



Kneaded pioglitazone poloxamer solid dispersions for enhancement of *In Vitro* dissolution and *In Vivo* bioavailability

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Abstract

Pioglitazone (PG) an oral hypoglycemic agent is characterized by low solubility in gastric fluid, low dissolution rate and inter-individual variability in bioavailability. The objective of this study was therefore to design optimized solid dispersion (SD) of PG with hydrophilic carriers eg Poloxamer 188 by kneading method in an attempt to enhance to the aqueous solubility and therapeutic efficacy of drug phase solubility study with increasing PXM (1:1-1:8) was done to study the influence of polymer concentration on solubility of PG. SD of PG and PXM in 1:1 to 1:8 w/w ratio were prepared by physically mixing and kneading method followed by dissolution studies. A comparative *in-vivo* study between optimized SD and PG was conducted on six healthy New-Zealand rabbits. The dissolution rate of PG from the kneaded dispersion was greatly enhanced as compared to those from physical mixture and pure drug. The *in vivo* studies indicated that the pharmacokinetic parameter following oral administration of the optimized SD and pure PG were significantly different. The peak serum conc. (C_{max}) for the kneaded SD and PG were found to be 596.33±0.84 ng/ml and 528.50±0.71 ng/ml resp. Whereas the time required to reach the peak serum conc. (T_{max}) for optimized SD was significantly shorter 1.5±0.04 hrs compared to that for PG 2.00±0.02 hrs. The relative bioavailability of SD under *in-vivo* test was found to be 120.98%. These results demonstrate that the use of a suitable hydrophilic carrier like PXM to formulate SDs by the kneading technique can rapidly accelerate the solubility and *in vitro* dissolution of a lipophilic drug like PG the suggested method also provides good signs of importance in the rate of absorption as well as bioavailability of Pioglitazone following oral administration.

Key-Words: Pioglitazone, Poloxamer 188, Kneading, Bioavailability

Introduction

Pioglitazone decreases insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output unlike sulfonyl ureas. Pioglitazone is highly selective agonist for peroxisome proliferator-activated receptor-gamma (PPAR γ)^{1,2} for this reason Pioglitazone is an oral antidiabetic agent that acts primarily by decreasing insulin resistance.³ Pioglitazone (RS)-5-(4-[2-(5-ethylpyridine-2-yl)ethoxy] benzyl thiazolidine-2,4 dione.⁴ It is used for treatment of type II diabetes mellitus⁵ however, the drawback of this potentially useful hypoglycemic agent is that it is highly hydrophobic and practically insoluble in water.^{6,7}

In general, rapid gastrointestinal (GI) absorption is required for oral hypoglycemic drugs, in order to prevent a sudden increase in blood glucose level after food intake in patient with diabetes mellitus.⁸ Slow absorption of a drug usually originates from either its poor dissolution from the formulation or poor permeability across the GI membrane. This eventually limits its oral bioavailability and therapeutic efficacy.⁹ For decades, various techniques have been used to improve the solubility and dissolution rate of poorly water soluble drugs. Among them, the solid dispersion method is the most frequently and effectively used one.^{10, 11, 12} Solid dispersion (SDs) of poorly water soluble drugs in hydrophilic carrier matrix have been reported to improve their solubility and dissolution rate.¹³ Moreover, they are also proven to enhance their bioavailability by increasing their dissolution.¹⁴ Since long, many investigators have studied SDs of poorly water-soluble drugs with various pharmacologically inert carriers to increase the dissolution and oral absorption of poorly water-soluble drugs; however, only a few systems are useful commercially.^{15,16} PXMs

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have been recently widely used as wetting and solubilizing agents as well as surface adsorption excipients. They have been employed to enhance the solubility, dissolution and bioavailability of many hydrophobic drugs using various techniques. For some drugs, the improvement in solubility using PXM was higher compared to the other meltable polymers such as PEGs and complex forming agents such as cyclodextrins. In the present study, PXM was thus empirically selected as a hydrophilic carrier for its excellent surfactant properties and oral safety.¹⁷ The formulation of Solid dispersion of Pioglitazone with poloxamer by kneading method. Overcome the problems of evident from the pharmacokinetic studies using a non tedious analytical method and holds good potential for commercial scale up.

