International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 330-349 www.ijprajournal.com ISSN: 2249-7781

Formulation Design, Charaterization and Antidiabetic Activity of Pioglitazone Nanosponges on *in-vivo* Animal Model

Shripathy D*[1], Shabaraya AR[2]

1,2,

Srinivas college of pharmacy Valachil, Mangalore, Karnataka, India

Submitted: 09-03-2023 Accepted: 18-03-2023

ABSTRACT

Nanosponges are the new and advanced drug delivery systems which have currently emerged as an outcome of rapid advances in nanotechnology. Nanosponges can be loaded with hydrophobic as well as hydrophilic substancesand they can easily improve the solubility of poorly water soluble molecules. Based on these advantages, some research was focused on the preparation and optimized nanosponges of Pioglitazone The nanosponges were prepared by the emulsion solvent evaporation method using high-speed homogenization using Ethyl cellulose (EC) and Polyvinyl alcohol (PVA). The chemical compatibility studies of Pioglitazone with excipients was carried out using FT-IR Spectrometer. It revealed that no interaction between the drug and excipients. Formulation study design was done using central composite design. The independent variables selected were Ethyl cellulose (A), Polyvinyl alcohol (B) and Stirring speed (C) and the dependent variable choosen were Particle size (Y1), percentage loading efficiency (Y2) commulative percentage of drug releaase(Y3).The particle size of prepared nanosponges in the range of 200-800 nm. The percentage yield was found to be between 70 to 80%. The formulation showed Cumulativepercentage drug release from 83 % to 90%. All the formulations followed zero-order release kinetics. Stability study of nanosponge formulation resulted in good stability.The antidiabetic study data were analyzed using one-way ANOVA followed by Dunette's test for significant differences among the various animal groups. The treated group caused a significant reduction of blood glucose level as compared to diabetic control ($p < 0.01$)OPNS1, and OPNS2 groups showed significant reduction ($p < 0.01$) in glucose levels at the end of study (21 days). In the case of O**PNS1**and O**PNS2**percent reduction of glucose level was higher than when compared with Pioglitazoe only ($p < 0.01$). However, on increasing dose **(OPNS2)** from 10 mg/kg to 15 mg/kg, no significant variation in blood glucose level was observed, therefore the Pioglitazone nanosponges could be administered in a dose of 10 mg/kg

KEYWORDS: Nanosponges, Pioglitazone emulsion solvent diffusion method

I. INTRODUCTION

Nanomedicine brings about the revolutionary improvement and development in the medical sciences [1] Applying nanotechnology in the medicines by employing nanoscale materials could be useful to monitor, control, construct and repair the biological systems [2]. In recent years, pharmaceutical scientists have explored nanotechnology for temporal and targeted drug delivery systems [3]. There have been various nanocarriers systems including metallic, polymeric-nanoparticles), nano-suspension, nano-tubes and nanosponges (NS) [4] extensively used for the effective treatment of infectious diseases, besides the commercial application in the consumer products.[5] Pioglitazone is a slightly hydrophobic small molecule (logP = 2.3; experimental value from Human Metabolome Database) commonly used in treatment, or progression control, of type 2 diabetes [6,7]. This drug acts by principally stimulating the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ), increasing, therefore, the sensitivity of peripheral tissues to insulin, reducing gluconeogenesis resistance in the liver and finally inhibiting macrophage activation. Application of Pioglitazone is seriously limited due to its low and pH-dependent solubility, low half-life in plasma due to rapid liver metabolism[8,9], In this regard, the emulsification-solvent evaporation methods were suggested for encapsulating hydrophobic drugs.[10] The purpose of the study was to formulate nanosponges containing Pioglitazone for the management of diabetes.

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II. MATERIALS

List of ingredients used:Pioglitazone (PIO), Ethylcellulose (EC), Polyvinyl alcohol (PVA) and Dichloromethane (DCM).

III. METHODS

PREFORMULATION STUDIES{11,12,13,14,15]

Absorbance maxima of Pioglitazone

Absorption maxima for Pioglitazone in phosphate buffer 7.2 pHwere determined by scanning the 20 mcg/ml concentration of drug solution within a range of 400 to 200 nanometres using a UV-Visible spectrophotometer

Standard Calibration curve of Pioglitazone in phosphate buffer 7.2 pH

Standard stock solutions A- weighed 50 mg of Pioglitazone was transferred into 100 ml volumetric flasks separately and then dissolved and made up to 100 ml with 0.1N HCl of pH 1.2 and phosphate buffer 7.2 pH, separately to get a concentration of 500 mcg /ml.

Standard stock solution-B

Accurately pipetted 4 ml from stock solution-A and transferred into 100 ml volumetric flask made up to 100 ml with of 0.1N HCl of pH 1.2 and phosphate buffer 7.2 pH, separately to result in a concentration of 20 mcg /ml of both the drugs

Dilutions

2,4,6,8 and 10 ml of standard stock solutions B were diluted with 10 ml phosphate buffer 7.2 pH, to obtain concentrations of 4,8,12,16 and 20 mcg/ml and absorbance was measured at λ max value using UV-Visible Spectrophotometer and plotted standard calibration curve.

Solubility detrminations

Solubility of Pioglitazone was determined in solvents: water, 0.1N HCl of pH 1.2, distilled water and phosphate buffer 7.2 pH separately. An excess amount of sample was added in 10 ml of solvent with sonication for one hour, at a temperature of 25 ± 0.5°C for 48 h, and sonicated using a sonicator (Electrolab^{TM}). Samples were filtered and assayed spectrophotometrically for drug content at absorbance maximum of respective drugs

Determination of melting point

The sample packed in a capillary tube was attached to the thermometer and held through a thread. The Thieles tube was heated using a Bunsen burner and the rate of temperature increase was carefully controlled. And the melting point was noted.

Fourier transforms infrared spectroscopy study (FTIR)

Drug polymer interactions were studied by using an FTIR spectrophotometer (Shimadzu, FT-IR-8400). FTIR analysis was carried out by the KBr pellet method. The sample was mixed with KBr and compressed into a disc in a manual press. The spectrum was scanned from 4000 to 400 cm^{1}

Differential scanning calorimetry / thermo gravimetric analysis (DSC/TGA)

The thermal behavior of the sample was determined by Simultaneous Differential Scanning Calorimetry/ Thermo Gravimetric Analysis-DSC/ TGA (TA Instruments SDT-Q600 Simultaneous TGA / DSC).

CHRETERIZATION OF PREPARED NANOSPONGES [16, 18, 19]

Particle size distribution and Zeta Potential

The particle size distribution and zeta potential were determined in water as a dispersion medium by laser diffraction size analyzer, Malvern Zetasizer (Model: ZS 200).

The Percentage Yield.

Determined by calculating accurately the initial weight of the raw materials and the last weight of the nanosponge obtained

$$\text{Percentage yield} = \frac{\text{Practical mass of nanosponge}}{\text{Theoretical mass (drug + polymer)}} \times 100$$

Weighed 50 mgof Pioglitazone loaded nanosponges were dispersed in 10 ml methanol and sonicated for one hour, centrifuged at 5,000 rpm for half an hour the supernatant was withdrawn and suitably diluted with phosphate buffer 7.2 pH.With the help of the standards curve. The Percentage Drug loading(%DL) was calculated by the following equation

$$\textbf{Percentage Drug loading} = \frac{\textbf{Practical drug content}}{\textbf{Theoretical drug content}} \times 100$$

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In -vitro drug release studies

The in-vitro release of nanosponges ofPioglitazone loaded nanosponges (dose equivalent) was placed in a dialysis bag secured with a clamp at each end and immersed in dissolution media. Dissolution is performed using USP type II dissolution test apparatus (Electro lab, India) in 900 ml phosphate buffer (7.2 pH) for 3, 4, 5, 6, 8, 12, 24, at 37 ± 0.5 ° C and stirring rate of 50 rpm. Samples (5 ml) were collected periodically and replaced with an equal volume of fresh dissolution medium on each occasion. the concentration of pioglitazone was determined spectrophotometrically using UV-Visible spectrophotometer (Jasco V530, Japan).

