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Development and Characterization of Pioglitazone Nanoparticles for the Effective Treatment of Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration between both authors. Author RAG and POK contributed to the research design and implementation, as well as the analysis of the findings and manuscript writing. Both authors read and approved the final manuscript.

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ABSTRACT

Pioglitazone is designated as a BCS class II medication since it is weakly water soluble.The goal of this study was to create starch nanoparticles for the administration of Pioglitazone in attempt to lessen dose-related side effects and maybe prolong its release in the treatment of diabetes.Using starch as a polymer, tween 80 as a stearic barrier, and citric acid to enhance stability, nanoparticles were constructed using the solvent evaporation technique. In-vitro characterization techniques for drug-polymer compatibility, size, surface morphology, encapsulation efficacy, and delivery properties were performed on framed nanoparticles, followed by *In-vivo* studies.The compatible nature of selected excipients for the manufacture of Pioglitazone nanoparticles was shown by FTIR findings.The results of the XRD analysis revealed that the generated Pioglitazone nanoparticles were non-crystalline in nature.The selected developed Pioglitazone nanoparticles were in cubic phase with average particle size of 160.5 ± 11.24–245.4 ± 15.96 nm with charge ranging from 10.5 ± 6.21-138.6 ± 5.31mV.The encapsulation efficiency of Pioglitazone nanoparticles produced ranged from 57.24 5.80 to 89.96 1.9%. The In-vitro drug release studies of Pioglitazone nanoparticles showed controlled drug release profile. Furthermore, *In-vivo* investigations on blood glucose profiles revealed that the created Pioglitazone nanoparticles for the treatment of diabetes mellitus had a substantial effect.

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1. INTRODUCTION

Diabetes mellitus (DM) is a condition which includes the disorders of carbohydrate metabolism leads to, with characteristic features of chronic hyperglycemia associated with defects in insulin secretion impairing glucose homeostasis.Diabetes mellitus (DM) is a type of endocrine condition marked by hyperglycemia (a high level of glucose in the blood), and is divided into Type 1 and Type 2 DM [1]. Diabetes is aptly called the "silent killer" because it has become one of the primary causes of kidney failure, eyesight loss, and cardiac arrest in India [2]. The World Health Organization (WHO) recorded 1.5 million fatalities worldwide each year, making it one of the leading causes of death in affluent countries.Diabetes is anticipated to affect 366 million people worldwide by2030, owing to a variety of reasons, including an increase in the percentage of people aged 65 and up, as well as a sedentary lifestyle [3]. Pioglitazone hydrochloride is a thiazolidinedione derivative that belongs to Biopharmaceutical Classification System class-II.They increase insulin sensitivity in muscle and liver by targeting peroxisome proliferator activated receptors in adipose tissue and liver [4]. Insulin sensitivity, glycemic control, dyslipidemia, hypertension, and microalbuminuria are all reported to improve with Pioglitazone in T2DM patients [5].The drug's pharmacokinetics and bioavailability are limited by its low aqueous solubility [6,7]. Nanoparticles are created from biocompatible and biodegradable materials such as polymers (natural or synthetic) and solid lipids. The drug packed inside nanoparticles is expected to be released from the lattice in the body via diffusion, swelling, erosion or degradation [8]. Biodegradable systems have the advantage of being nontoxic, bio-compatible, biodegradable, and water soluble over non-degradable systems [9]. Starch natural biopolymer is also employed in the delivery of drugs and bio-actives. The availability of simple and cost-effective methods for producing starch and starch derivative nanoparticles is always a priority [10]. The current study intended to develop Pioglitazone nanoparticles using natural biodegradable polymer starch for effective diabetic mellitus treatment.

2. MATERIALS

Solvent/chemicals potassium dihydrogen phosphate, sodium hydroxide, dimethyl sulphoxide, Tween 80, starch and methanol were purchased from Sigma Aldrich, Bangalore, India. Milli Q water was filtered through 0.22 µm filter and used throughout the analysis.

3. Methodology

3.1 Compatibility Analysis

The compatibility of starch, Pioglitazone, Pioglitazone nanoparticles was checked by analysing FTIR spectra using FT- IR spectrometer (JASCO FT/IR 6300) at wavelength ranging from 4000- 400 cm^{-1}. Potassium bromide pellet press technique was adopted for sample preparation.

3.2 Synthesis of Pioglitazone Nanoparticles

Under magnetic stirrer at 25°C for 30 minutes, a weighed amount of sodium hydroxide (750 mg) in 35 ml water was treated with starch 2.5g (7.2mM).Add 200mg Tween 80 dissolved in 10ml distilled water to the above entire content, together with different amounts of Pioglitazone , then 5 ml distilled water containing 500 mg citric acid, and leave under magnetic stirrer for 30 minutes at 25°C.The resultant drug-encapsulated cross-linked starch nanoparticles were next purified by centrifugation (4500 rpm for 10 minutes) and washed twice with 80/20 absolute ethanol/water to eliminate unreacted compounds. Absolute ethanol is used for the final wash, which is then dried at 25°C.

