Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Suppl 2, 2014

Research Article

SMEDDS FORMULATION: DEMONSTRATION OF ENHANCED BIOAVAILABILITY OF PIOGLITAZONE IN RATS

HYMA.P, [1] ABBULU.K.[2] SUNIL S JALALPURE [3]

[1]Dept of Pharmaceutics, Jyothishmathi College of Pharmacy, Rangareddy, Andhra Pradesh, [2]Mallareddy College of Pharmaceutical Sciences, Secunderabad, [3]BSRC,KLE University, Belgaum, Karnataka, India. Email: rk_hyma@yahoo.com

Received: 18 Dec 2013, Revised and Accepted: 10 Feb 2014

ABSTRACT

Objective: The present study was aimed at kinetic evaluation of Pioglitazone (Pio) in a novel Self micro-emulsifying drug delivery system (SMEDDS) for enhanced oral administration. Poor water solubility of the drug has envisaged this work to prove with kinetic data the enhanced solubility by using novel SMEDDS systems.

Methods: SMEDDS of Pio consisted of cotton seed oil as oily phase, tween 80 as the surfactant and PEG 400 as the co- surfactant containing 15 mg/ml of Pio. The optimized formulation selected by the aid of pseudo-ternary phase diagrams; confirmed increase in drug release by in vitro dissolution studies, with enhanced drug diffusion through biomembranes demonstrated by ex vivo intestinal diffusion studies, with SEM and TEM studies of reconstituted SMEDDS as a proof of nano size range and spherical shape of the droplets was selected for the kinetic study in rats.

Pio SMEDDS formulation was administered orally to one study group and plain drug suspension to the other group. The plasma drug concentration was estimated by validated RP-HPLC method after successful plasma spiking of the drug.

Results: An increase in Cmax and AUC values ($p< 0.05$) for the SMEDDS formulation depicts the increase in bioavailability of otherwise poorly soluble drug on comparison with plain drug formulation. A 3 month stability studies at accelerated conditions (40^0C & 75% RH) showed no change in physical appearance, droplet size and dissolution rate of the drug.

Conclusion: Thus SMEDDS formulation was found to be instrumental in improving oral bioavailability and thus therapeutic efficacy of Pioglitazone.

Keywords: Tween 80, Phase diagrams, TEM, Plasma spiking, Biomembranes.

INTRODUCTION

Pioglitazone is a thailzolidine derivative which decreases insulin resistance by its action at PPAR-γ receptors.[1,2]. Its very poor water solubility has limited its efficacy and bioavailability. Hence a novel self microemulsifying drig delivery system (SMEDDS)[3] was improved and evaluated for enhanced bioavailability of pioglitazone. SMEDDS systems comprises of oils, surfactants and co-surfactants as excipients intimately mixed to form a fine oil in water emulsion upon dilution with G.I fluids to achieve nanosized drug droplets for excellent solubility [4,5].

Many studies were conducted on pioglitazone as formulations in solid dispersions[6,7], inclusion complexes [8,9] and fast dissolving formulations [10]. However this study emphasizes on SMEDDS formulation of pioglitazone and evaluation of kinetic parameters by conducting bioavailability studies in rats [11,12]. Wherein the otherwise poorly water soluble drug was demonstrated to show increase in bioavailability as confirmed by the kinetic data obtained from the study.

MATERIALS AND METHODS

Formulation design of SMEDDS containing pioglitazone

According to solubility analysis and phase diagrams studies, the formulations were prepared by initially dissolving pioglitazone in PEG400 (cosurfactant) at 60◦ C in an isothermal water bath, cottonseed oil was then added and mixture was cooled to ambient temperature, then tween 80 (surfactant) as shown in table 1,was added and the final mixture was sonicated to get a clear solution. The formulation was equilibrated at ambient temperature for at least 48 hours and examined for signs of turbidity (or) phase separation [13].

The liquid SMEDDS were solidified by adsorbing them onto microcrystalline cellulose which serves as carrier. In all the formulations, the drug concentration was constant as 15 mg/mL of liquid SMEDDS.