Evaluation of drug release kinetics

To investigate the mechanism release from nanosponges ofPioglitazone.The release data was analyzed for zero order, first order, Higuchi model, and Korsmeyer-Peppas model. The data was presented in graphical representation and regression analysis was performed. Mt versus t (zero-order), Log cumulative percentage of the drug remained versus t (first-order) was calculated by linear regression analysis

Stability studies on optimized formulation

The accelerated stability studies were carried out on optimized nanosponges ofPioglitazone using a sealed vial and placed in stability chambers maintained at 25 °C ± 2 °C/60% RH ± 5% RH and 30 °C ± 2 °C/65% RH ± 5% RH The formulations subjected to stability tests were analyzed for zero, one, three and six months for its % CDR and % Drug content.

IN-VIVO ANIMAL STUDY[20,21]

Streptozotocin-induced antidiabetic activity of optimized Pioglitazone nanosponges

Routes of drug administration

The vehicle, standard drug, and test drugs were administered orally with the help of anoral feeding needle (infant feeding syringes).

Experimental animals

Healthy Swiss albino rats (180-200g) of either sex was used for the experiments. They were maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5%, and 12 h light/ dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to a standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. (Ref: SCP/IAEC/ F150/P105/2016 dated 6/08/2016). All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Streptozotocin-Induced Anti-Diabetic Activity

Fasting blood glucose was determined after depriving food for 16h with free access to drinking water. Hyperglycemia was induced by a single i.p injection of 65 mg/kg of STZ in citrate buffer, freshly prepared and injected within 5 minutes of preparation to prevent degradation. After administration of STZ, the animals had free access to feed and water ad libitum. The development of hyperglycemia in rats was confirmed by fasting blood glucose estimation 48 h post-STZ injection wherein the animal fasted overnight again for blood collection from the tail vein. The rats with fasting blood glucose levels of above 200 mg/dl at 48 h after STZ injection were considered diabetic and included in the study.

Experimental design [22,23]

Pioglitazone Nanosponges

Animals will be randomly divided into 4 groups of 6 each and assigned as below.

Group I: Vehicle control (Citrate buffer).

Group II: Diabetic control (Streptozotocin 65mg/Kg). (i.p)

Group III: Diabetic Rats + Pioglitazone (10mg/Kg)

Group IV: Diabetic Rats + Pioglitazone NanospongesOPNS1 (10 mg/kg)

Group V: Diabetic Rats + Pioglitazone NanospongesOPNS2(10 mg/kg) andOPNS2 (15 mg /kg)

Collection of blood and serum samples:

The above treatment was carried out in each group of animals for 30days. Bloodsamples were withdrawn under mild anesthesia from the tail tip of the overnight fastedanimals on the 1st, 7th, 14th and 21st day. Fasting blood glucose was measured usingsingle touch glucometer

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IV. RESULTS AND DISCUSSIONS

Absorbance maxima of Pioglitazone

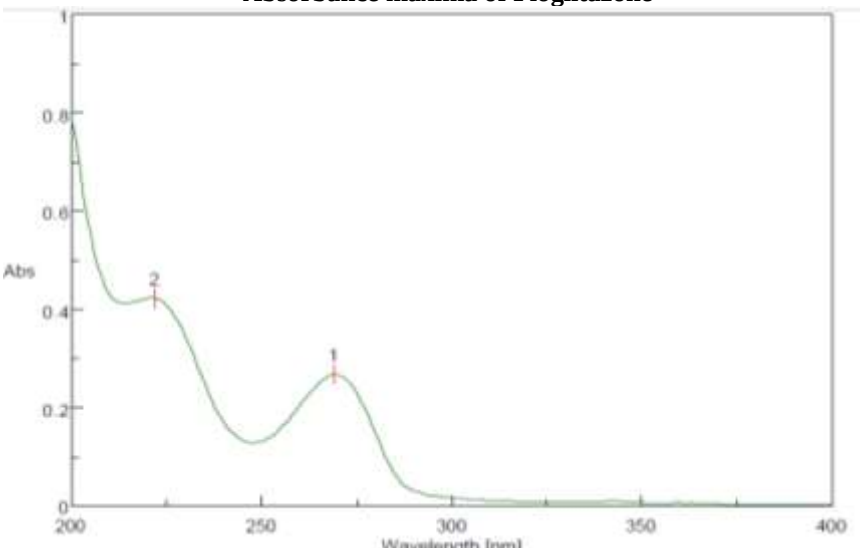

Fig.1 UVspectra of Pioglitazone in inPhosphate buffer 7.2 pH

Table 1: Absorbance values of Pioglitazone in Phosphate Buffer 7.2 pH

Sl.No.	Concentration in mcg/ml	Absorbance*	Standard* Deviation (±)
1	5	0.198	±0.021
2	10	0.342	±0.006
3	15	0.543	±0.078
4	20	0.692	±0.089
5	25	0.875	±0.014
6	30	0.997	±0.045

* SD- Average of three determinations

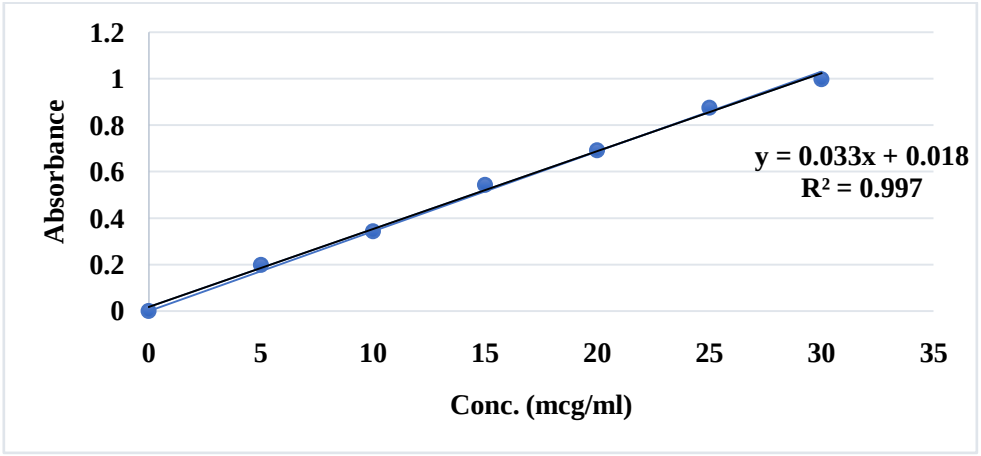

Fig. 2: Calibration Curve of Pioglitazone in Phosphate Buffer 7.2 pH

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Table2 : Solubility study results of Pioglitazone

Drug	Medium	Tem.^{0}C	Concentration mg/ml
Pioglitazone	**0.1N HCl 1.2 pH**	**25**	**0.068**
	Phosphate Buffer 7.2 pH	**25**	**0.057**
	WATER	**25**	**0.023**