3.3 Particle Size and Zeta Potential Analysis

Particle size distribution and zeta potential of Pioglitazone nanoparticles was measured using Zeta sizer (Nano ZS90 series, Malvern Instruments, UK) by diluting Pioglitazone nanoparticle at 1:10 ratio using Milli Q water.

3.4 Morphology

The developed Pioglitazone nanoparticles were visualized for its morphology using scanning electron microscope.

3.5 XRD analysis

The amorphous nature of Pioglitazone nanoparticles in comparison to Pioglitazone was

checked using powder X-ray beam diffractometer at a voltage 40 KV and current of 20 mA..

3.6 UV Spectrophotometric Method

UV Spectrophometric method for the estimation of Pioglitazone was performed at the wavelengths of 288 nm**.** The linearity of Pioglitazone was performed at the selected wavelength conditions.

3.7 Encapsulation Efficiency

Pioglitazone nanoparticles were treated with phosphate buffer saline (PBS). Centrifuged at 10000 rpm for 15 minutes. Absorbance of the supernatant was measured using UV spectrophotometer at the wavelengths of 288 nm (Pioglitazone) and the amount of drug un-entrapped in the supernatant was calculated as drug entrapment efficiency.

3.8 *In-vitro* Release Studies

The *in-vitro* release of Pioglitazone from the Pioglitazone nanoparticles was checked using the dialysis bag method. The release studies were performed by placing 1 mL of Pioglitazone nanoparticles formulation with 1 mL of phosphate buffer and the sink condition was maintained by placing the dialysis bag (Mol. Wt. cut off 12,000 - 14,000 Da; pore size, 0.2 µm) in a glass beaker containing 100 mL phosphate buffer pH 7.4 kept under magnetic stirring condition at 37˚C. Periodically, 2 mL of sample was withdrawn and replaced with 2 mL of phosphate buffer (pH 7.4) at different time intervals. The amount of Pioglitazone released from the Pioglitazone nanoparticles was measured by UV-Visible spectrophotometer at the wavelengths of 288 nm (Pioglitazone).

3.9 Anti Diabetic Activity

By single intraperitoneal injection of freshly prepared solution of Streptozotocin (25 mg/kg BW) in physiological saline after overnight fasting for 12hrs Diabetes mellitus is induced in wistar rats**.** Hyperglycemias in rats is confirmed by plasma glucose estimation 72 hrs posts Streptozotocin injection. The rats with fasting plasma glucose level of >160-200mg/dl were used for this experiment.

3.9.1 Experimental procedure

Rats were divided into 3 groups after the induction of Streptozotocin diabetes. In the experiment 6 rats were used in each group. Group-I acts as (Normal control) consist of normal rats given with 10ml/kg of normal saline, orally. Group-II as (Toxic control) Diabetic control received 25mg/Kg of Streptozotocin through I.P. Group-III acts as Diabetic control received Pioglitazone nano preparation at a dose of (2.7mg/Kg I.P) for 28 days.

3.9.2 Sample collection

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, were determined. Blood was collected from the eyes (venous pool) by sino-ocular puncture in EDTA coating plasma tubes for the estimation of blood parameters.

3.9.3 Biochemical parameters estimation

The biochemical parameters such as Blood glucose, Plasma insulin, Total haemoglobin, were determined by standard protocol.The part of liver for each test was perfused with ice cold 0.15M KCl and 1mM EDTA solution and homogenized twice its weight of ice cold buffer (0.01 cysteine and 1mM EDTA in 0.1 ml Tris-HCL, pH 7.4) and centrifuged for 20 min at 4^0C.

4. RESULTS AND DISCUSSION

4.1 FT‑IR Spectroscopic Analysis

Pioglitazone nanoparticles showed major bands at 3316 cm^{-1}, 3266 cm^{-1}, 3268 cm^{-1} could be attributed to hydrogen bonded O-H group stretching of starch molecule. Bands nearer to 2922 cm^{-1} can be attributed to unsymmetrical stretching of C–H (CH_2 group) of starch molecules. The absorption band at 1609 cm^{-1}, 1638 cm^{-1} and 1639 cm^{-1} was due to the presence of bonded water in starch. The peak at 1331 cm^{-1} and 1364 cm^{-1} represented the angular deformation of C–H (CH_3 group) of starch molecules (Fig. 1). The peak at 926 cm^{-1}, 929 cm^{-1} and 993 cm^{-1} was related to the C–O–C of a-1,4 and a-1,6 glycosidic linkage. Other peaks, at 1147 cm^{-1} and 1076 cm^{-1} were associated with C–O bond and C–C bond of starch nanoparticles.