Material and Methods

Pioglitazone was obtained from Medley Pharmaceuticals Ltd. Daman as a gift sample, Poloxamer 188 and 407 were obtained from Alembic pharmaceutical Pvt. Ltd. Badodara. All other chemical used were of analytical grade. Ultra double distilled water (milipore) was used throughout the study. Animal studies were approved and conducted in accordance to the Institutional Animal ethical Committee.

Preparation of physical mixture

A physical mixture (PM) of PG with PXM 188 in 1:1 ratio was prepared by thoroughly mixing the accurately weighed quantity of drug and carrier in by using glass mortar and pestle for 5 min. This mixture was then subsequently passed through mesh no. 40 and stored in a desiccator for 48 hrs.

Preparation of Solid dispersions

The Kneading method (KM) was used for the preparation of solid dispersion. Eight different drugs: Carrier ratios (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8) were used. PG 1 to PG 8 corresponds to preparations containing PXM 188 and PG 9 to PG 16 correspond to preparations containing PXM 407. Pioglitazone and PXM 188 or 407 were weighed according to these weighed ratios. PG and PXM were triturated using a small volume of methanol to give a thick paste, which was kneaded for 30 minutes and then dried at 40° C in an oven. The dried mass was then pulverized¹⁸

Determination of Solubility

Phase solubility was performed as described by Higuchi and Connors. Excess amount of solid dispersion were added to 25 ml phosphate buffer (pH 7.4) taken in a stoppered conical flasks, and mixture were shaken for 24 hrs in a rotary flask shaker. After shaking to achieve attain equilibrium, 2 ml aliquots were withdrawn at 1 hr intervals and filtered through What man filter paper no 40. The filtrate was

analyzed spectrophotometrically at 270 nm. Shaking was continued until three consecutive reading were the same.¹⁹

Determination of drug content

The drug content was calculated by dissolving solid dispersion equivalent to 15 mg drug into a 100 ml volumetric flask and dissolved in minimum amount of methanol; and the volume was made up to the mark with using phosphate buffer (pH 7.4) and then filtered through whatman paper no 40 with, filter and assayed for drug content using UV double beam (Shimadzu) spectrophotometer at 270 nm. Three replicates were prepared, and the average drug contents were estimated in the prepared solid dispersion.²⁰

Actual drug content was calculated for all batches using the equation:

$$\text{Drug content (\%)} = \frac{\text{PG}_{\text{act}} \times 100}{\text{PG}_{\text{sd}}}$$

Where PG_{act} is Actual PG Content in weighed quantity of Solid dispersion, and PG_{SD} is theoretical amount of PG in SD.

In-vitro drug release

Accurately weighed preparations equivalent to 15 mg of Pioglitazone were added to 900 ml of dissolution media (7.4 phosphate buffer) contained in USP dissolution apparatus II (Electro lab, TDT-08L) and stirred at a speed of 50 rpm at 37 ± 0.5°C. Five milliliter aliquots were withdrawn at 5, 10, 15, 20, 30 minutes and replaced by 5 ml of fresh dissolution media (37°C). The collected samples were analyzed after suitable dilution at 270 nm using UV-visible spectrophotometer against the blank. The dissolution of pure Pioglitazone was done similarly. The release profile data was analyzed for cumulative percent dissolved at different time intervals and for dissolution efficiency at 5 and 15 minutes.²¹

In-vivo Pharmacokinetic study²²

Based on the in-vitro dissolution profile, an optimum solid dispersion Pioglitazone:PXM 188 (1:5w/w) prepared by kneading technique was selected for comparison of in-vivo performance against plain Pioglitazone

Study design

6 albino New Zealand rabbits of average weight 2.5 ± 0.3 kg were used for the study. The rabbits were divided into 2 groups of 3 rabbits each with cross over technique (n=6). All rabbits were fasted overnight with ad libitum access to water during the experiment and the animals were fed 8 hours after the oral dose. One group of animals received a single dose of PG (15 mg/2ml), formulated as a suspension containing sodium carboxy methyl cellulose (equivalent to 0.5% w/w of the drug). The second group was

administered a solution containing solubility enhanced kneaded PG: PXM (1:5w/w) at the same dose. The suspensions were administered orally through a sterile pediatric feeding tube (size 8) followed by 2 ml of distilled water to wash off any drug remaining in the feeding tube and upper alimentary tract. 1mL of blood sample was collected using 22 gauge needle from the shaved marginal ear vein into heparinized Eppendorf micro-centrifuge tubes at time intervals of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 up to 8 hrs.. Xylene was applied to the marginal ear vein before withdrawal, which causes blood vessel to dilate. The blood samples were immediately centrifuged at 6000 rpm for 10 min to separate plasma and stored at -40° C until further analysis.