Table 3: Melting point of Pioglitazone

Melting point	Reported	Observed
Pioglitazone	183 to 184 ^{o}c	185 ^{o}c

DRUG POLYMER INTERACTION STUDY BY FTIR

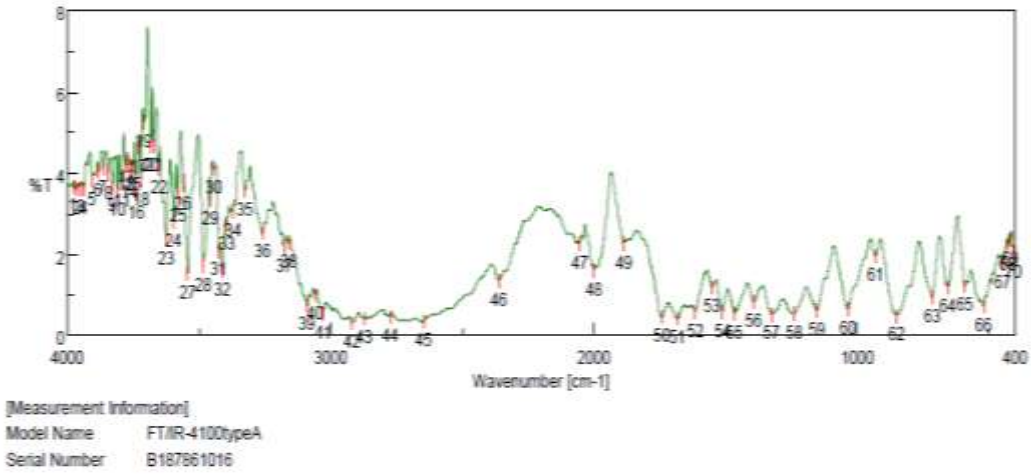

Fig. 3: FT-IR spectra of Pioglitazone

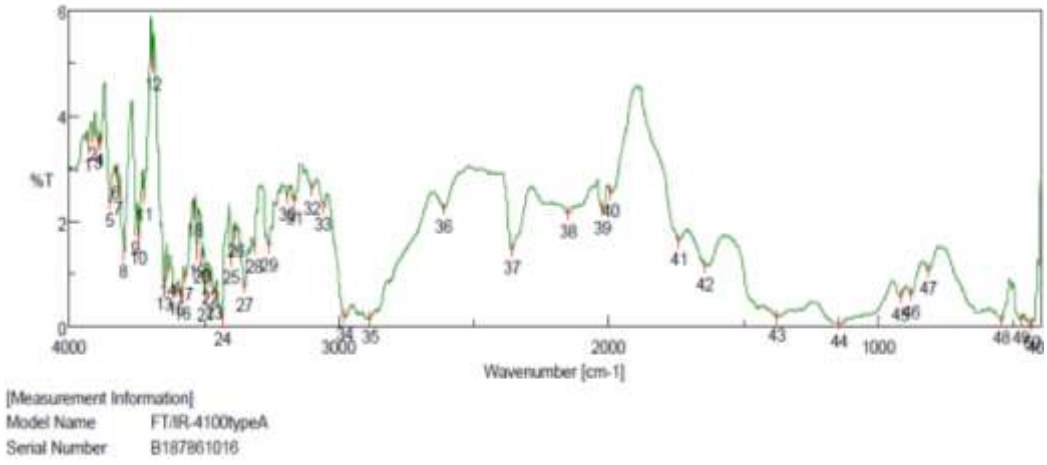

Fig.4:FT-IR spectra of Ethyl Cellulose

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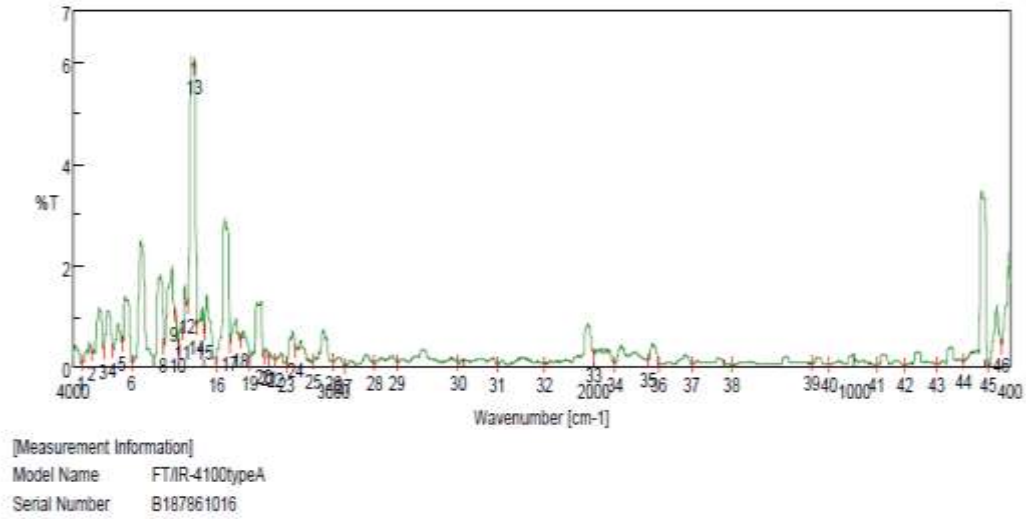

Fig.5: FT-IR spectra of PVA

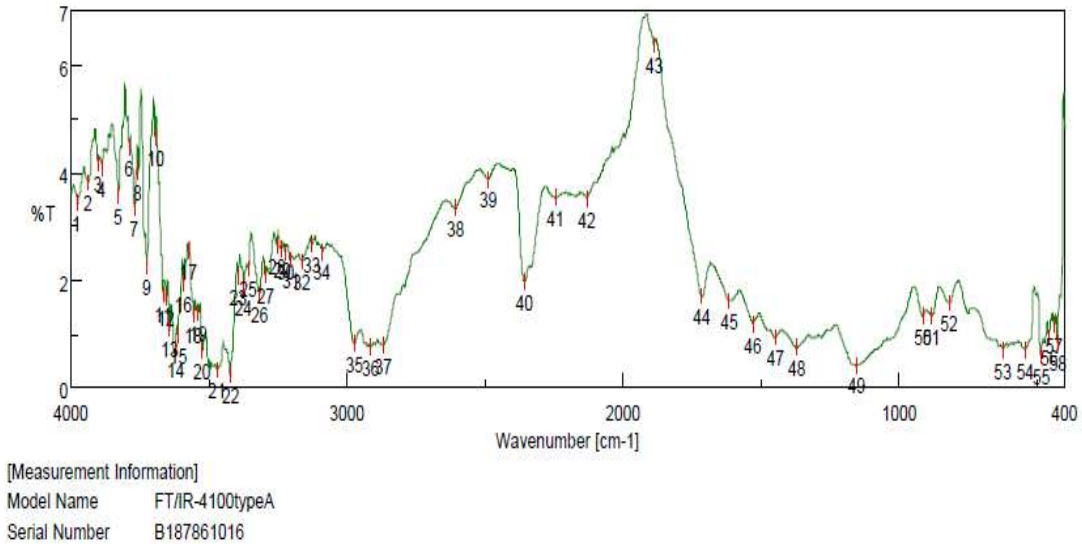

Fig.6: FT-IR spectra of preliminary formulation containing Pioglitazone byEmulsion Solvent Diffusion Method

Table 4: Peaks observed in FT-IR spectra of Pioglitazone

Description	Pioglitazone cm-1	Polymers with Pioglitazone . cm-1
C-H stretching	3084, 2996	3045
C=C stretching	16101,680,	1620
C=O stretching	1650,1743	1755
C-S stretching	1225,1243	1263

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DSC-TGA ANALYSIS

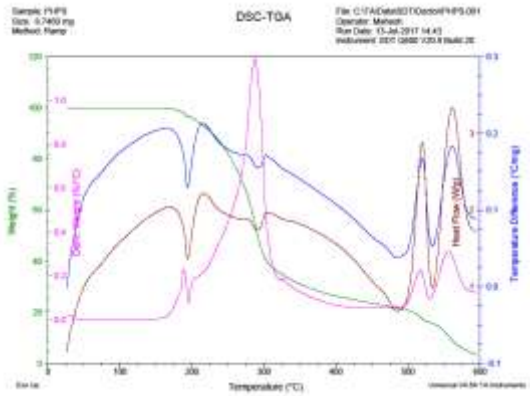

Fig.7:DSC-TGA analysis of Pioglitazone

FORMULATION OF PIOGLITAZONE NANOSPONGES BY EMULSION SOLVENT DIFFUSION METHOD

Table 5: Designing of experiments

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Independent variables	Level used (actual, coded)			
	Low (mg)	High (mg)	Low coded	High coded
Factor (EC) -X_1	100	300	-1.00	1.00
Factor (PVA) -X_2	0.75	2.00	-1.00	1.00

