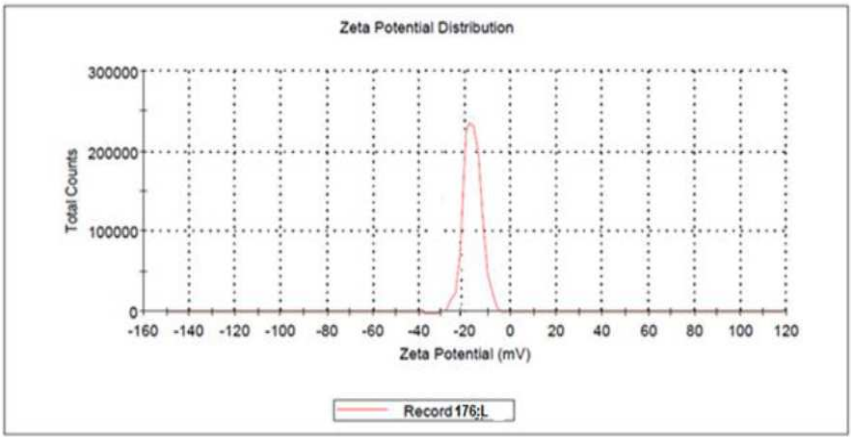

Figure. 8. Zeta potential report of F1 formulation

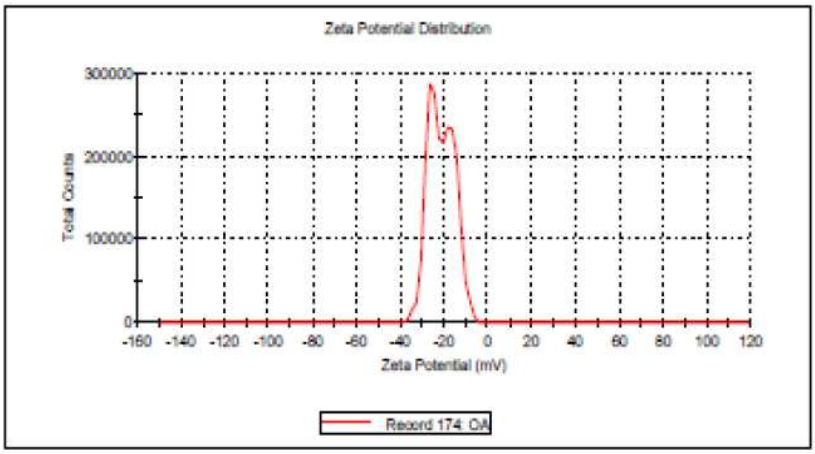

Figure. 9. Zeta potential report of F7 formulation

3.9.8 *In vitro* release studies

In vitro drug release studies were performed for stable SEDDS formulations and pure drug filled in hard gelatin capsules and was performed in simulated gastric fluid (pH 1.2) and in 1% SLS. The % cumulative drug release of

Pioglitazone hydrochloride from all SEDDS formulation was significantly higher than comparatively with pure Pioglitazone Hydrochloride. It indicates that the release rate of Pioglitazone hydrochloride from SEDDS was high due to the physical state (small droplet) that permits the faster diffusion of the drug into aqueous medium. Higher dissolution rates of formulations compared to pure drug is due to in formulation drug will be in dissolved form where as pure drug will be in crystalline form. Hence, more time was needed for drug release from crystalline form than from oil (Liquid or dissolved state) formulation in SGF and 1% SLS (Fig. 12.0 and Fig. 13.0). The release rate of SEDDS formulation F1 (Mean droplet size 76.91 nm) was faster followed by F7 (102.6nm) than remaining F2, F3, F8, F9. The decreased size of micro emulsion was increasing the release rate of drug this is may be due to decreased size and increase in surface area. The concentration of the drug in SGF and 1% SLS were calculated from the respective standard graph (Fig. 10.0 and 11.0).