4.2 Development of Pioglitazone Nanoparticles

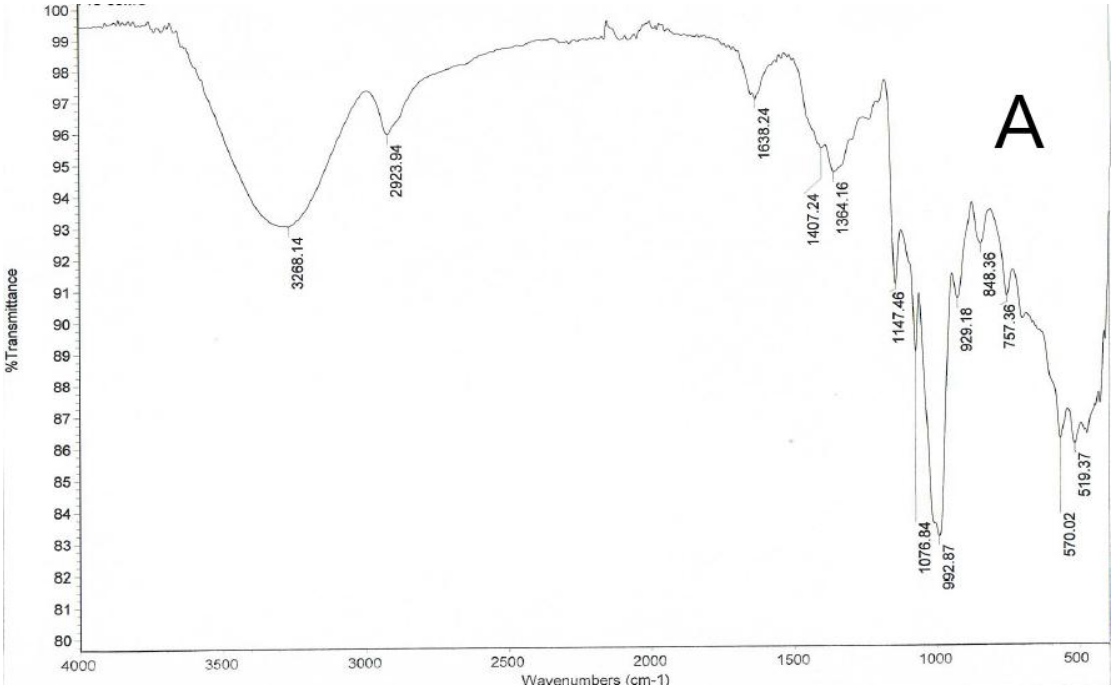
The Pioglitazone nanoparticles were prepared using solvent evaporation technique. Herein, starch, tween 80 and citric acid were used for the preparation of Pioglitazone nanoparticles. Here in aqueous solution of tween 80 acts as a “stearic barrier” and this supports the production of smaller particles with uniform size distribution. In addition, citric acid used in the formulation may support the stability of the developed Pioglitazone nanoparticles. By varying the starch concentration and Pioglitazone concentrations different trials were prepared and these trials were checked for its size, zeta potential and drug content.

Previously Noha Badawi et al; 2020 developed pomegranate extract loaded solid lipid nanoparticles by hot homogenization followed by ultra-sonication technique. From the studies they concluded that stearic acid as lipid, Tween 80 as surfactant, as well as sonication with short time and high amplitude can be selected for the formulation development to elicit minimum particle size, maximum zeta potential, highest entrapment, and sustained drug release behavior [11].

4.3 Particle Size and Zeta Potential Analysis of Pioglitazone Nanoparticles

The particle size for different Pioglitazone nanoparticles were in the range of 160.5 ± 11.24– 245.4 ± 15.96 nm. Whereas, the zeta potential ranges from 10.5 ± 6.21-138.6 ± 5.31mV (Table. 1 and Fig. 2). The zeta potential value indicates that the developed Pioglitazone nanoparticles are positively charged. Results conclude that the particle size of Pioglitazone nanoparticles gets increased on increasing the starch concentration. Among the different trials the nanoparticle prepared at 1:1 ratio showed the lowest particle size of 160.5 ± 11.24 nm.

The commercial applications of nanoparticles are mass produced, uncapped, have large size distributions [12–14], they are associated with aggregate formation or may gets dissolved when exposed to natural systems releasing ions. The surface chemistry, compared with the uncoated nanoparticles are due to the presence of a surface coating [15]. The nonspecific interactions with cells and proteins may reduces the toxicity of nanoparticles which may be achieved by coating of nanoparticles with biocompatible/organic polymers which ultimately increases the dispersion/stability, of nanoparticles [16,17].