Evaluation of Pio SMEDDS

The prepared formulations were evaluated for physical appearance, dynamic light scattering was used to characterize nanostructuring, TEM[14] and SEM studies[15]were done for surface characteristics, dissolution studies [16]and Ex-vivo intestinal studies were done to estimate drug release and diffusion [17].

In vivo studies

HPLC analysis of Pio in rat plasma:

The concentration of Pio in rat plasma was determined by validated HPLC method using C18 column, with water and acetonitrile as mobile phase (50:50) at 1ml/min flow rate, detected at 225nm of uv detection. 200µl of plasma containing drug was mixed with 200µl of acetonitrile, vortexed for 5 min, centrifuged at 5000 rpm for 15 min. the supernatant of 20µl was injected into HPLC [18,19]

Animals

Healthy male wistar rats weighing 300-350g were selected and housed with institutional guidelines, fasted overnight and had free access to drinking water. All the experiments were performed after receiving approval of the institutional animal care committee of KLE university Belgaum [20].

Experimental design

Animals were separated into two experimental groups, the first group consisted of rats given SMEDDS pio and the second group contained rats given a suspension of plain pio (0.25% CMC Na). The drug was administered orally without sedation into the stomach at a dose of 10 mg/kg body weight.

Blood samples (app 0.5ml) were collected from retro orbital plexus pre dose for plasma drug spiking and the 15min, 30min, 1,2,4,6,8,12 & 24hrs after drug administration into heparinised tubes. The blood was centrifuged at 5000rpm in cooling centrifuge and plasma was separated, stored at -20^0C until further use [21].

Data analysis

The pharmacokinetic parameters were calculated by using one compartment model and peak time (t_{max}), peak level Cmax were estimated by PK solver. Area under the whole blood concentration

time curve (AUCtot) was also calculated by the trapezoidal rule for the mean whole blood levels.

All results were expressed as mean ±SD. Differences between two related parameters were assessed by student t test or one way anova by using graph pad prism software [22,23].

Stability studies

Chemical and physical stability of solid and liquid Pio SMEDDS were assessed at storage conditions of 40 ±2⁰ C/75 %RH. The samples were evaluated at 1,2,3 months for physical appearance and drug content and particle size was estimated at end of three months [24,25].

RESULTS AND DISCUSSION

Formulation design of SMEDDS containing pioglitazone:

Table 1: Formulation of Pio SMEDDS

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	G1	G2	G3	G4	G5
%Tween 80	54.1	50.8	51.6	55.4	50.5	55.2	54.4	56.2	56.1	54.7	55.9	62.5	61.2	42.5	34.7
% of PEG-400	27	30.4	25.2	22	23.9	17.6	17.2	32.5	12.3	11.3					
%of cottonseed oil	18	18.6	23	22.4	10.5	27.1	28.3	31.5	31.4	33.9	16.1	6.2	30	36.1	48.2
% Glycerol											27.9	31.2	8.6	21.2	16.8

Evaluation of Pio SMEDDS

The formulations when evaluated for phase separation and visibility grade were found to be stable with no phase separation and had visibility grade A. The emulsification time was found to be below 108 sec, with a cloud point of 80⁰C, upon dilution with 6.8 phosphate buffer and distilled water there was no observed precipitation or cloudiness indicating the stability of SMEDDS on dilution. The targeted particle size was achieved within nanosized range as shown in table 2 & fig 1, with zeta potential being negative and PDI within the range. The optimized formulation when subjected to in vitro dissolution study on comparison to marketed formulation showed faster dissolution rate within 60 min as shown in fig 2, the SEM photograph of solid SMEDDS was clear evidence that the liquid SMEDDS was completely adsorbed onto the solid carrier which shows clear particles with roughened surfaces as shown in fig 3, the ex-vivo rat intestinal diffusion study has clearly depicted that the diffusion of the drug has been increased on comparison with plain drug formulation as seen in fig 4,the TEM photograph of optimised formulation seen in fig 5 is a clear evidence of decreased particle size as round particles in nanosize range can be noticed in the figure.

