IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2016 Vol.:8, Issue:1

Formulation and Evaluation of Nanosuspension of Antidiabetic Drug

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Submission: 7 December 2016
Accepted: 12 December 2016
Published: 25 December 2016

Keywords: Eudragit L 100, Nanoparticles, Antidiabetic, Pioglitazone

ABSTRACT

The objective of our study is to load Type 2 antidiabetic drug, Pioglitazone in Eudragit L100 and Eudragit S100 nanoparticles in order to enhance bioavailability and to reduce dose frequency. Eudragit was dissolved in Methanol at various concentrations; drug was solubilized in above eudragit solution kept over stirrer at room temperature for a period of 5 minutes. The Poloxamer 407 aqueous solution with 1% concentrations. Then added drug-polymer solution into aqueous solution. Followed by high-speed mechanical stirring for 5 hrs. The resulting eudragit nanoparticles suspension was centrifuged at 5,000rpm for 4hrs, after freeze drying the nanoparticles were collected. SEM studies show that formulation L2 having optimum nanosized particles. Zeta potential shows good positive potentials. It shows good entrapment efficiency. And good release profile follows Higuchi release kinetics. From all these results it concludes that formulation L2 is the best formulation and which is recommended for future studies like Nano dry powder suspension preparation.

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INTRODUCTION [1-6]

Pioglitazone is an oral antidiabetic drug having reported low solubility. Low solubility shows that the drug is poorly absorbed in its absorption site. As a fractional amount of the total dose is absorbed and shows lower therapeutic efficacy. Other major concentration of drug remains as it is. After the specified gastrointestinal transit period the drug needs to be eliminated. The time taken by the drug after its absorption till its elimination causes several side effects such as renal damage, heart failure, nausea, vomiting and increase the risk of balder cancer. The absorption of pioglitazone can be improved if the solubility will be improved. If the solubility improves there will be better patient compliance and improved therapeutic efficacy of drug which is highly desired for pioglitazone. Therefore, to provide this drug in a more accessible and patient complaint form and to overcome such problems with reduced side effects, the present study it was decided to increase solubility of drug by formulating nanoparticles and to incorporate into nanosuspension. On the basis of these considerations, in this study, effort has been made to formulate pioglitazone loaded nanoparticulate formulations incorporated into nanosuspension technology. After cursory search of literature, it was found that few antidiabetic drugs such as Gliclazide, Glipizide, Repaglinide, Glibenclamide have been developed into suitable nanoparticulate drug delivery systems with excellent results to fulfill its purpose. In view of the above, it was planned to develop nanoparticulate drug delivery system of pioglitazone by using suitable biodegradable polymer with an aim to provide better-controlled release profile and efficacy of this therapeutic agent.

MATERIAL AND METHODS

Pioglitazone HCL purchase from Wockhardt, Aurangabad and Eudragit S100, Eudragit L 100, Poloxamer F128 (407), Methanol Purchase from Chempure chemical Ltd. India,

METHOD OF PREPARATION OF NANOPARTICLES:-

1) Emulsification – Solvent – Method[7] :-

The drug and polymer dissolved in a volatile water-immiscible organic solvent such as Dichloroethane, Chloroform. Then using a Homogeniser organic phase is then emulsified as nanodroplets in an aqueous phase then nanoparticles are 6 form due to polymer are precipitate and organic phase is evaporated.

Formulation of the pioglitazone loaded polymeric nanosuspension tablet:

Table no.1: Formula For Loaded Polymeric Nanosuspension Tablet

BATCH	DRUG(mg)	POLYMER(mg)		SURFACTANT POLOXAMER 407 (%)	DISTILLED WATER(ml)
		Eudragit L100	Eudragit S100		
L1	30	30	-	1	30
L2	30	60	-	1	30
L3	30	90	-	1	30
S1	30	-	30	1	30
S2	30	-	60	1	30
S3	30	-	90	1	30

Evaluation of Pioglitazone loaded Polymeric Nanosuspension [9,10]

1) Solubility Studies:-

Solubility of drug can be calculated by using Calibration equation method using UV-visible spectrophotometer

2) Particle size and zeta potential values of Pioglitazone loaded polymeric nanosuspension preparation:-

The particle size is also evaluated by a Transmission electron microscope. A sample of drug loaded NPs was suspended in water and sonicated for 30 seconds. One drop of this suspension was placed on a carbon-coated copper TEM grid and stained with 1% uranyl acetate for 10 minutes, then dry. Image was visualized.