Table 6: Nanosponges containing Pioglitazone was prepared by Emulsion Solvent Diffusion method

			X1-Factor 1	X2-Factor 2	Response-Y1	Response Y1	Response Y1
Std	Run	Space Type	A:EC	B:PCA	Percentage Loding Efficiency (%LE)	Cummulative Percentageof Drug Relese (%CDR)	Particle Size (nm)
PN1	8	Factorial	100	1			
PN2	4	Factorial	300	1			
PN3	3	Factorial	100	2			
PN4	6	Factorial	300	2			
PN5	1	Axial	58.57	1.5			
PN6	2	Axial	341.42	1.5			
PN7	5	Axial	200	0.75			
PN8	7	Axial	200	2.25			
PN9	9	Center	200	1.5			

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PARTICLE SIZE ANALYSIS

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	248.8	Peak 1:	380.9	100.0	199.6
PdI:	0.402	Peak 2:	0.000	0.0	0.000
Intercept:	0.662	Peak 3:	0.000	0.0	0.000
Result quality	Good				

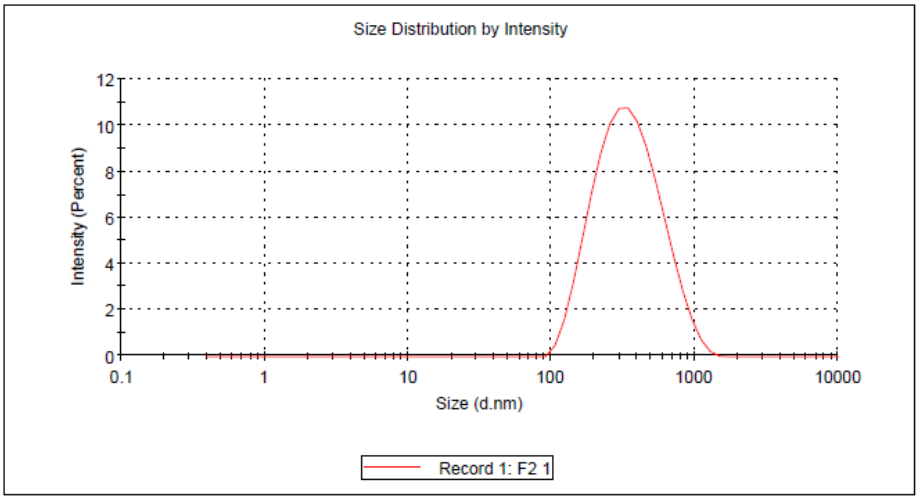

Fig. 8: Particle size of formulation PN1

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	307.2	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	803.1	Peak 1:	688.5	92.9	189.7
PdI:	0.321	Peak 2:	5188	7.1	481.3
Intercept:	0.865	Peak 3:	0.000	0.0	0.000
Result quality	Good				

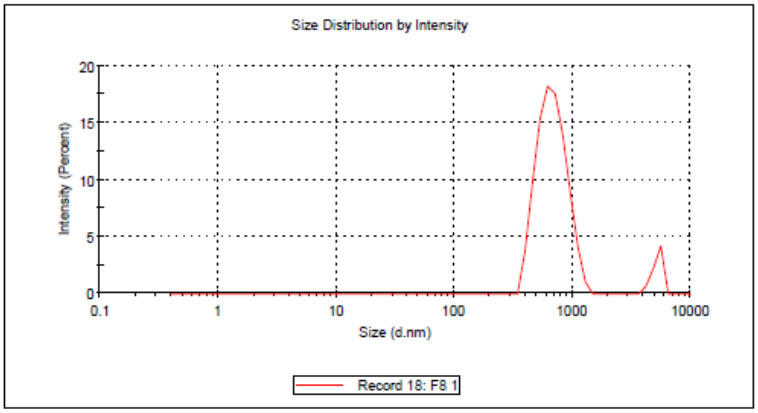

Fig.9: Particle size of formulation PN2

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System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	482.3	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	548.0	Peak 1:	463.0	100.0	75.50
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	0.682	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

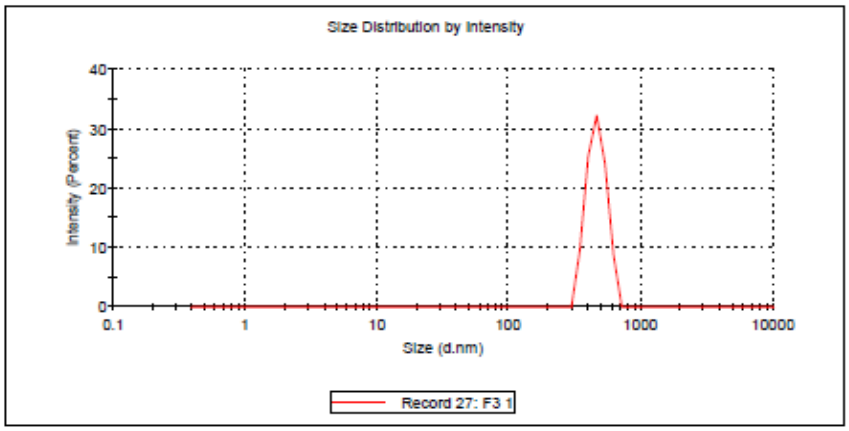

Fig. 10: Particle size of formulation PN3

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	495.6	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	10

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	921.6	Peak 1:	893.9	100.0	160.0
PdI:	0.456	Peak 2:	0.000	0.0	0.000
Intercept:	0.690	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

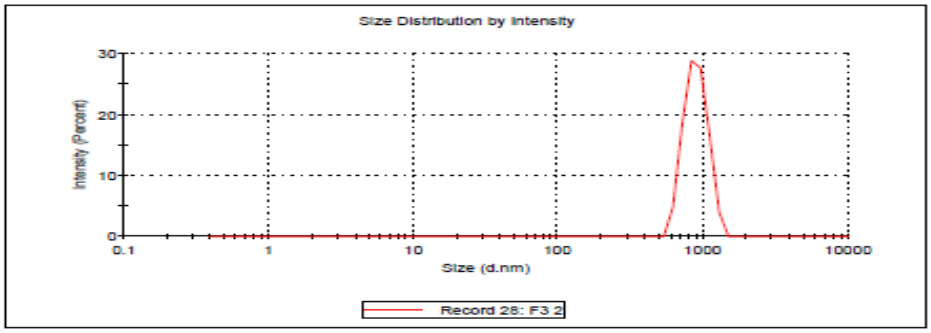

Fig. 11: Particle size of formulation PN4

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System

Temperature (°C):	25.0	Duration Used (s):	70
Count Rate (kcps):	220.7	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	756.9	Peak 1:	645.0	100.0	95.75
PdI:	1.000	Peak 2:	0.000	0.0	0.000
Intercept:	0.731	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

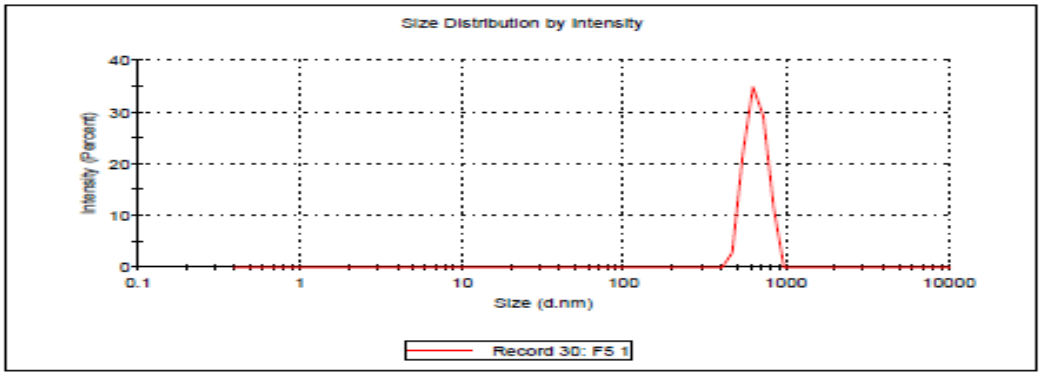

Fig. 12: Particle size of formulation PN5

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	409.6	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	11