Extraction of Pioglitazone from plasma

The plasma samples were spiked with known concentrations of PG in acetonitrile so as to obtain plasma concentrations of 100ng/ml, 200ng/ml up to 600ng/ml and 10.0 μ g/ml. To 0.5 ml of the spiked plasma sample taken in a polypropylene centrifuge tube, 0.5 ml of acetonitrile was added and the samples were vortexed for 30 seconds to precipitate plasma proteins. 2 ml of chloroform was added and the samples were vortexed again for 2 minutes to extract PG into the organic layer. The mixture was then centrifuged for 15 minutes at 3000 rpm. Then, 1 ml of the organic layer was transferred to a clean glass vial and evaporated in a vacuum oven whose temperature was maintained constant at $40\pm 1^{\circ}$ C. The dry residue was reconstituted with 1ml of acetonitrile, diluted with 1 ml of internal standard (1.5 μ g/ml rosiglitazone in acetonitrile) and vortexed for 30 seconds. The resulting solution having a final internal standard concentration of 400ng/ml, was filtered through 0.45 μ syringe filter (Millipore) and 100 μ l of the sample was injected and analyzed for PG content using the HPLC method mentioned below.

Analysis of Pioglitazone by High Pressure Liquid Chromatography (HPLC)²³

The concentration of PG in the plasma samples was analyzed by a standardized reverse phase HPLC method. The system consisted of aAgilent 12:20 Binary system with UV-detector, in-line degasser and an auto-sampler programmable coupled to a personal computer. Data and system management was handled by chromatography manager software. The separation was performed at 45° C using a C 18 column [250 x 4.6 mm], supplied by Agilent Technologies, USA. The mobile phase comprised of buffer: acetonitrile (55:45% v/v) and was run at a flow rate of 1.0ml/min. The aliquots were loaded in an auto sampler tray in glass vials, 20 μ l sample was injected and the eluting peaks

were monitored at a λ_{max} of 270 nm. The developed HPLC method was validated for linearity (100 to 600 μ g/ml), repeatability, and precision.

Pharmacokinetic Parameters Estimation and Statistical Analysis

Results from HPLC analysis were plotted as drug concentration in plasma vs. time. Non compartment pharmacokinetic parameters including Tmax, Cmax and AUC were estimated by Kinetica 5.0 computer program. The AUC values for each curve were calculated from time zero to the last data point using the trapezoidal rule with extrapolation to infinity. The AUC_{0- ∞} values obtained from curve were used to calculate the relative bioavailability Results of in vivo experiments are reported as mean \pm S.D Statistical tests of significance were performed using Graph Pad Prism 5.0 software. The variables were compared with a oneway ANOVA., A P-value less than 0.05 were considered significant.

Results and Discussion

All physical mixtures and solid dispersion (SD) were easy to prepare and reproducible.

Solubility studies

The solubility profile of PG was found to be 0.006 mg/ml, and drug release was found to be only 32.18% during in vitro dissolution study, suggesting a strong need to enhance the solubility and dissolution of PG. Therefore, a solid dispersion technique using PXM 188 and PXM 407 was employed for solubility and dissolution enhancement of PG in the present investigation. The improvement in solubility was observed with for all solid dispersion, Increase in weight fraction of surface-active carrier resulted in an increase in the solubility of all dispersions. Maximum solubility enhancement was found in 1:5 ratio of PG: PXM 188 prepared by the kneading method.i.e. 0.356 mg/ml. Enhancement in saturation solubility was found to be in order of PXM 188> PXM 407.(table 1)

Drug content estimation

The drug content of PG solid dispersion (SD) was found to be in range 97.62 to 99.92 and these values are within the acceptable range., as given in Table 1, indicating uniform drug distribution in all the solid dispersion (SD) (table 1)

In-vitro Dissolution studies

The in vitro release profile of PG from PXM 188 and PXM 407 solid dispersion (prepared by the kneading method) and physical mixture formulations are shown In vitro dissolution studies in Figures 1 and 2, and the graph for the comparison of the cumulative percent release is illustrated in Figure 3. According to observations, drug release was increased with increasing the concentration of both the grades of

Poloxamer (i.e., PXM 188 and PXM 407) up to a certain limit, and after that then it almost becomes constant. Drug release from SDs and physical mixtures was faster than that from the pure drug. The dissolution of drug from solid dispersion was found to be faster than that from physical mixtures; this may be due to the molecular and colloidal dispersion of drug in hydrophilic carrier matrix of Poloxamer.