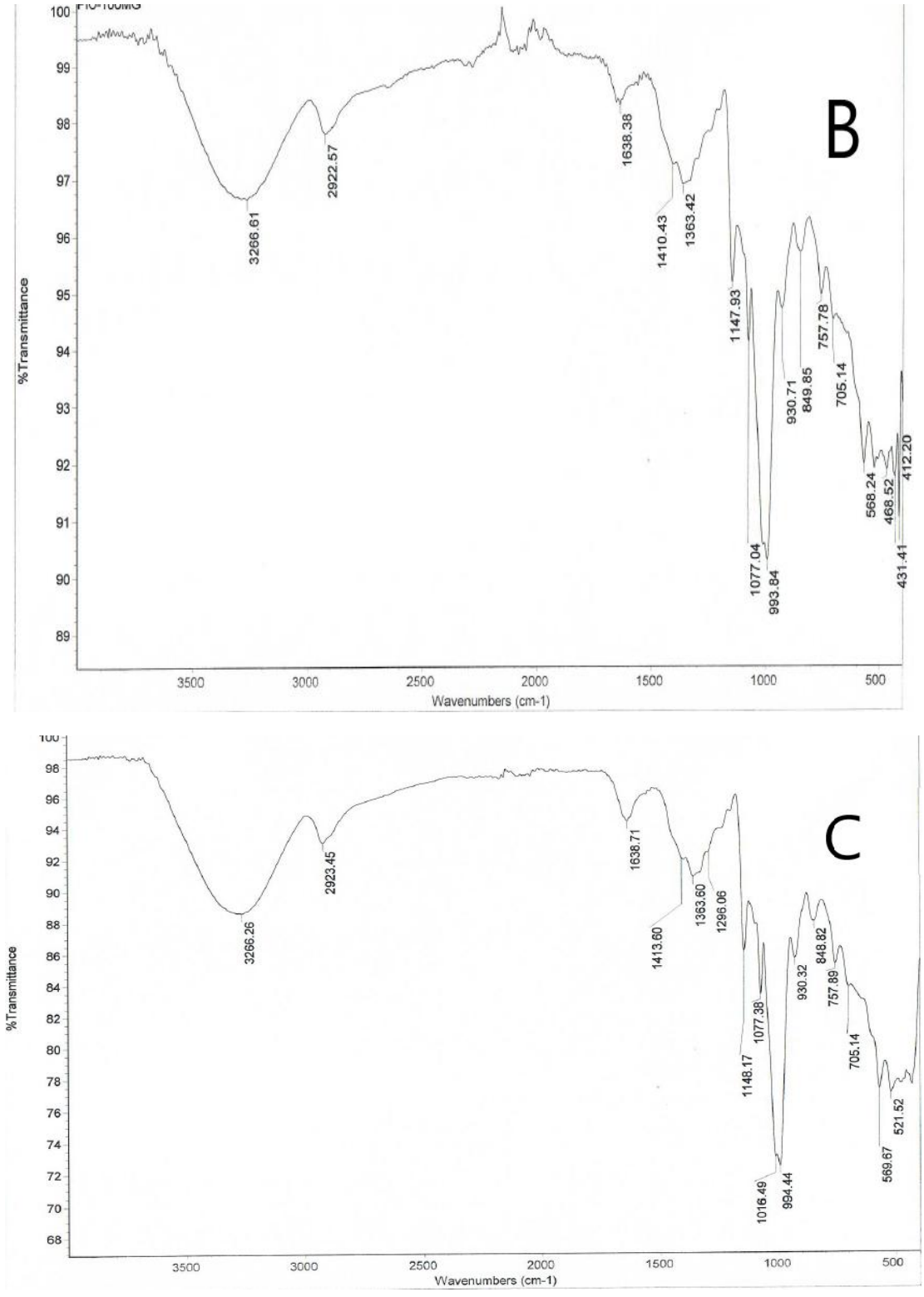


Fig. 1. FT-IR spectra of Pioglitazone nanoparticles(1:1) (B) Pioglitazone nanoparticles (1:2)(B) and Pioglitazone nanoparticles (1:3)(C)

Table 1. Formulation layout for the development of pioglitazone nanoparticles

Formulation Code	Concentration (Starch: Pioglitazone)	Average particle size (nm) ± SD	Zeta potential (mV) ± SD	Pioglitazone Encapsulation efficiency (%) ± SD
PG-NPs - 1	1:1	160.5 ± 11.24	10.5 ± 6.21	69.39 ± 4.41
PG-NPs - 2	1:2	240.4 ± 34.25	20.7 ± 6.06	89.96± 1.9
PG-NPs - 3	1:3	245.4 ± 15.96	138.6 ± 5.31	57.24 ± 5.80