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	201.0	Peak 1:	208.5	100.0	64.64
PdI:	0.313	Peak 2:	0.000	0.0	0.000
Intercept:	0.909	Peak 3:	0.000	0.0	0.000
Result quality	Good				

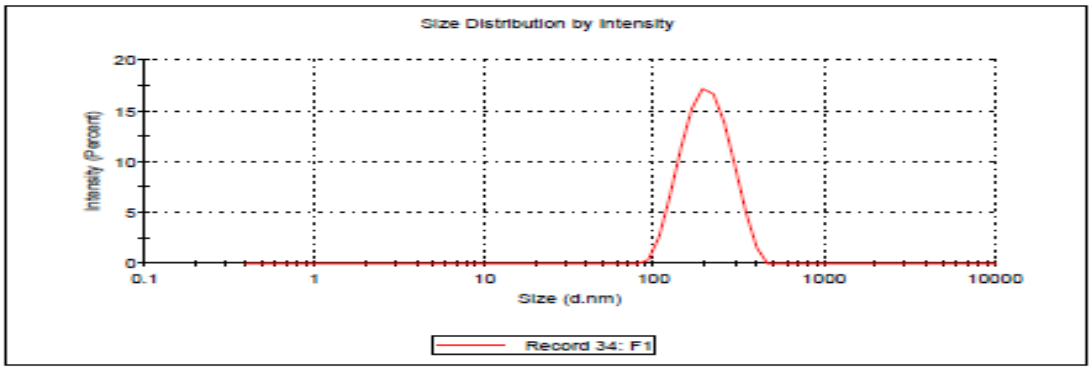

Fig. 13: Particle size of formulation PN6

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System

Temperature (°C):	25.0	Duration Used (s):	70
Count Rate (kcps):	229.5	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	230.2	Peak 1:	322.1	84.2	119.4
PdI:	0.602	Peak 2:	57.90	11.8	14.51
Intercept:	0.906	Peak 3:	5226	3.9	458.7
Result quality	Good				

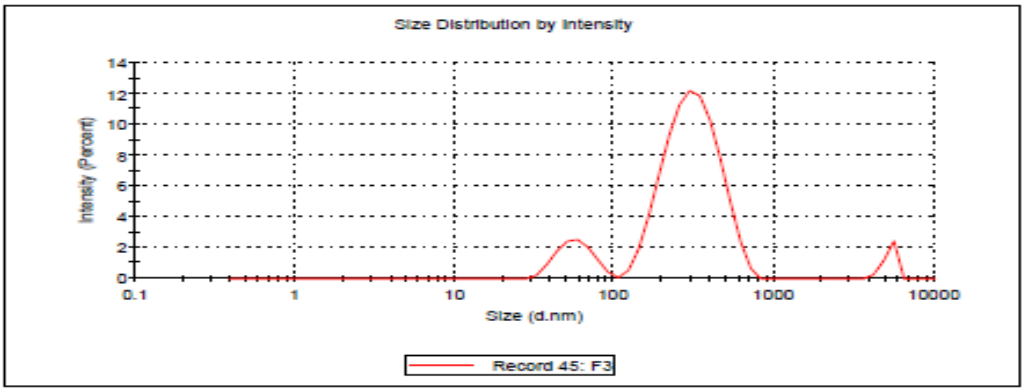

Fig. 14: Particle size of formulation PN7

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	216.9	Peak 1:	238.8	71.1	140.2
PdI:	0.446	Peak 2:	1307	28.9	630.8
Intercept:	0.909	Peak 3:	0.000	0.0	0.000
Result quality	Good				

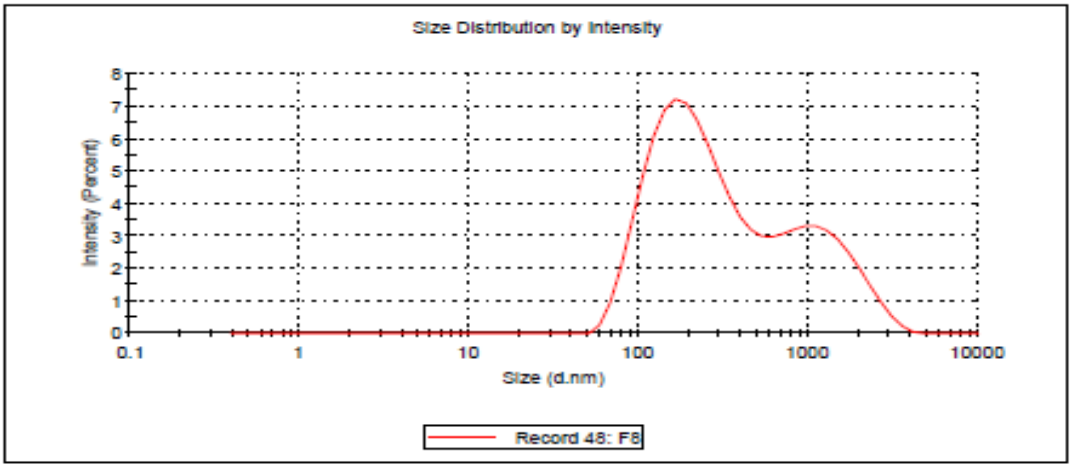

Fig. 15: Particle size of formulation PN8

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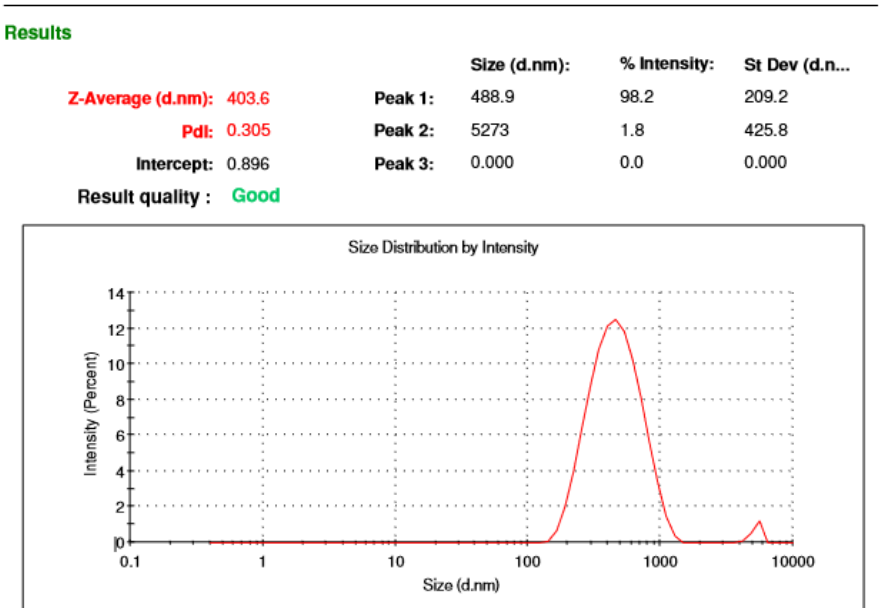

Fig. 16: Particle size of formulation PN9

(%LE)Cummulative Percentage of Drug Relese (%CDR) and percentage yield(%YL) of Pioglitazone nanosponges

Table 7: The particle size analysis, zeta potential, polydisperse index(PDI), Percentage Loding Efficiency