In-Vivo Pharmacokinetic Study

The oral administration of 15 mg dose of PG or an equivalent of its 1:5 kneaded SD to 6 healthy rabbits and the mean pharmacokinetic parameters after administration of the drug and the solid dispersion are listed in table no.1. It is evident that there exists a clear difference between the biological performance of both the pure drug and the solid dispersion under test, which reflects itself by the significant difference ($p < 0.05$) in the peak concentrations between both of them, determined using one way ANOVA. The maximum plasma level (C_{max}) of 528.50 ± 0.71 ng/ml was attained after 2.0 ± 0.02 hours. On the other hand, the solid dispersion resulted in significantly rapid appearance of PG in plasma, showing C_{max} value of 596.33 ± 0.84 ng/ml after 1.5 ± 0.04 hours. While area under curve of pure drug AUC_{tot} 1679.5 and SD AUC_{tot} of 2030.66 The relative bioavailability of the kneaded solid dispersion was found to be 120.98% depicting an improvement in the efficacy of the drug. (figure 4) and (figure 5).

With the increasing number of drug candidates which are poorly soluble, solid dispersions with hydrophilic carriers play an increasingly important role in pharmaceutical development, which was depicted in the present study. Successful solubilization of PG was achieved using the Kneading technique and poloxamer (water soluble carrier). Based on the rapidly improved in vitro dissolution and in vivo absorption rate, it is very much evident that such formulations would be highly advantageous for the use of oral drug therapy, with faster onset of action and better therapeutic efficacy.

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Table 1: Evaluation of physical mixtures and solid dispersion SDs of pioglitazone

Formulation Code	%Drug content	Solubility mg/ml	DE ₅	DE ₁₅
PG Pure	--	0.006	17.51±0.35	32.18±0.07
PM 1	98.95	0.014	19.71±0.04	36.18±0.11
PM 2	98.92	0.013	17.60±0.11	36.23±0.22
PG 1	97.62	0.074	41.08±0.12	59.05±0.87
PG 2	97.90	0.125	41.62±0.22	59.10±0.05
PG 3	97.92	0.185	41.96±0.15	59.23±0.18
PG 4	98.33	0.202	42.16±0.19	59.48±0.37
PG 5	99.92	0.356	48.14±0.18	63.43±0.23
PG 6	98.93	0.242	45.26±0.28	59.62±0.54
PG 7	99.44	0.307	45.60±0.35	60.16±0.45
PG8	99.63	0.324	46.44±0.25	60.53±1.16
PG9	99.24	0.126	34.19±0.39	55.34±0.36
PG10	97.59	0.167	34.59±0.29	55.44±0.22
PG11	97.96	0.212	34.83±0.14	55.83±0.14
PG12	98.67	0.235	40.79±0.19	56.35±0.22
PG13	98.91	0.281	41.00±0.10	57.15±0.06
PG14	99.82	0.345	44.38±0.16	62.23±0.16
PG15	99.26	0.320	42.63±0.16	58.91±0.11
PG16	99.11	0.314	42.32±0.08	59.34±0.11