3) Differential scanning calorimetry (DSC):

DSC study was carried out for Pioglitazone HCL. The DSC studies indicated the sharp peak at 192^0C, which is the melting point of Pioglitazone HCL drug and indicating crystal nature of drug.

4) X-ray Diffraction Study (XRD):

The powder x-ray diffraction analysis was carried out for Pioglitazone HCl. Powder x-ray diffraction study indicates sharp distinctive numerous peaks which indicate crystalline nature of Pioglitazone HCl.

5) Percentage (%) drug content and Percentage (%) entrapment efficiency:

a) Percentage (%) drug content –

The 1 ml formulation was taken and dissolved in 9 ml methanol. From this solution, 1 ml withdrawn and diluted with phosphate buffer 7.4 to make up volume 10 ml. Then this solution was sonicated for 1 to2 hrs by using sonicator. Then filter through Whatman's filter paper (No.41). Analysed at 269 nm by using UV double beam spectrometry. The % drug content calculated by formula:-

$$EE\ (\%) = \frac{(\text{Total drug content} - \text{Free dissolved drug}) \times 100}{(\text{Drug amount used})}$$

FW – Amount of drug found in total formulation.

IW - Initial amount of drug in the formulation.

b) Percentage (%) entrapment efficiency:

Take 2 ml of formulation in Nessler's tube (10 ml). These solution was centrifuged in centrifuge machine at 2000 – 3000 rpm for 4 to hrs. Then supernatant liquid was filtered through Whatman's filter paper (No.41) and diluted with phosphate buffer solution 7.4 Up to 10 ml. These solution was analyzed at 269 nm using UV double beam spectrometry. The % entrapment efficiency was calculated by following formula:-

$$EE\ (\%) = \frac{(\text{Total drug content} - \text{Free dissolved drug}) \times 100}{(\text{Drug amount used})}$$

6) Surface morphology (by using optical light microscopy):

The microscopic image of pioglitazone loaded polymeric nanosuspension shows the surfactant is attached to the polymeric surface which is use to stable the polymeric nanoparticles

7) pH – determination by using pH meter:-

pH of formulation can be measured by using Digital pH meter.

8) In-vitro drug release test and dissolution velocity:-

The In-vitro drug release of pioglitazone was studied by using Dialysis membrane – 110 bags (cut –off 3500 Da) system. The dissolution medium used was freshly prepared by 0.1N HCL and phosphate buffer 7.4. The dialysis membrane -110 bags previously soaked overnight in the dissolution medium. One end of bag is tied. Then 5 mg drug was placed in bag and another end of bag is tied. These bag is suspended in 200 ml dissolution medium. Temperature maintained at 37 ± 1^{0}C. The dissolution medium was stirred at 100 rpm speed. Each 3 ml solution was withdrawn at hourly interval time and replaced by equal volume of receptor medium then diluted with the phosphate buffer 7.4 and analyzed by UV-Visible double beam spectrometry.

9) Data Analysis:-

The In-vitro drug release data of nanosuspension calculated by using various kinetic model like Zero order, First order, Matrix and Korsmeyer-Peppas model.

10) Accelerated Stability studies:-

The sample of nanosuspension was keep in glass vial for 3 months at room temperature (40°C). After 3 months the sample was observed by using different parameter like physical appearance, sedimentation, % drug content, % drug entrapment, in vitro, particle size was performed using zeta potential/particle mastersizer 2000.

RESULTS AND DISCUSSION

1. Solubility Studies:-

Solubility of drug can be calculated by using Calibration equation method using UV-visible spectrophotometer.

Table no.2- solubility profile

Solvents	Inference
Water	151µg/ml
Chloroform	67 mg/ml
Methanol	211 mg/ml

2) Particle size and zeta potential values of Pioglitazone loaded polymeric nanosuspension preparation:-

Table no.3: - Particle size and zeta potential

Batch no.	Drug-to-polymer ratio	Average Particle size* nm	Zeta potential* Mv
Pioglitazone : Eudragit S100			
S2	1 : 2	821± 12.35	7.63 ± 0.008
Pioglitazone : Eudragit L100			
L2	1 : 2	751 ± 11.34	9.03 ± 0.020

3) Pioglitazone loaded nanosuspension preparation:-

Table no.4: % Drug content and % percentage entrapment efficiency of pioglitazone loaded nanosuspension