Code	Particle Size (nm)	Poly dispersive index (PDI)	Zeta potential (mV)	Percentage Loding Efficiency (%LE)	Percentage Yield (%YL)	Cummulative Percentage of Drug Relese (%CDR)
PN1	248	0.626	-13..20	53%	72%	96.02%
PN2	803	0.648	-24.39	74%	60%	97.67%
PN3	548	0.603	-08.49	49%	75%	96.35%
PN4	495	0.451	-11.22	67%	63%	95.18%
PN4	756	0.526	-13.34	45%	55%	98.78%
PN5	201	0.446	-12.89	73%	68%	94.61%
PN7	230	0.620	-09.54	66%	58%	95.91%
PN8	216	0.408	-11.78	57%	71%	96.04%
PN9	403	0.210	-13.78	59%	72%	92.86%

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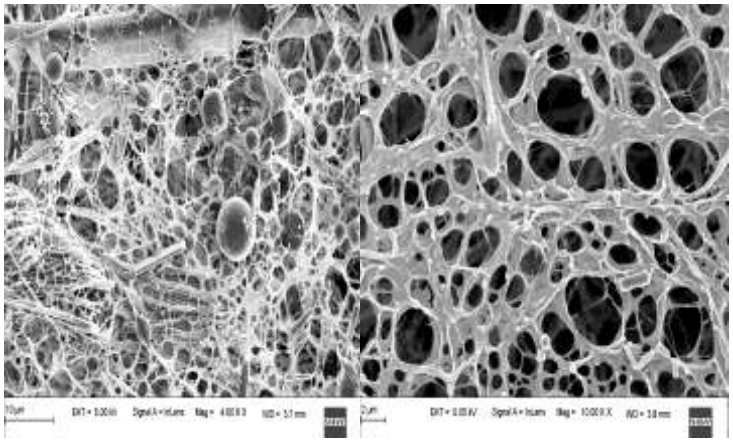

Fig17: SEM image of formulation PN1

IN- VITRO DISSOLUTION STUDIES OF NANOSPONGES OF PIOGLITAZONE INPHOSPHATE BUFFER 7.2 pH

Table8:Cummulativepercentage drug release of Pioglitazone Nanosponges prepared by emulsion solvent diffusion method in Phosphate Buffer 7.2 pH

Time(hr)	% CDR *								
	PN1	PN2	PN3	PN4	PN5	PN6	PN7	PN8	PN9
1	38±1.34	38±1.56	38±0.56	38±1.45	37±1.47	33±0.45	26±1.05	31±1.87	34±0.41
2	45±1.21	43±1.01	41±0.89	43±1.39	41±1.48	38±0.67	29±1.01	35±1.91	38±0.97
3	60±1.04	55±1.80	49±0.87	47±1.87	48±1.93	48±1.34	32±1.21	39±1.57	41±1.37
4	66±0.23	58±1.30	60±1.23	59±1.27	47±0.94	52±1.47	37±1.42	41±0.94	44±1.67
5	68±0.34	66±1.42	69±1.89	62±.0.90	51±2.76	58±1.39	40±.1.57	46±1.87	48±1.89
10	72±1.43	75±1.91	71±1.45	68±0.56	54±1.30	66±1.32	61±0.51	51±1.94	59±1.34
15	75±1.98	82±1.99	78±2.45	72±1.78	72±1.29	72±1.62	72±0.92	68±0.23	69±1.72
20	80±0.34	88±1.89	80±1.98	82±1.39	78±1.73	83±1.29	83±1.39	78±0.83	78±1.56
24	85±1.89	93±0.23	86±1.34	92±1.34	83±1.36	92±1.43	91±1.34	89±1.01	90±1.43

*** SD of n=3**

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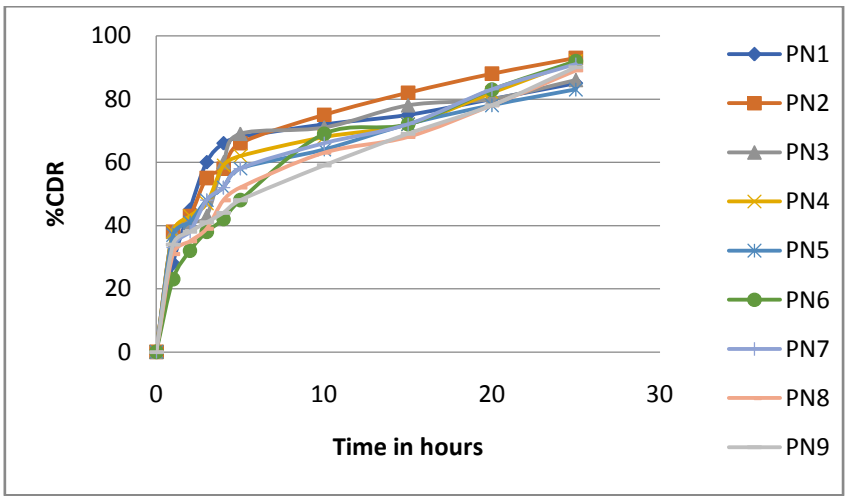

Fig. 18: In-vitro Dissolution Profile of Pioglitazone Nanosponges prepared by emulsion solvent diffusion method in Phosphate Buffer 7.2 pH

KINETICS OF DRUG RELEASE

Table 9: Comparison of correlation coefficient (r^2) and rate constant of zero-order kinetic modelsnanosponge prepared by emulsion solvent diffusion method

Formulation code	Zero-order		First-order	
	r^2	k_0	r^2	K_1
PN1	0.996	8.45	0.868	0.328
PN2	0.987	8.34	0.899	0.327
PN3	0.993	8.76	0.889	0.327
PN4	0.999	8.34	0.803	0.327
PN5	0.991	9.07	0.806	0.327
PN6	0.993	8.43	0.880	0.327
PN7	0.918	8.92	0.845	0.327
PN8	0.929	8.21	0.809	0.327
PN9	0.997	8.69	0.876	0.327

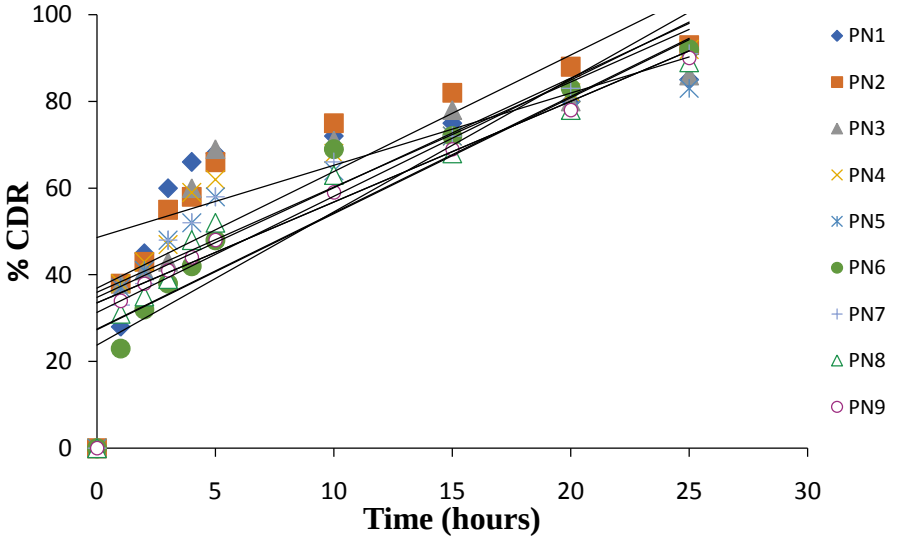

Fig.19: Zero order kinetic models of nanosponge

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Table 10: Effect of Pioglitazone NanoSponge (OPNS1 &OPNS2) on blood glucose level in STZ induced diabetic rats

Group	Blood glucose level (mg/dl)				
	Before diabetic induction	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal Control	88.32±1.38	88.84±1.08	89.04±0.98	89.67±1.56	89.65±2.17
Diabetic control DC	88.45±4.45	258.18±3.62	263.54±4.67	261.09±5.15	261.98±5.92
Pioglitazone (10mg / Kg) PIO	88.06±4.36	259.43±7.65	229.32±5.23**	199.74±6.29**	165.60±5.76 **
Pioglitazone OPNS21**(10 mg/kg)**	88.42±2.13	258.36±3.65	215.62±5.32 **	184.87±2.63**	144.65±3.24 **
Pioglitazone OPNS2 **(10 mg/kg)**	88.92±3.17	259.26±2.69	205.62±6.40 **	174.87±2.21**	134.65±3.80 **
Pioglitazone OPNS2 **(15 mg/kg)**	88.23±2.88	258.45±2.63	204.21±5.91 **	173.87±2.54**	134.05±1.94 **