Table 2: Droplet size, zeta potential and polydispersity index of optimized SMEDDS of pioglitazone on dilution with water

Formulations	Particle Size(nm)	Zeta Potential(V)	PDI
F4	10.75	-5.07	0.150
F5	90.26	-2.38	0.236

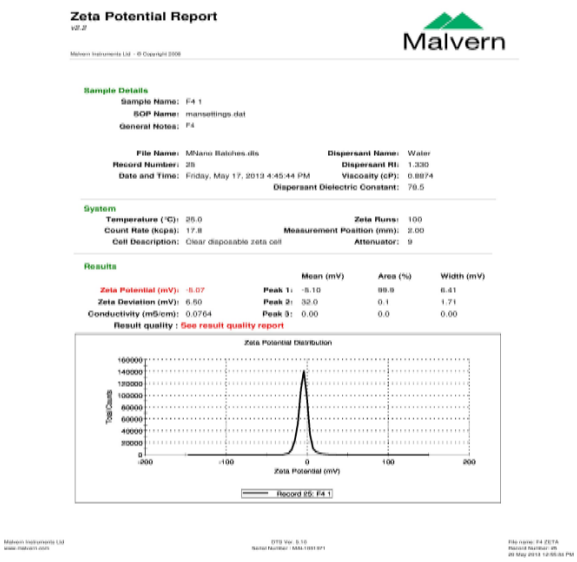

Fig 1: Particle size of optimized formulation.

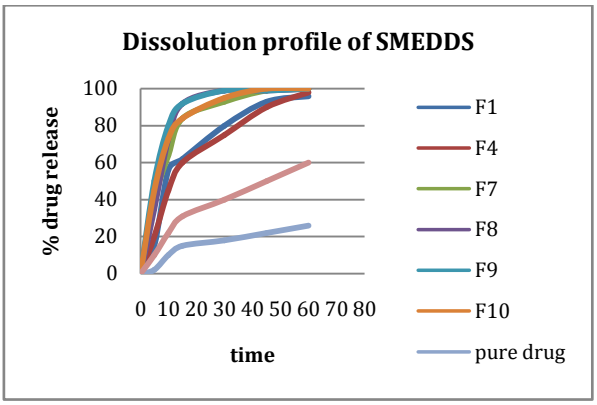

Fig .2: *In vitro* dissolution study of SMEDDS formulation

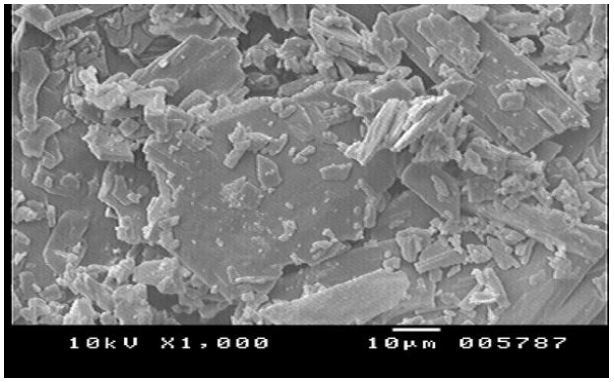

Fig. 3: SEM photograph of SMEDDS formulation

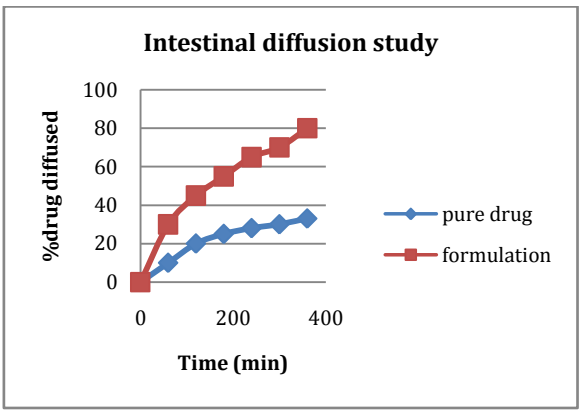

Fig. 4: *Ex-vivo* intestinal study of SMEDDS formulation

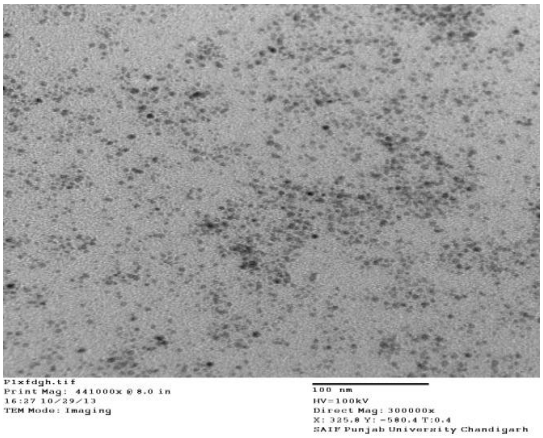

Fig. 5: TEM photograph of optimized formulation.