Batch no.	Drug-to-polymer ratio	% Drug content*	% Drug entrapment efficiency*
Pioglitazone : Eudragit S100			
S1	1 : 1	84.64± 0.67	67.86± 0.15
S2	1 : 2	83.21± 0.27	70.36±0.49
S3	1 : 3	86.78± 0.29	68.56± 0.38
Pioglitazone : Eudragit L100			
L1	1 : 1	80.71± 0.63	65.70± 0.28
L2	1 : 2	84.28± 0.54	73.93± 0.42
L3	1 : 3	85.71± 0.63	71.43± 0.96

*** All values are expressed as Mean ± SD, n = 3.**

4) Surface Morphology:-

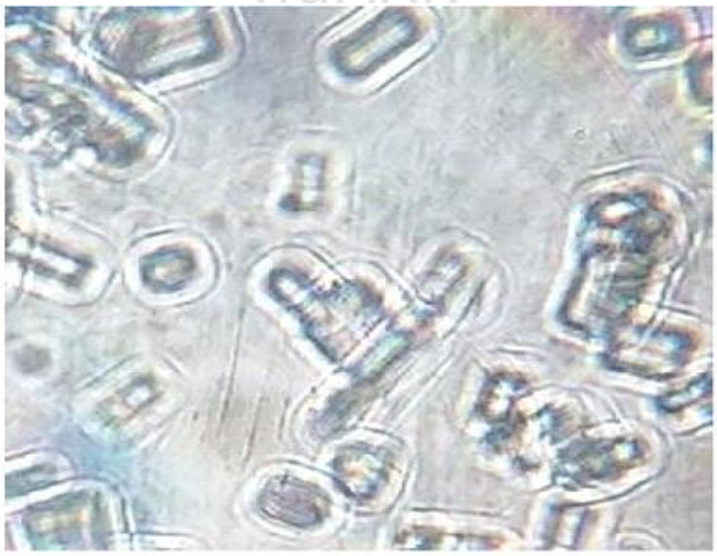

Fig:1 Microscopic image of Pioglitazone Loaded Polymeric nosuspension

5) pH – determination by using pH meter:-

Table no 5 - pH of Pioglitazone loaded polymeric nanosuspension preparation

Batch no.	pH*
S1	6.76 ± 0.034
S2	6.87 ± 0.031
S3	6.57 ± 0.033
L1	5.88 ± 0.021
L2	6.15 ± 0.045
L3	6.28 ± 0.028

6) In- Vitro drug release test and dissolution velocity:

Table no.6: In-vitro Pioglitazone release from the Eudragit S100 & Eudragit L100 loaded nanosuspension formulations

Sr. No.	Time In Mins	% Drug Release Of Optimized Batch
1.	720	95.52

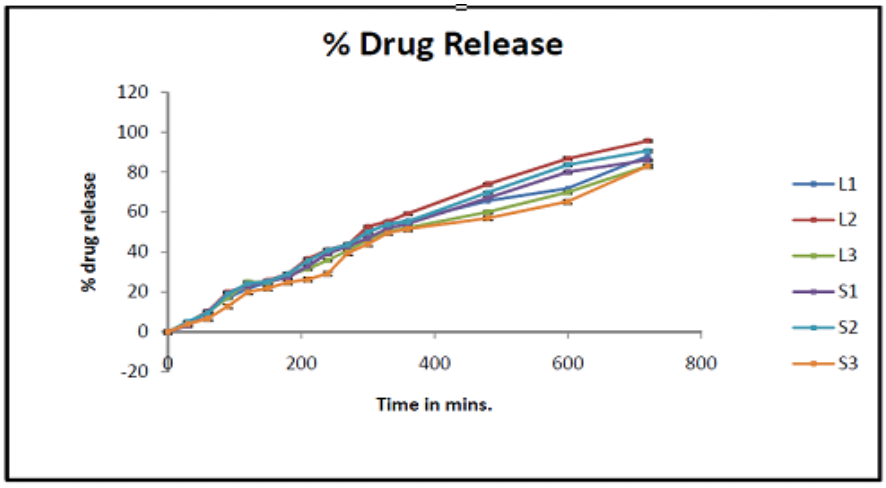

Fig no 2 -Comparative In-vitro drug release

7) Data analysis:-

Table no 7: Release kinetic models for Pioglitazone loaded nanosuspension

Batch	Mathematical models (r^2 values)				Best fit model
	First order	Zero order	Higuchi's matrix	Peppa's plot	
L1	0.999	0.984	0.998	0.993	First order
L2	0.989	0.994	0.996	0.993	Higuchi's order
L3	0.986	0.984	0.987	0.972	Higuchi's order
S1	0.995	0.988	0.998	0.986	Higuchi's order
S2	0.992	0.986	0.997	0.980	Higuchi's order
S3	0.969	0.963	0.971	0.965	Higuchi's order