Values are mean ± SEM (n=6) one way ANOVA followed by Dunette's test. Where ** represents significant at p< 0.01 as compared with diabetic control group

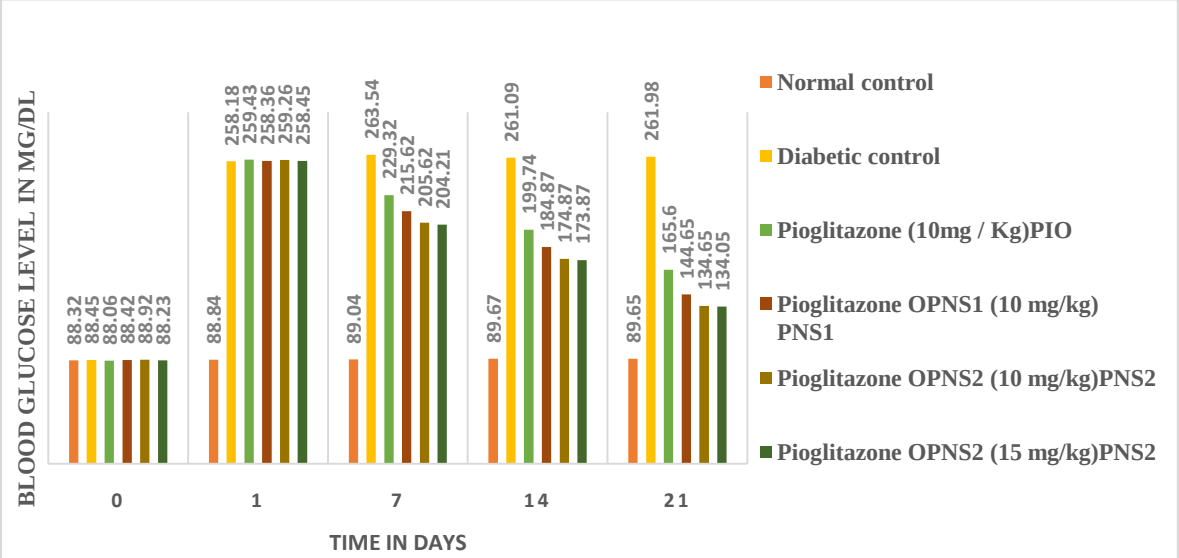

Fig.20: Effect of Pioglitazone Nanoponges (OPNS1 &OPNS2) on blood glucose level in STZ induced diabetic rats

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EFFECT ON BODY WEIGHT

Table11 :Body weight in STZ induced diabetic rats

Group	Body weight (g)				Change in body weight (%)
	Day 1	Day 7	Day 14	Day 21	
Normal	185.45±5.38	187.50±4.86	189.55±6.92	192.45±4.66	3.77
Diabetic control DC	185.50±6.32	164.65±5.67	160.85±6.25	154.25±5.25	-16.84
Pioglitazone PIO (10 mg/kg)	186.31±4.12	180.61±2.61 *	179.56±5.48**	179.05±4.65**	-3.37
Pioglitazone OPNS1 (10 mg/kg)	185.65±2.58	181.30±2.26**	180.22±4.67**	181.55±5.46**	-2.25
Pioglitazone OPNS2 (10 mg/kg)	185.35±2.30	185.30±2.26**	183.22±4.67**	182.55±5.46**	-1.51
Pioglitazone OPNS2 (15 mg/kg)	185.10±1.34*	185.60±2.34**	183.11±2.97**	181.25±1.46**	-1.41

Values are mean ± SEM (n=6) one way ANOVA followed by Dunette's test. Where ** represents significant at $p< 0.01$ as compared with diabetic control group

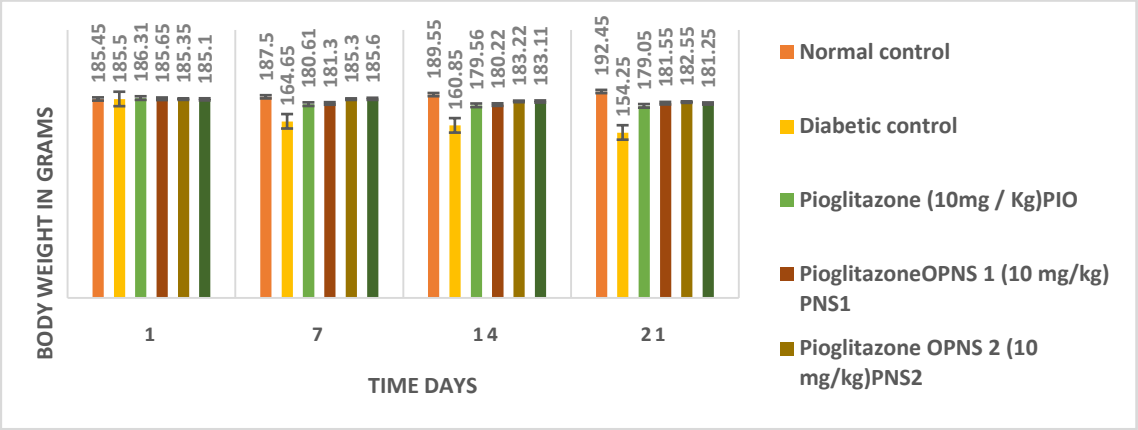

Fig.21: Effect on body weight in STZ induced diabetic rats

Histopathology studies of the pancreas in STZ induced diabetic rats:

Fig. 22: a) Diabetic control (DC) **Fig. 22: b) Pioglitazone (PIO)**

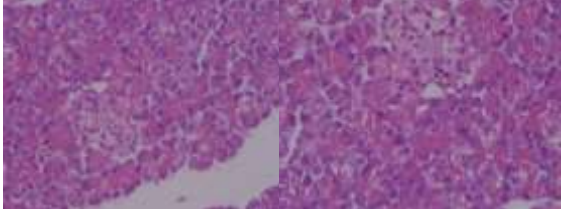

Fig.22c) :Pioglitazone Nanosponge 1(OPNS1)Fig.22 d)Pioglitazone Nanosponge 2 (OPNS2)

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V. RESULTS AND DISCUSSIONS

Scanning for Absorbance Maximum

The UV spectrum of Pioglitazone shows prominent absorbance maxima at wavelength 269 nm when scanned between 200-400nm using 0.1N HCl andPhosphate buffer 7.2 pH, The peak obtained is shown in Fig.1

Standard Graph of Pioglitazone

Calibration Curve of Pioglitazone ein 0.1 HCl of 1.2 pH showed a R^2 value of 0.9972 .The standard graph range was found to be within beers-lamberts' range of concentration i.e 05 -30 mcg/ml (Table 1and Fig.2)

Solubility Study of Pioglitazone

Solubility of Pioglitazone in 0.1N HCl of pH 1.2 was 0.068 mg/ml , and phosphate buffer pH 7.4.0.057 mg/ml.whereas it showed poor solubility in water and (Table 2)

Melting Point of Pioglitazone

Melting point of was found to be e 185 ^oC.Which complied with I. P. standards, indicating the purity of the drug sample (Table 3)

Drug Polymer Intraction Study by FTIR Spectra

FTIR spectra of Pioglitazone and polymers are shown in Fig 3,4,5 and 6 . FTIR spectra of the Nanosponges of Pioglitazone showed characteristic peaks at, 3045 cm-1 cm^{-1} corresponding to C-H stretching, 1620 cm^{-1} due to C=C stretching, 1755cm^{-1} showing C=O stretching,1263 cm^{-1} showing C-S stretching. which confirms matching of peacks with pure Pioglitazone , peaks and confirms the compatibility of the drug with polymers (Table 4)

DSC-TGA Thermogram Study

DSC-TGAthermogram (Fig 7) of pure Pioglitazone , EC, PVA, and preliminary nanosponges containing Pioglitazone respectively. DSC Thermogram of pure Pioglitazone showed a sharp endothermic peak at 188^{0}C The TG curve shows that major degradation occurs above 220^{o}C. The TGA curve shows an initial 2.2% loss corresponds to moisture content.