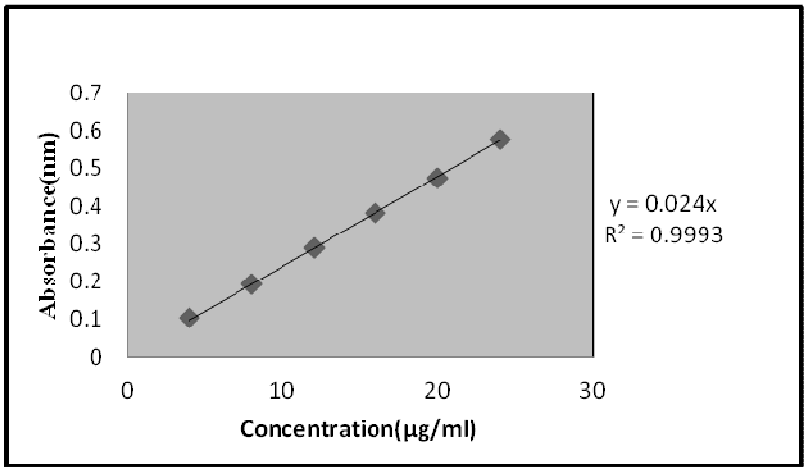

Figure. 10. Linearity plot of Pioglitazone HCl in 0.1N HCl (pH 1.2)