In –vivo studies: Animal studies of Pioglitazone SMEDDS on comparison with plain drug suspension demonstrated the enhancement of bioavailability of the drug as seen in table 3 & fig 6 & 7. An increase in the Cmax value with decreased Tmax of SMEDDS formulation indicates that the absorption of the drug has increased greatly, the AUC values show a fourfold increase, which is due to the improved oral bioavailability of the drug attributed to decreased particle size and increased solubility.

Table 3: *In vivo* pharmacokinetic studies of Pio SMEDDS IN RATS

Formulation	Cmax(µ/ml)	Tmax(hrs)	AUCtot(µg/ml/h)
Plain drug	6.2±2.76	4.3±0.29	134.2±2.76
Pio SMEDDS	79.03±5.53	1.46±0.120	511.5±61.1

Data are mean ±standard deviation; Cmax: peak concentration; tmax: peak time; AUCtot: area under the concentration-time curve calculated by trapezoidal rule.

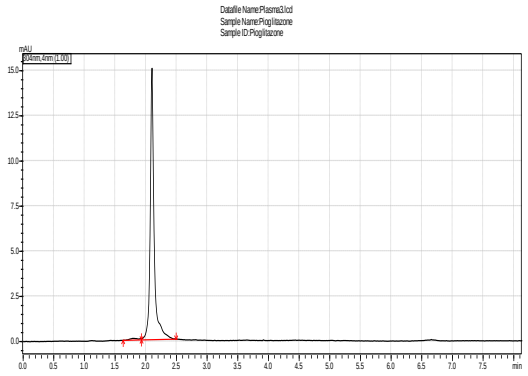

Fig .6: HPLC chromatogram of plasma spiked

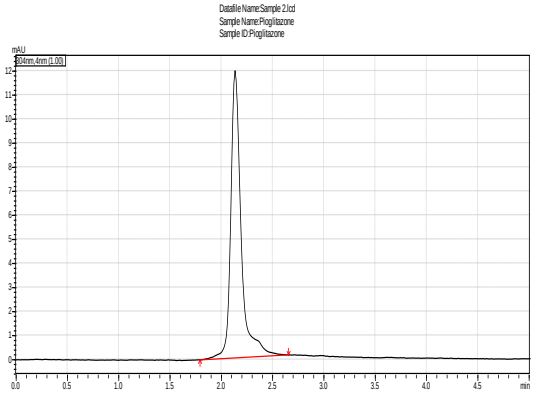

Fig. 7: HPLC chromatogram of Pioglitazone SMEDDS With pioglitazone

Stability studies of Pio SMEDDS: The stability studies conducted on optimized SMEDDS formulation has clarified that the liquid SMEDDS were stable for 3 months, with no physical changes and drug content when evaluated at the end of each month. The particle size of the formulation has shown no much change, with PDI and zeta potential too not much varied which is an authentication of good stability of the prepared formulations.

Table 4: Stability studies of Pio SMEDDS

Time(months)	Physical appearance	Drug content(%)	Particle size(nm)	PDI	Zeta potential(mV)
0	Clear liquid	100.04±0.25	90.26	0.236	-2.38
1	Clear liquid	99.98±0.32	-	-	-
2	Clear liquid	99.91±0.38	-	-	-
3	Clear liquid	99.92±0.36	95.6	0.245	-2.43

CONCLUSION

The studies conducted on the selected formulations demonstrated the stability of the system as emulsion did not develop phase separation, drug precipitation and exhibited excellent in vitro dissolution profiles compared to pure drug and commercially available pioglitazone formulations. The droplet size analysis and zeta potential values confirm the reduction in particle size to nanometer range which definitely improves solubility hence the dissolution rate and absorption of otherwise poorly soluble drug. The ex vivo intestinal study was performed to demonstrate the permeability characteristics of the drug and its SMEDDS formulation. However, optimized formulation was seen to possess good permeability across the membrane which was due to effective partitioning of the drug and lowered interfacial tension credited to high concentration of surfactant (60–70%) in the formulation.