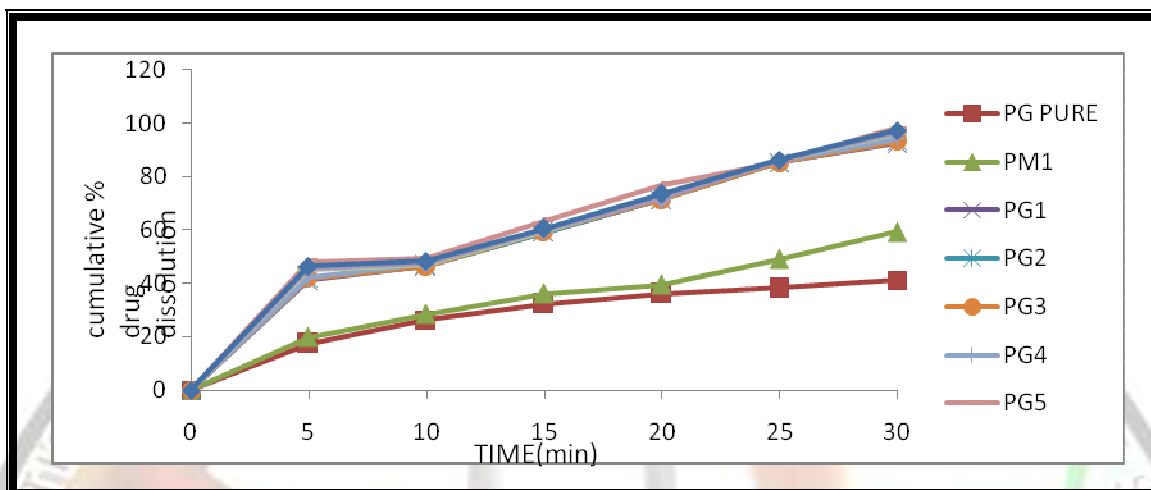


Fig.1: *In-vitro* drug dissolution of PG in pH 7.4 phosphate buffer from solid dispersion and physical mixture of PG-PXM 188 systems

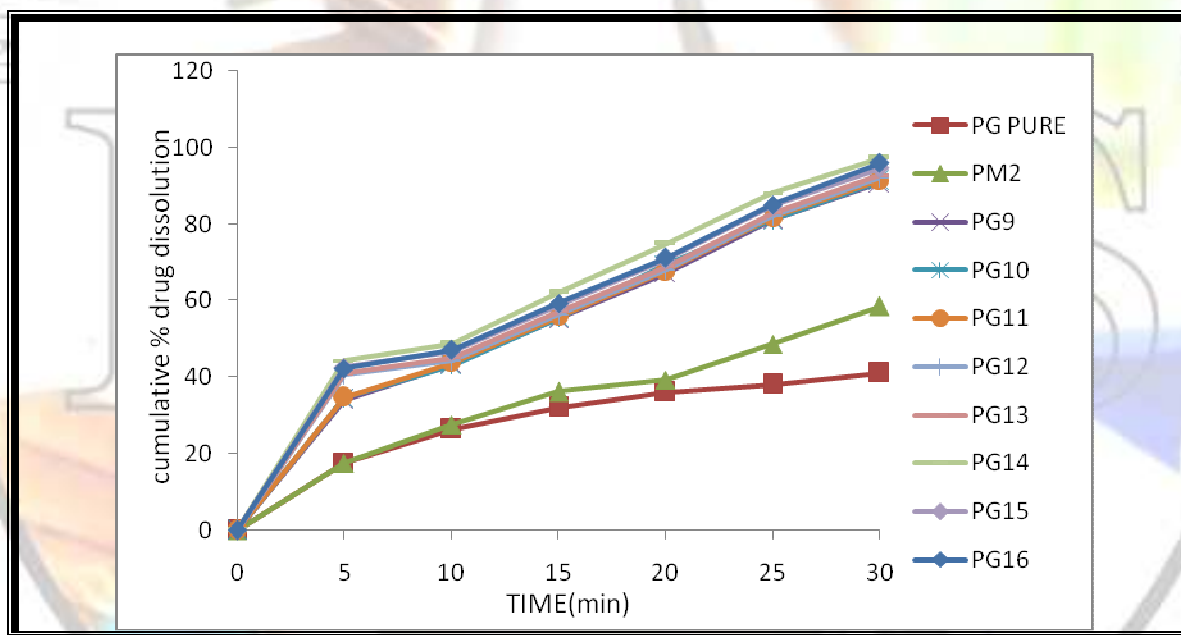


Fig. 2: *In-vitro* drug dissolution of PG in pH 7.4 phosphate buffer from solid dispersion and physical mixture of PG-PXM 407 systems