8) Accelerated stability studies:-

Table no 8: Accelerated stability studies data interpretation. Batch L2

Evaluation Parameters	Before stability storage	After three month
		Temperature 40±2 ℃ & 75 ± 5 % RH
Physical appearance	Clear	Clear
Sedimentation	Not Observed	Not Observed
% drug content	84.28%	83.78
% entrapment efficiency	73.93%	73.84
Particle size* nm	751	751
pH	6.15	6.11
% *in-vitro* release	95.57%	94.89

EXPERIMETAL WORK:-

A) UV- Visible spectrophotometric characterization:

Calibration curve of Pioglitazone

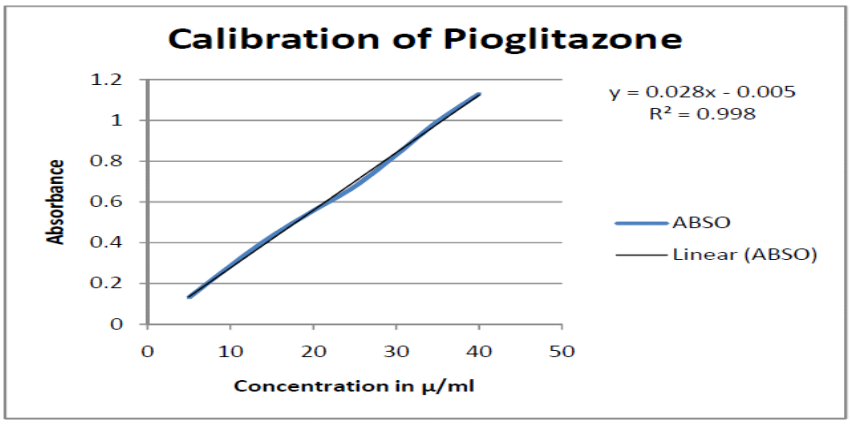

Fig no 3: Calibration curve of Pioglitazone in phosphate buffer 7.4

B) Interpretation of IR spectrum:

Shows peaks observed at different wave numbers and the functional group associated with these peaks.

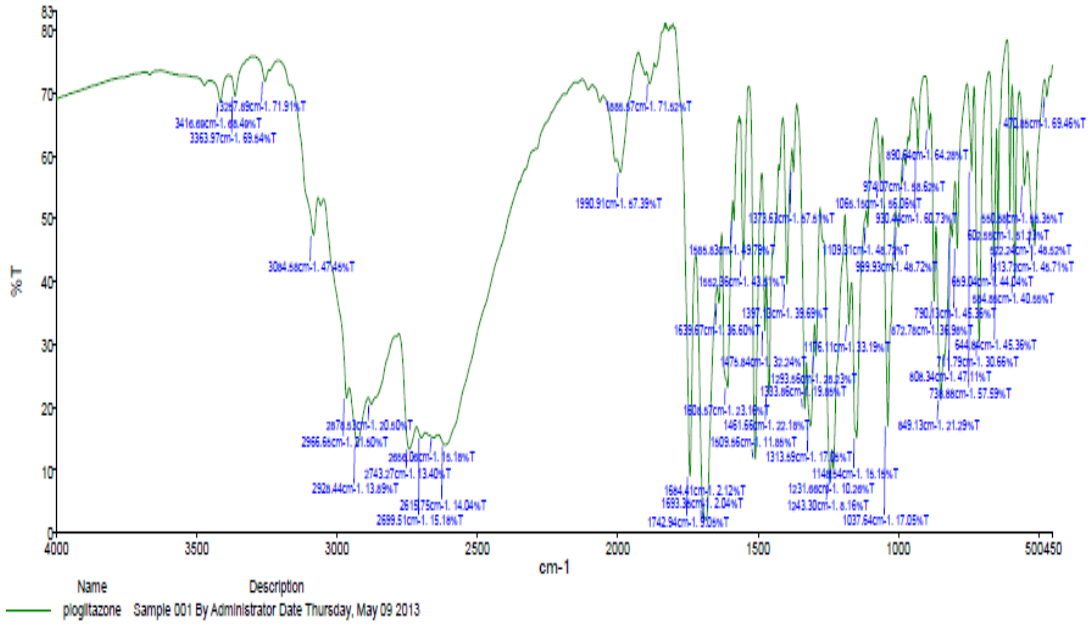

Fig no: 4 (a) Structure of Pioglitazone; (b) IR spectrum of Pioglitazone

Table no: 9 Characteristic frequencies in IR spectrum of Pioglitazone

Functional Group	IR value
-NH Strech	3363 cm^{-1}
-CH Strech for CH_2 Group	2966, 2928 cm^{-1}
Asymetric C=O	1742, 1693 cm^{-1}
C=C Strech	1608, 1509 cm^{-1}
CH_2 deformation	1475 cm^{-1}