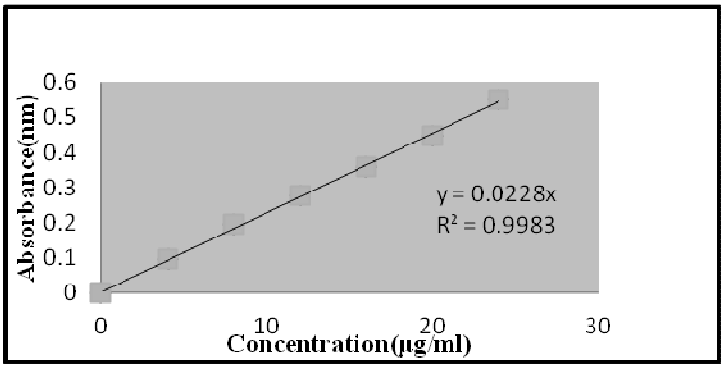

Figure. 11. Linearity plot of Pioglitazone Hydrochloride in 1% SLS

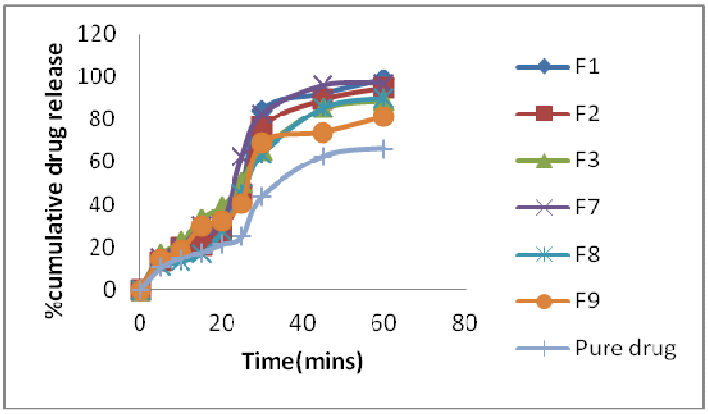

Figure. 12. Dissolution profile of various formulations in 0.1N HCl

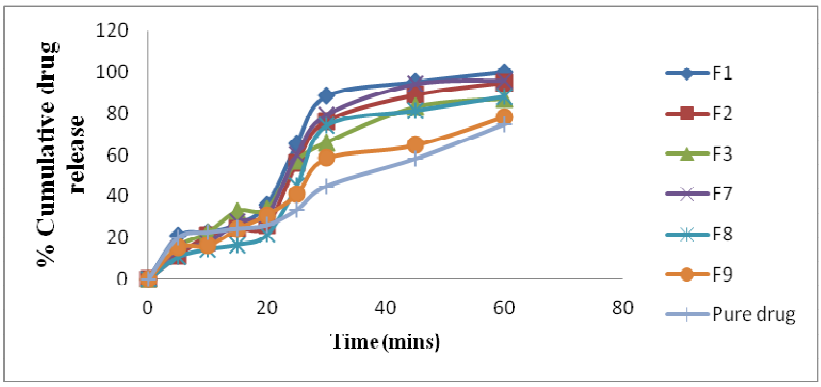

Figure. 13. Dissolution profile of various formulations in 1% SLS

3.9.9 FTIR (Fourier Transforms Infrared Spectroscopy) studies

FT-IR analysis of all the excipients used in the formulation and the drug were studied for the interaction of the excipients and the drug in the final formulation. The basic peaks of Pioglitazone hydrochloride were observed at 1053 cm-1, 2902 cm-^{1}, 934 cm-^{1} and same peaks were observed without any deviation in the spectra of Labrafac PG, Tween 80 & Oleic acid, and Tween 80 containing formulations (Fig. 14 to Fig. 18). This shows that there was no drug - excipient interactions.