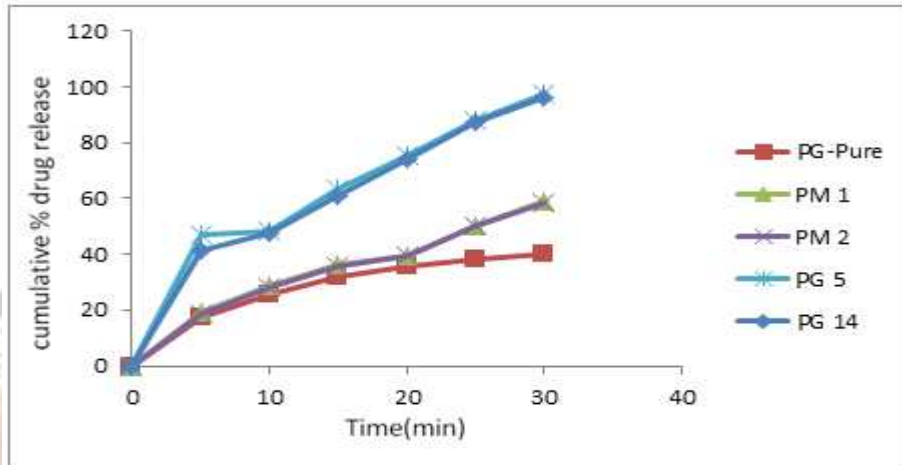


Fig. 3: Comparative dissolution profile of PG in pH 7.4 Phosphate buffer from physical mixture and solid dispersions prepared used in PXM188 and PXM407

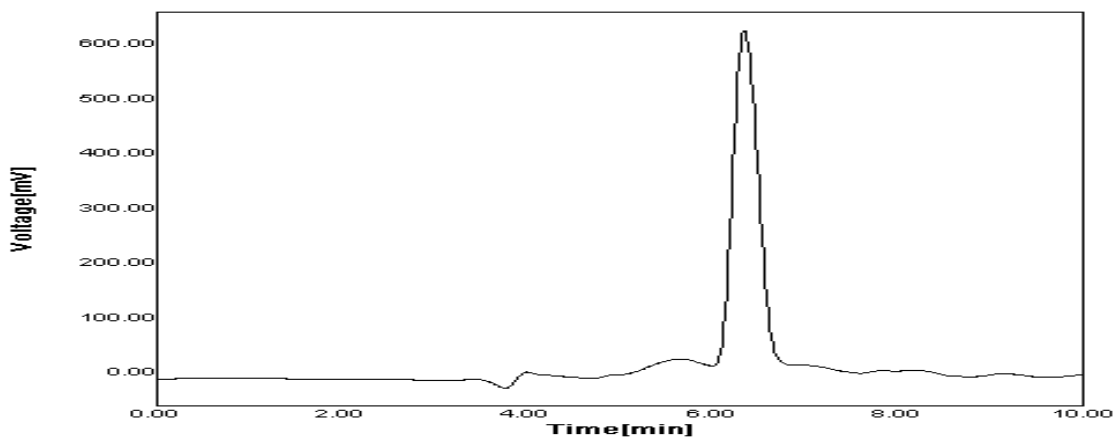


Fig. 4: Chromatogram of pioglitazone

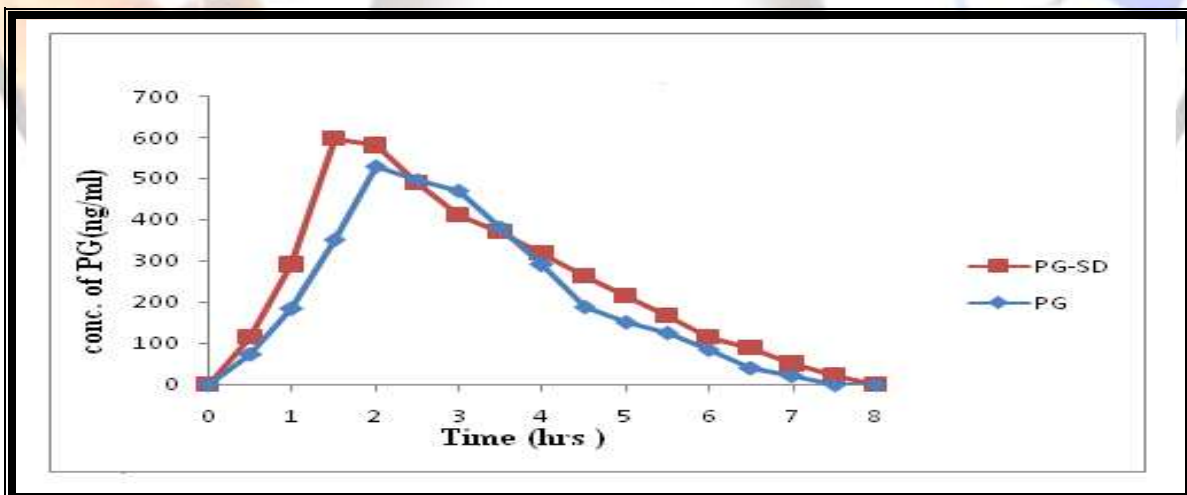


Fig. 5: Plasma concentration v/s time profile of PG and solid dispersion PG