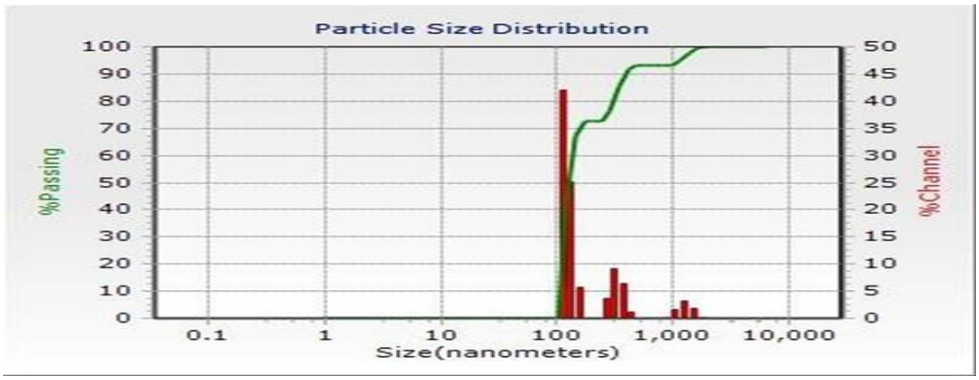


Fig. 2. A typical particle size data of pioglitazone nanoparticles

4.4 Morphology of Pioglitazone Nanoparticles

The morphology of Pioglitazone nanoparticles was checked using scanning electron microscope and found the particles are spherical shaped with aggregate nature (Fig. 3). Whereas the metallic nanoparticles play key roles in various applications such as solar cells, sensors and catalysis due to their plasmonic property, chemical stability, and larger surface to volume ratio [18,19]. Whereas the bimetallic nanoparticles can offer further opportunities for the enhanced performance due to their electronic heterogeneity, multi-functionality and specific site response with more than one element in an individual nanostructure.

4.5 XRD Analysis of Pioglitazone Nanoparticles

XRD diffraction spectra of Pioglitazone nanoparticles exhibit sharp diffraction peaks between the 2θ values (18, 19, 20, 23, 25, 32) with an average peak intensity of nearer to 700 to 1000 %, especially the peak at 2θ value (19) shows higher peak intensity of nearer to 1000 % which indicates the crystalline nature of Pioglitazone nanoparticles. The absence of major peaks for Pioglitazone nanoparticles may be due to the non- crystalline nature of Pioglitazone nanoparticles which may be encapsulated within the nanoparticles. Whereas the XRD diffraction spectra of Pioglitazone pure drug exhibit sharp diffraction peaks between the 2θ values (18, 19, 20, 23, 25, 32) with an average peak intensity of nearer to 1000 % (Fig. 4 and 5).

4.6 Encapsulation Efficiency of Pioglitazone Nanoparticles

Higher drug encapsulation efficiency is desired to achieve better therapeutic effect on target site. The amount of Pioglitazone incorporated into the Pioglitazone nanoparticles was determined in terms of encapsulation efficiency for three different formulations and is showed in Table and found to be 57.24 ± 5.80 to 89.96± 1.9%. Among the different trials the Pioglitazone nanoparticles developed at the ratio of 1:2 showed the higher encapsulation efficiency of 89.96± 1.9%.

4.7 *In-vitro* Release Studies

The *In-vitro* release profile of Pioglitazone nanoparticles (1:1) analyzed by UV Spectrophotometer showed an release of 2.65% at 72 h, which indicates a sustained release profile (Fig. 6). Whereas Pioglitazone nanoparticles (1:2) also showed a sustained release profile of 7.83% at 72 h, the Pioglitazone nanoparticles (1:3) showed a sustained release profile of 31.76% at 72 h as shown in Fig.6. The

results indicates that Pioglitazone nanoparticles (1:3)shown superior release profile compared to Pioglitazone nanoparticles (1:1) and Pioglitazone nanoparticles (1:2).

4.8 *In vivo* Studies

The levels of initial and final blood glucose, and change in body weight , in normal rat, and treatment control animals in each group was shown in Table. 2. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with nanopartcles of Pioglitazone at a dose of 2.7 mg/kg increased the body weight non-significantly as compared to normal control animals. Fasting blood glucose level was significantly increased 185.30 ± 4.60 in diabetic animals as compared to normal animals. However the level of fasting blood glucose, returned to near normal range in diabetic rats treated with nanopartcles of Pioglitazone at a dose of 2.7mg/kg respectively. Table no: 3 illustrates the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rat and treatment control animals in each group. The levels of total hemoglobin, and plasma insulin levels were decreased significantly whereas glycosylated heamoglobin levels were increased significantly as compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin and plasma insulin, returned to near normal range in diabetic rats treated with nanopartcles of Pioglitazone l at a dose of 40mg/kg respectively.