C) Differential scanning calorimetry (DSC):

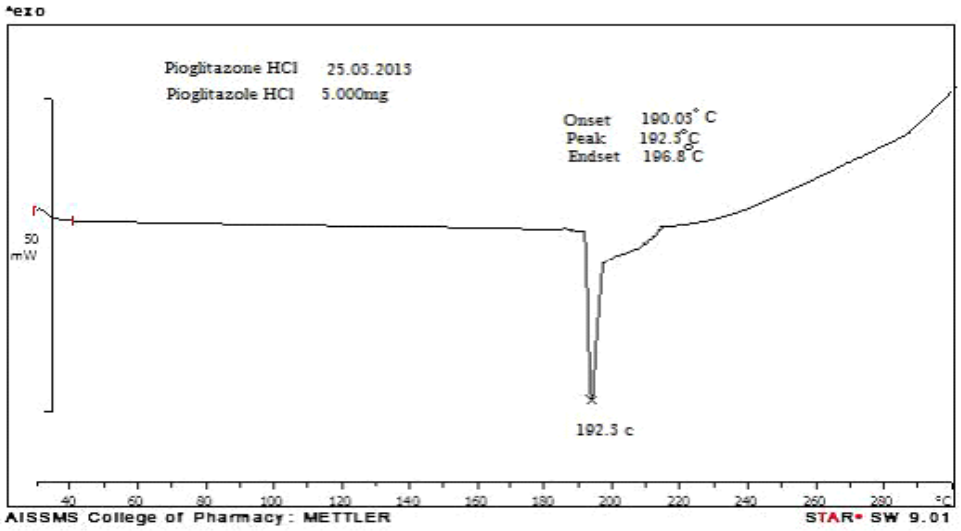

Fig no: 5 DSC

D) X-ray Diffraction Study (XRD):

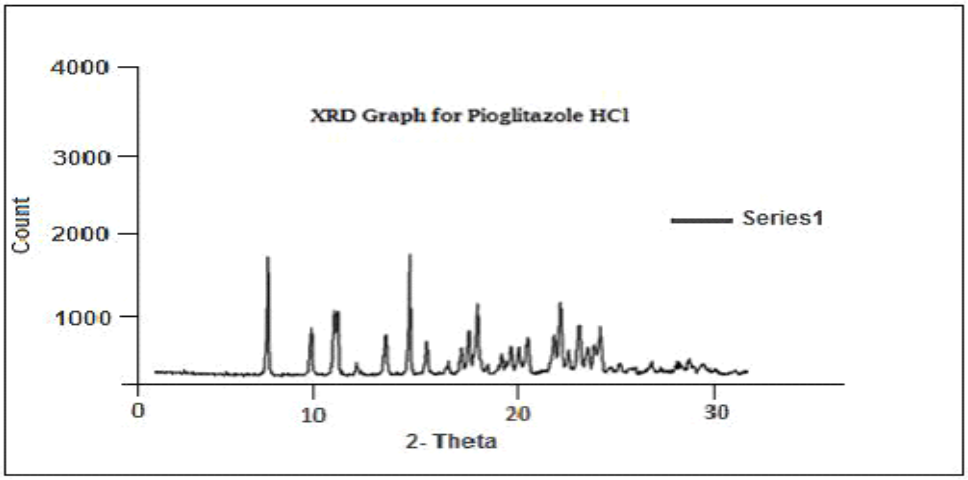

Fig no: 6 XRD

CONCLUSION

It was found that the suspension formulation containing nanonized pioglitazone with the potential of eudragit L100 and eudrajit S100 which is prepared by emulsification-solvent evaporation method give rapid dissolution profile.

The nanosuspension of pioglitazone were found to produce instant drug release. The finding indicates that the nanosuspension technology was suitable for improving solubility, dissolution rate and duration of action and oral bioavailability of poorly soluble drug.

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