The in-vivo study was carried out to determine whether the developed SMEDDS formulation could enhance the absorption of Pio after oral administration. The pharmacokinetic parameters deduced from the plasma concentration time profiles of Pio obtained from SMEDDS formulation produced a significantly greater improvement of drug absorption than the plain drug. The SMEDDS formulation gave significantly higher AUC & Cmax than pure drug; as seen the AUC was about 500 folds higher than pure drug. The higher absorption of Pio in self micro emulsifying formulations might be attributed to larger surface area obtained after oral administration of SMEDDS formulation and this promoted the direct absorption as micro emulsion droplets. The developed formulations were found to be physically and chemically stable for 3 months as confirmed by drug content and particle size values. In conclusion, the current study of pioglitazone SMEDDS should be a desirable formulation for antidiabetic drug therapy associated with steroid-induced diabetes too. The in vitro and ex vivo results showed significant release and permeability of the drug compared to plain drug. Therefore, the findings of the current study may be evolved into an effective therapeutic technology for the treatment of diabetes mellitus.

ACKNOWLEDGEMENTS

The author would like to thank Basic sciences research center(BSRC) KLE university Belgaum for providing necessary facilities to carry out animal studies, SAIF Punjab university for TEM analysis, manipal college of pharmaceutical sciences for particle size analysis.

REFERENCES

1. Sajal kumar saha, A.K.Azad chowdhary, Sitish Chandra bachar, Sreedam Chandra das, Rahul H Khuddus, Md Aftabuddin.Comparative in vitro-in vivo correlation analysis with pioglitazone tablets. Asian pac J trop dis 2013;3(6):487-491.
2. GillPS, Dunn C.J. Pioglitazone drugs 2000;60(2):333-343.