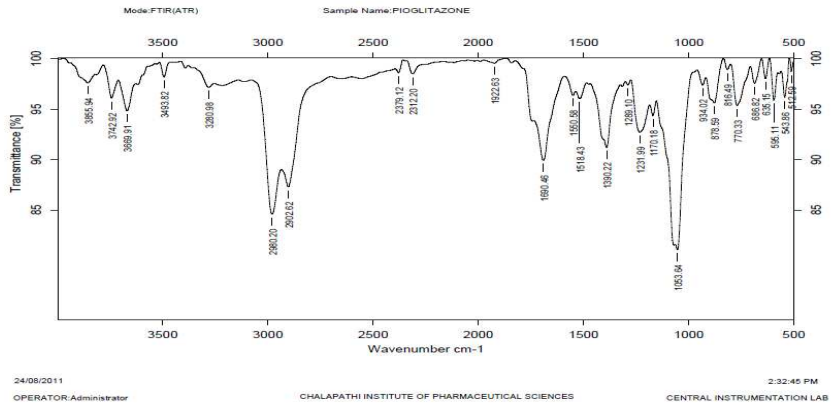

Figure. 14. FTIR spectra of Pioglitazone Hydrochloride

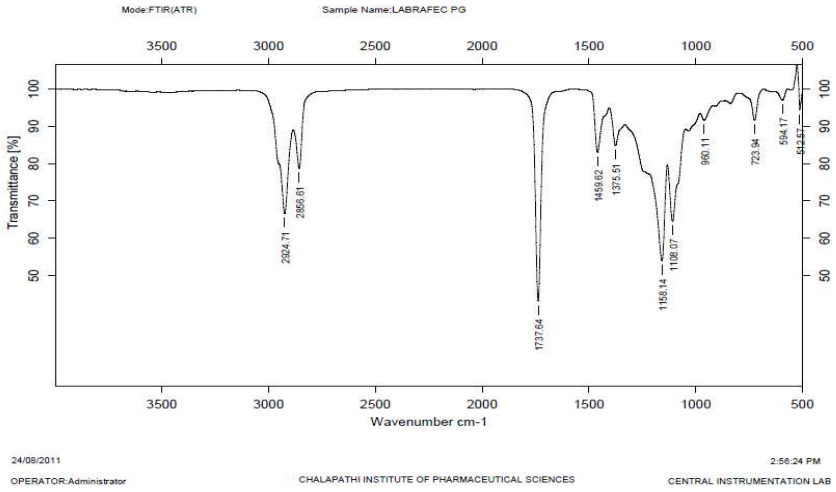

Figure. 15. FTIR spectra of Labrafac® PG

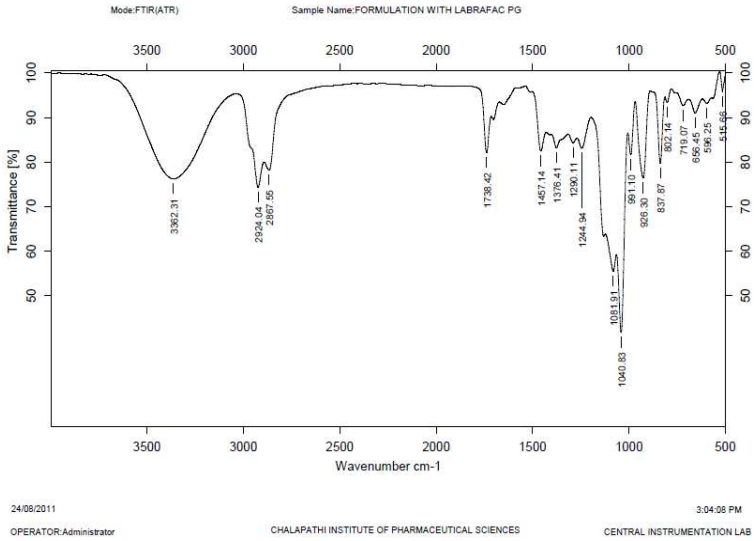

Figure. 16. FTIR spectra of formulation (F1) with Labrafac® PG

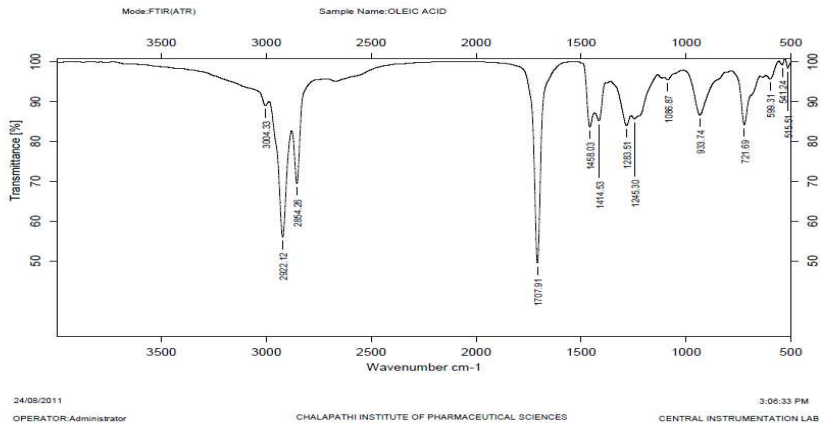

Figure. 17. FTIR spectra of Oleic acid

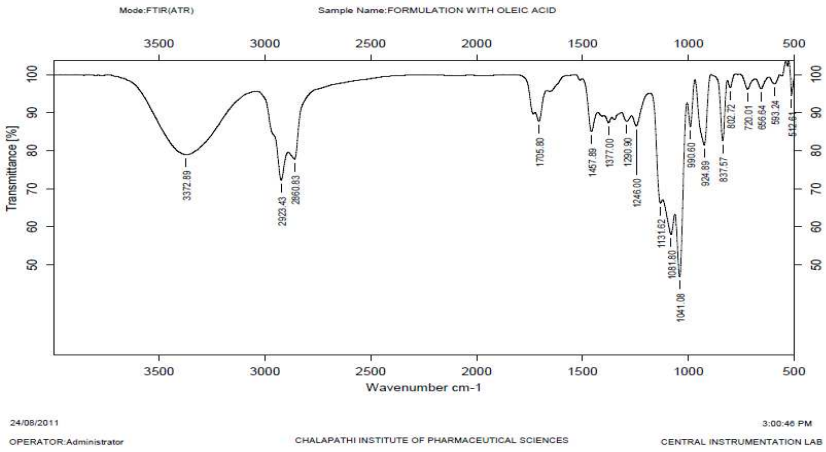

Figure. 18. FTIR spectra of (F7) with oleic acid

CONCLUSION

SEDDS formulations were successfully designed by constructing pseudo ternary diagrams and evaluated. Dissolution rate limited absorption of Pioglitazone was surmounted by producing droplets of nano size range, by which solubility and dissolution rate is, enhanced which in turn helps in improving the bioavailability.

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