Dessining Of Expariments

Experimental design, the factor combinations (table 5) have resulted in 9 different formulations batches of nanosponges(Table 5) different batches of nanosponges were formulated and evaluated for these the responses., The significance of model and model terms generated were analyzed by analysis of variance (ANOVA). In polynomial equations, positive sign before the factor shows the linear correlation between response and factor, while the negative sign shows the inverse relation between the same.

Response seuface methodology is used and nine formulations PN1 to PN 9 was prepared (Table). Percentage loading effencicy of prepared PN1 to PN 9 was between 45% to 74 % and the Percentage yield between 55%-to 75%

The particle size range of formulation for PN1 to PN 9 was from 201nm to 803 nmand the zeta potential between -0.08 to -13.78 t mv. which indicated stable formulation (Table and Fig8 to 16)The SEM analysis showed spongy morphology of nanosponges (Fig 17)

In- vitro Dissolution Studies in Phosphate buffer 7.2 pH

Dissolution medium phosphate buffer 7.2 pH using USP type II dissolution apparatus

In- vitro Dissolution studies in phosphate buffer 7.2 pH (up to 24 hours) showed the Cummulative percentage release for formulation PN1 to PN 9 ranges from 85±1.89%, 93±0.2386±1%.34, 92±1.34%,83±1.36%, 92±1%.43,91±1.34%, 89±1.01%,90±1.43% The formulation PN2,PN6,PN7 and PN9 t formulation which showed % CDR above 90% respectively (Table 8 & Fig. 18).

Kinetics of drug release

Kinetics of drug release from the nanosponges containing Pioglitazone prepared by emulsion solvent diffusion with high speed homogenization method is subjected to mathematical treatment. The values of R^2 obtained and presented in Table 9, Fig19 The best fit model with highest correlation coefficient values (R^2) for the formulation codes PN1 to PN9 is between 0.991 to 0.999 indicating that the release is best fits to zeroorder kinetics release model.

EVALUATION OF ANTIDIABETIC ACTIVITY OF PIOGLITAZONE NANOSPONGES ON STREPTOZOTOCIN INDUCED DIABETIC RATES

Effect on Blood Glucose Level

Initially fasting blood glucose (FBG) level was found within the range of 80-90mg/dl in all the groups at baseline . Treatment with STZ in normal saline (50mg/kg, i.p.) had increased the FBG level more than 200mg/dl after 48 h. Changes in FBG

International Journal of Pharmaceutical Research and Applications
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level in different groups after repeated dose of drug administration are tabulated in Table10 and represented in Fig 20. Diabetic control group has showed significant increase in fasting blood glucose during the study period. Pioglitazone (10 mg/kg) significantly ($p<0.01$) reduced FBG after repeated administration as compared to diabetic control group. Whereas treatment with OPNS1 and OPNS2 still bettered the results compared to treatment with only with Pioglitazone on 1st, 7th, 14th and 21st day

Effect on body weight

Body weight of animals in all groups was recorded at 1st, 7th, 14th and 21st day. Highest change (decrease) in body weight during study period was found to be in diabetic control group. Pioglitazone and OPNS1 and OPNS2 treated group animals normalised the body weight as compared to diabetic control group.(Table 11 and Fig.21)In the present study, diabetes was induced by using Streptozotocin (STZ). Streptozotocin is a broad-spectrum antibiotic, induces diabetes in a wide variety of animal species by damaging the insulin-secreting cells of the pancreas.

Pioglitazone is a second-generation Sulphonyl urea derivative, oral hypoglycaemic agent and found to be effective in diabetic rats that retain functioning of islet β-cells. Hence the principal mechanism of action is to stimulate the production and secretion of insulin by the β-cells of the pancreas. This drug may lower down the output of glucose from the liver by an insulin-independent mechanism.

In the present study, it is found that the blood glucose levels in STZ treated rats were significantly increased as compared to normal rats. The animals treated with the standard drug Pioglitazone and those treated with Pioglitazone Nanoponge OPNS1 and OPNS2 form showed a significant reduction in glucose levels.

The results indicate that the Pioglitazone inNanoponge form (O**PNS1**and O**PNS2)** resulted in lead to an increase in the effect of Pioglitazone . The blood glucose level after oral administration of Pioglitazone and optimized Pioglitazone nanosponge formulation OPNS1and OPNS2 in STZ induced diabetic rats is presented in Table 102 and Fig.20. which represents blood glucose levels at 1st day (initial reading) and after the study i.e., 21 days (final reading) for control, untreated, and treated groups of animals (n = 6).

The data were analyzed using one-way ANOVA followed by Dunette's test for significant differences among the various animal groups. The treated group caused a significant reduction of blood glucose level as compared to diabetic control ($p < 0.01$). It is clear from the data that the blood glucose levels of diabetic control (DC) animals continued to increase till the completion of the study, whereas, in Pioglitazone , OPNS1, and OPNS2 groups showed significant reduction ($p < 0.01$) in glucose levels at the end of study (21 days). In the case of O**PNS1**and O**PNS2**percent reduction of glucose level was higher than when compared with Pioglitazoe only ($p < 0.01$). However, on increasing dose **(OPNS2)** from 10 mg/kg to 15 mg/kg, no significant variation in blood glucose level was observed, therefore the Pioglitazone nanosponges could be administered in a dose of 10 mg/kg

Histopathology studies of the pancreas in STZ induced diabetic rats:

Normal control: Normal rats showing normal acini and normal cellular population in islets of Langerhans and absence of both damage to islets and hyperplasia (Fig22b)

Diabetic control: Suggests extensive damage to the islets of Langerhans and reduced islet size in STZ induced animals.(Fig 22a)

Standard: Diabetic rats treated with Pioglitazone showing complete restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia. (Fig 22b)

Nano Sponge form of Pioglitazone : OPNS1 and OPNS2 treated diabetic rats showing restoration of normal cellular population size of islets of Langerhans and cells are partially preserved (Fig 22c and 22d)

VI. CONCLUSIONS

Absorbance maxima Pioglitazone was at wavelength 269 nm in Phosphate buffer 7.2 pH, The standard graph range was found to be within beers-lamberts' range of concentration, with a R^2 value of 0.9972. solubility in phosphate buffer of pH 7.4.was 0.057 mg/ml Melting point of was complied with I. P. standards, indicating the purity of the drug sample. Characteristic FTIR peaks ofPioglitazone and Nanosponges of Pioglitazone matched and confirmed the compatibility .The percentage loading effencicy of formulation PN1 to PN 9 ranges from 45% to 74%, and the percentage yield between 55%to 75% . Particle size of formulation PN1 to PN 9 ranges from 201nm to 803 nm and the zeta potential between -0.89 to -

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13.78 mv. In- vitro dissolution studies the %CDR was between 83% to 90% for formulation PN1 to PN9. The drug release kinetics of all formulation with r^2 value found to be between 0.991 to 0.999 in zero order model .Nanospoges of Pioglitazone (10 mg/kg) significantly reduced FBG after repeated administration as compared to diabetic control group. Pioglitazone Nanosponge formulation OPNS1, and OPNS2 groups showed significant reduction ($p < 0.01$) in glucose levels at the end of study . Histopathology studies of the pancreas in STZ induced diabetic rats showed that Pioglitazone nanosponges OPNS1 and OPNS2 treated diabetic rats restored the of normal cellular population size of islets of Langerhans.

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