3. Shweta gupta, Sandip chavhan, Kruthika K Sawanth..Self-nanoemusifying drug delivery systems for adefovir-dipivoxil : Design, characterization, in vitro & ex vivo evaluation. Colloids & Surfaces A: Physiochem Eng Aspects. 2011;392:145-155
4. L.Wang, J.Dong. Design & optimization of a new self – nanoemulsifying drug delivery system. J Colloid Interface Sci.2009; 330:443-448.
5. Marietta Von Suesskind-Schwendi, Michael gruber, Didier Tourad, Werener kunz, Christ Schmid, Stephen W Hirt, Karle lehle. Pharmacokinetics of a self-microemulsifying drug delivery system of tacrolimus.Biomed Pharmacother 2013;67(6):469-73
6. Nian-qui shi, Yong-sheng lei, Li-Ming Song, Jin Yao, Xue-bing Zhang, Xing –lin Wang. Impact of amorphous & semicrystalline polymers on the dissolution & crystallization inhibition of pioglitazone solid dispersions. Powder technol. 2013;247:211-221.
7. S. Soltanpur, W.E Jr Acre, A.Jouyban. Solubility of pioglitazone Hcl in aqueous solutions of ethanol, propylene glycol, and N-methyl 2 pyrrolidone at 298.2 degrees K. AAPS Pharm sci tech. 2009;10:1153-1157.
8. Elbary AA, Kassem MA, Abou Samra MM, Khalil RM. Formulation and hypoglycemic activity of pioglitazone – cyclodextrin inclusion complexes. Drug discovery therapy. 2008; 2(2):94-107.
9. V.Pandit, R.Gorantla, K.Devi, RS Pai and S.Sarasija. Preparation and characterization of pioglitazone inclusion complexes.J young Pharm.2011; 3(4):267-274.
10. Vinay pandit, Roopa S.Pai, Kusum devi, Sarasija suresh. In vitro In vivo evaluation of fast dissolving tablets containing solid dispersion of pioglitazone. J.Adv Pharm Technol Res.2012; 3(3):160-170.
11. Krishnamoorthy balakumar, Chellan Vijayaraghavan, Natarajan tamil selvan, Ranganathan Hari Prasad, Siyad abdu. Collois & surfaces A Biointerfaces. 2013 ;(112):337-343.
12. DG Fatouras, DM karpf, F.S Nielsen, A.Mullertz. Clinical studies with oral lipid based formulations of poorly soluble compounds. Ther.clin.risk.2007; 3:591-604.
13. Ahmed S.Zidan, Omaima A.Sammour, Mohammed A.Hammad, Nagia A Megrab, Muhammad J.Habib, Mansoor A khan. Quality by design: Understanding the formulation variables of a cyclosporine A self nanoemulsified drug delivery systems by box-benhken design and desirability function. Int.J.Pharm.2007;332:55-63.
14. Nanfa Sernkaew, Wichin Ketjinda, Prapaporn Boonme, Narubodee Phadoongsombut, Ruederkorn wiwanttanapatapee. Eur J Pharm Sci. 2013; 50:459-466.
15. Xiao-Lan Wei, Ying –Rui Han, Li-Hui Quan, Chun-Yu Liu, Yong-Hong laio. Oily nanosuspensions for long acting intramuscular delivery of curcumin didecanoate prodrug: Preparation, characterization and in vivo evaluation. Eur J Pharm Sci.2013;49:286-293.
16. Yogeshwar G.Bacchav, Vandana B.Patravale. SMEDDS of glyburide: Formulation, In vitro evaluation & stability studies. AAPS Pharm Sci Tech.2009;10(2):482-487.
17. S.E.Eltayeb, Zhiguisu, Yongping Shi, Sai li, Yanyu Xiao, Qineng Ping. Preparation & optimization of transferin modified artemether lipid nanospheres based on orthogonal design of emulsion formulation and physically electrostatic adsorption. Int.J.Pharm.2013; 452:321-332.
18. T. Radhakrishna, D.Sreenivas, G.Om Reddy. Determination of Pioglitazone Hcl in bulk and pharmaceutical formulation by HPLC & MEKC methods. Jnl of Pharm & Biomed Analysis.2002; 29:593-607.
19. Kenji Yamashita, Hiromi Murukami, Teruaki okuda, Michio Motohashi. High performance liquid chromatographic determination of Pioglitazone and its metabolites in human serum and urine.Jnl of Chrom B.1996; 677:141-146.
20. Sudhir N Umathe, Pankaj V.dixit, Vijendra kumar, Kuldeep U Bansod, Manish M Wanjari. Quercetin increased pretreatment increases the bioavailability of pioglitazone in rats: Involvement of CYP3A inhibition.Biochemical Pharmacology. 2008; 75:1670-1676.
21. Suke, S.G, Harsh Negi, P.K.Mediratta, B.D Banerjee, K.K Sharma. Anti-arthritic and anti- inflammatory activity of combined pioglitazone and prednisolone on adjuvant-induced arthritis. Eur J Pharmacol (2013), http://dx.doi.org/10.1016/j.ejphar.2013.09.019.
22. Ajeet K Singh, Akash chuarasia, Anshumathi awasthi, Guatam mishra, Dinesh Asahi, Roop K Khar and Rama mukherjee. Oral bioavailability enhancement of exemestane from self-microemulsifying drug delivery system(SMEDDS). AAPS Pharm Sci Tech.2009; 10(3): 906-916.
23. Pradeep R.Patil, S.Pravan, R.H Shobha rani, Ananth R Paradhkar. Bioavailability assessment of ketoprofen incorporated in gelled selfemulsifying formulation: A technical note. AAPS Pharm Sci Tech.2005;6(1):E9-E13.
24. Sanjog Jain, Amit K Jain, Milind Pohekar, Kuashik Thanki. Novel self-emulsifying formulation of quercetin for improved in vivo antioxidation potential: Implications for drug induced cardiotoxicity and nephrotoxicity. Free Radical Biology & medicine. 2013; 65:117-130.
25. International conference on harmonization guidance for industry Q1A (R2): Stability testing of new drug substances and products geneva international federation of pharmaceutical manufacturers & associations: 2000.