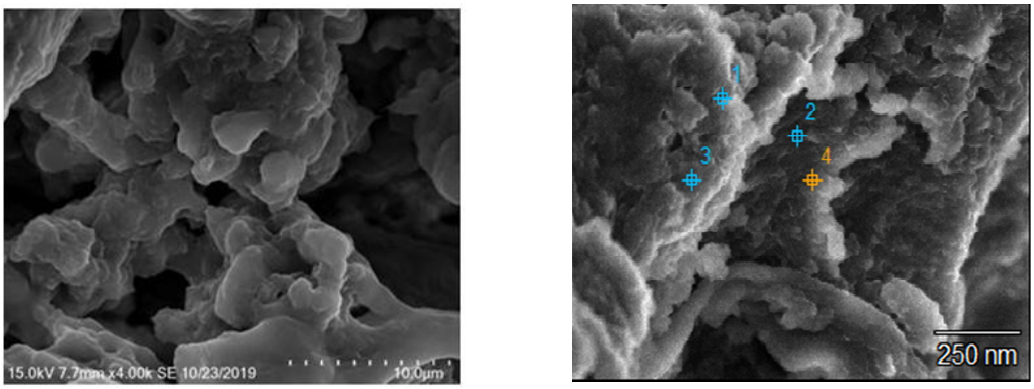


Fig. 3. SEM image of Pioglitazone nanoparticles

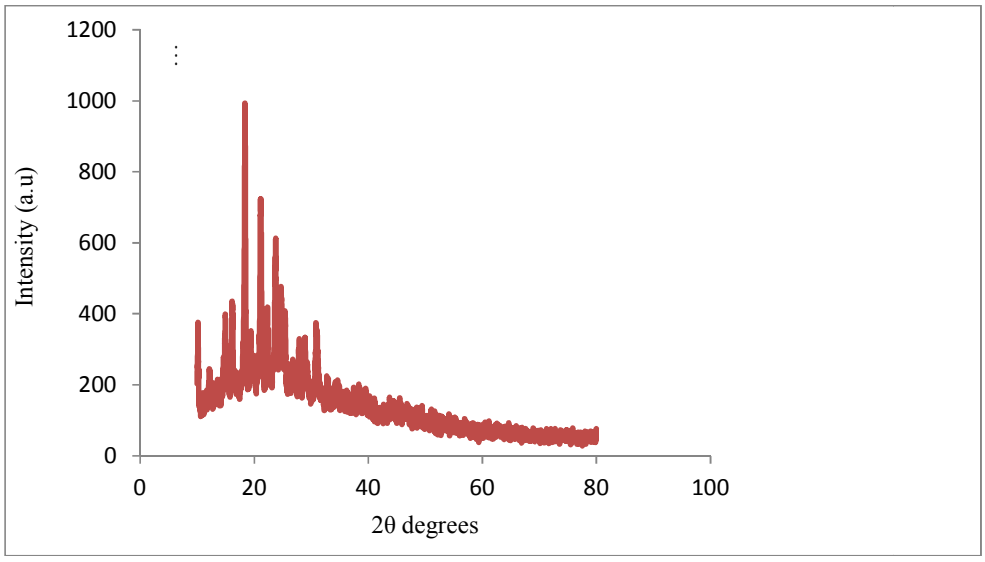


Fig. 4. XRD spectra of pioglitazone

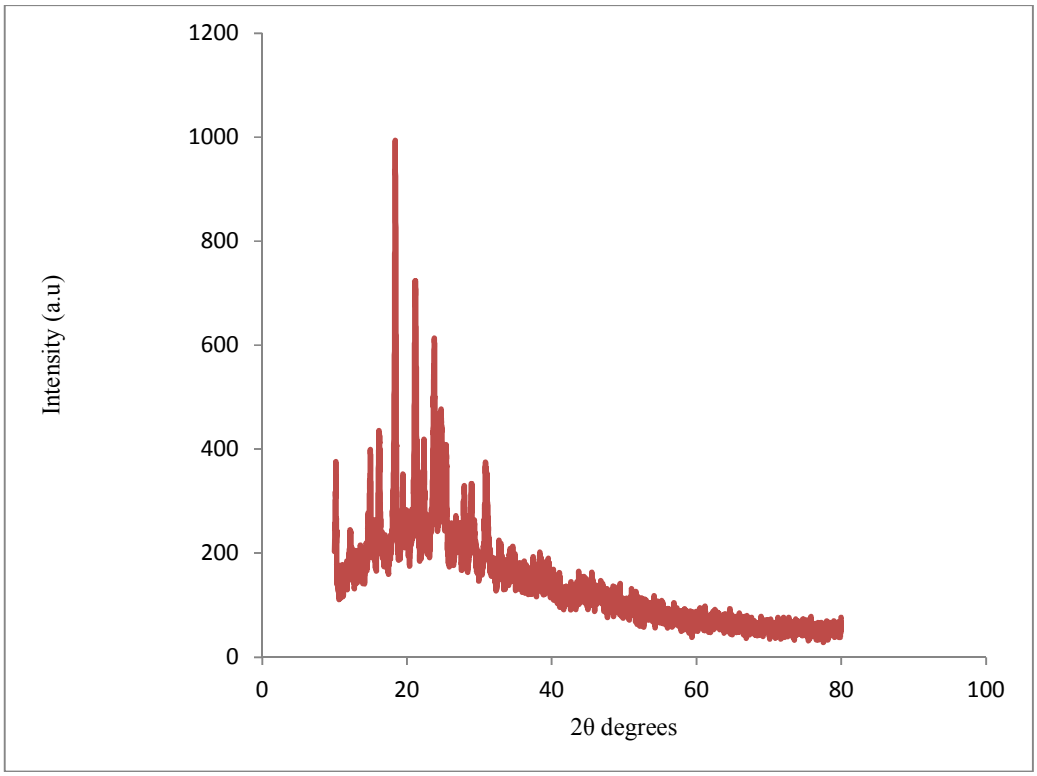


Fig. 5. XRD Spectra of Pioglitazone nanoparticles

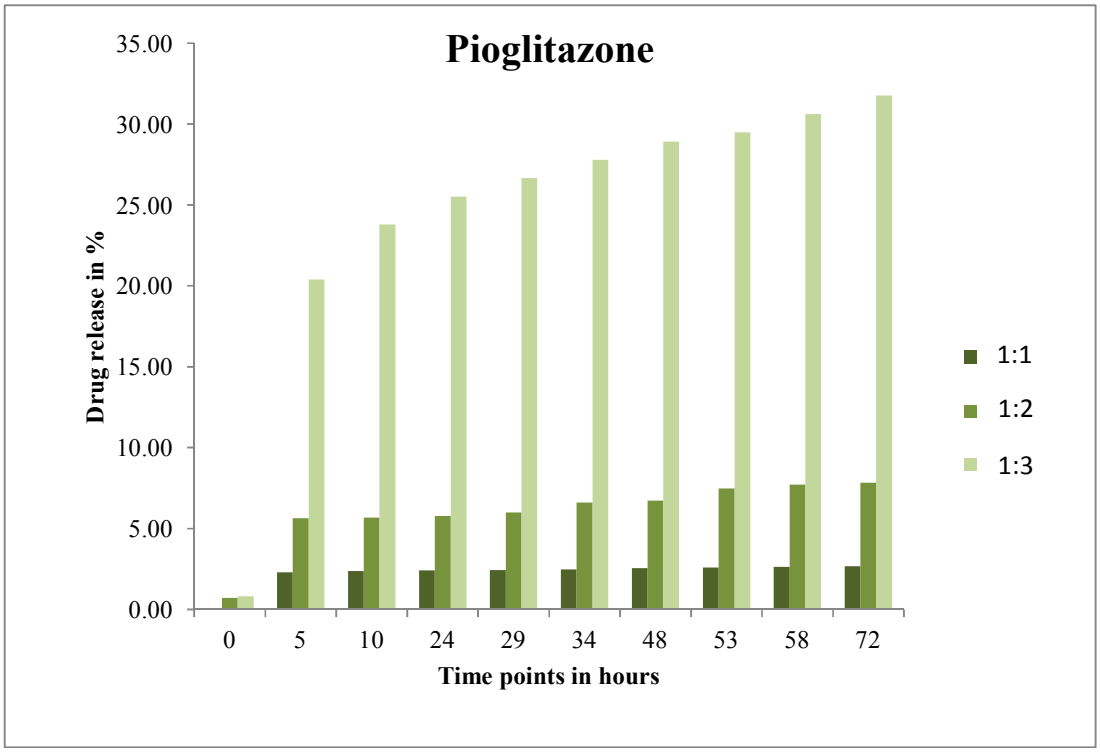


Fig. 6. *In vitro* release profile of pioglitazone nanoparticles

Table 2. Effect of *nano particles*on initial and final body weight and blood glucose in normal and treated animals

Group	Body weight (g)		Blood glucose (mg / 100ml) Initial	Blood glucose (mg / 100ml)
	Initial	Final		Final
G1	205 ± 5.20	240 ± 5.55	85.70 ± 2.30	93.60 ± 2.45
G2	225 ± 5.30	180 ± 4.80**(a)	92.20 ± 2.35	185.30 ± 4.60**(a)
G3	208 ± 5.18	230 ± 5.25	90.40 ± 2.38	112.50 ± 3.05**(b)

*Values are expressed as mean ± SEM.Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.** (a) Values are significantly different from normal control G1 at P<0.001.** (b) Values are significantly different from Diabetic control G2 at P<0.01*

Table 3. Effect of *nanoparticle preparation* on plasma insulin, hemoglobin and glycosylated hemoglobin in normal and treated animals

Groups	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA_1 (%)	Plasma Insulin (µU/ml)
G1	13.60± 1.25	0.40 ± 0.07	30.10 ± 2.20
G2	8.80 ± 0.80**(a)	0.96 ± 0.16**(a)	13.50 ± 1.40**(a)
G3	13.45 ± 1.16**(b)	0.42 ± 0.08**(b)	32.80 ± 2.45**(b)

*Values are expressed as mean ± SEM. Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests. ** (a) Values are significantly different from normal control G1 at P<0.001. ** (b) Values are significantly different from Diabetic control G2 at P<0.01*

Previously with reference to the treatment of Diabetes mellitus Sohrab Alam et al; 2016 developed Pioglitazone loaded nanostructured lipid carriers for the treatment of type 2 diabetes with an aim to investigate the bioavailability improvement by transdermal delivery using high-pressure homogenization followed by ultrasonication. Their prepared Pioglitazone loaded nanostructured lipid carriers showed a size of 166.05 nm, drug loading of 10.41%.. The in vivo pharmacokinetic study of developed Pioglitazone loaded nanostructured lipid carriers showed 2.17 times enhancement with respect to its bioavailability study and pharmacodynamics. They observed a lowering in the blood sugar level in a sustained pattern with a shelf life of 1.83 years [20]. Bindu Madhavi et al; 2013 developed Pioglitazone and Piperine loaded BSA nanoparticles to combat the adverse effects of Pioglitazone by combining with herbal principle, Piperine using modified desolvation and coacervation method by cross linking, with glutaraldehyde. Here in nanoparticles coloaded with Pioglitazone and Piperine showed a loading efficiency of 81.5 ± 1.7 with a size range in nano and drug release followed a sustained pattern upto 24 h [21].

Inorder to offer an improved bioavailability with reference to Diabetes mellitus treatment Appana Chowdary et al; 2015 improved the oral bioavailability of poorly water soluble Pioglitazone by Nanoparticles using Polaxomer 188, Tween 80 and Poly Vinyl Pyrrolidine as stabilizers which in turn improves the solubility of Pioglitazone . and better action [22]. Pioglitazone hydrochloride nanoparticles using polycaprolactone (PCL) as polymer by improving its bioavailability upon post oral administration which might increases the solubility in gastrointestinal fluids was developed by Adhokshaja Canchi et al; 2017 and percentage entrapment efficiency of developed Pioglitazone hydrochloride nanoparticles was found to be 62.23 to 76.39 %, drug content of 2.09 to 3.56 mg with a controlled release up to 12h [23]. Excess glucose in the blood interacts with haemoglobin during diabetes. As a result, total haemoglobin levels are reduced in Streptozotocin-induced diabetic rats.

Since Diabetes mellitus is a life-threatening metabolic syndrome over the past few decades, the incidence of diabetes has climbed exponentially. Several therapeutic approaches have been undertaken, but the occurrence and risk still remain unabated. The developed nanoparticulate systems may be effective against diabetes and associated vascular complications via acting on several therapeutic targets which may afford improved pharmacokinetic and biopharmaceutical attributes.

5. CONCLUSION

The solvent evaporation approach was used to successfully manufacture Pioglitazone -loaded Starch nanoparticles. The size and shape of the produced nanoparticles were examined using particle size distribution, zeta potential, and SEM.The drug's inclusion within the nano carrier was confirmed by the analysis.The created nanoformulations demonstrated sustained drug release, which may increase medication retention time in blood circulation.Animal studies support the formation of Pioglitazone in nanoparticles, which may have therapeutic benefits by decreasing blood glucose.This research will be relevant in future studies aimed at developing Pioglitazone-loaded nanoparticles with excellent encapsulation efficiency for the effective treatment of type 2 diabetic mellitus.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal experimentation was performed in accordance with the Institutional Animal Ethics Committee (IAEC) guidelines (IAEC approval No-245/PO/RCB/B1/S/18/CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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