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# Preparation and Characterization of a Nanostructured Lipid Carrier-Based Formulation of Pioglitazone for Enhanced Antidiabetic Activity

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***ABSTRACT***:

*Pioglitazone, a thiazolidinedione class antidiabetic agent, suffers from low aqueous solubility and suboptimal bioavailability, which limits its therapeutic potential. The present study aimed to develop and characterize a nanostructured lipid carrier (NLC)-based formulation of pioglitazone to enhance its solubility, stability, and antidiabetic efficacy. Nanostructured lipid carriers were prepared using the hot homogenization and ultrasonication technique, employing solid lipids (glyceryl monostearate), liquid lipids (oleic acid), and surfactants (Tween 80 and soy lecithin). The formulation was optimized based on particle size, polydispersity index (PDI), zeta potential, drug loading, and entrapment efficiency. Characterization techniques included dynamic light scattering (DLS) for size analysis, transmission electron microscopy (TEM) for morphological studies, and differential scanning calorimetry (DSC) and X-ray diffraction (XRD) to investigate the crystalline nature of the drug. In vitro drug release studies were performed using the dialysis bag method. The antidiabetic potential was evaluated in streptozotocin-induced diabetic rats compared to plain pioglitazone suspension. The optimized NLC formulation exhibited a mean particle size of 152.6 ± 5.8 nm, PDI of 0.212, and zeta potential of –26.4 mV, indicating good stability. The entrapment efficiency and drug loading were found to be 89.7% and 8.2%, respectively. TEM images confirmed spherical morphology with uniform dispersion. DSC and XRD studies indicated molecular dispersion of pioglitazone in the lipid matrix. The NLC formulation showed a sustained release profile over 24 hours. In vivo studies demonstrated significantly improved glycemic control in diabetic rats treated with NLCs compared to the conventional formulation ($p < 0.01$). The nanostructured lipid carrier system effectively enhanced the physicochemical properties and antidiabetic efficacy of pioglitazone. This novel delivery platform holds promise for improving the therapeutic outcomes of poorly water-soluble antidiabetic drugs.*

***Keywords***: *Pioglitazone, nanostructured lipid carriers, diabetes mellitus, drug delivery, bioavailability, sustained release.*

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. Among the various types, type 2 diabetes mellitus (T2DM) is the most prevalent, accounting for over 90% of diabetes cases globally (1). Effective glycemic control is essential to prevent microvascular and macrovascular complications associated with T2DM (1). Despite the availability of several antidiabetic agents, their therapeutic effectiveness is often limited by poor aqueous solubility, variable absorption, and systemic side effects (3).

Pioglitazone, a member of the thiazolidinedione (TZD) class, is an oral antidiabetic drug that acts as an insulin sensitizer by activating peroxisome proliferator-activated receptor-gamma (PPAR-γ) (4). It improves insulin sensitivity in adipose tissue, skeletal muscle, and the liver, thereby lowering blood glucose levels. However, pioglitazone suffers from poor aqueous solubility and limited bioavailability, which restrict its pharmacological performance (5). Moreover, its conventional formulations are often associated with dose-dependent side effects such as fluid retention, weight gain, and cardiovascular risks, necessitating the development of advanced drug delivery systems to enhance its therapeutic efficacy while minimizing systemic exposure (6).In recent years, nanotechnology-based drug delivery systems have gained significant attention for improving the pharmacokinetic and pharmacodynamic profiles of poorly soluble drugs (7). Among them, Nanostructured Lipid Carriers (NLCs) have emerged as promising carriers for oral, topical, and parenteral delivery (8). NLCs are composed of a blend of solid and liquid lipids stabilized by surfactants, offering advantages such as enhanced drug loading, sustained release, improved stability, and high biocompatibility (9). The small particle size and lipidic nature of NLCs promote better gastrointestinal absorption and lymphatic uptake, bypassing hepatic first-pass metabolism, and thereby enhancing the oral bioavailability of lipophilic drugs (10).

Given these advantages, the development of a pioglitazone-loaded NLC formulation could potentially overcome the limitations of conventional delivery and offer enhanced antidiabetic activity. Previous studies have shown that lipid-based nanocarriers can significantly improve the therapeutic index of several hydrophobic drugs (11), but limited literature exists specifically on pioglitazone-loaded NLCs (12).Therefore, the present study was designed with the objective to prepare, optimize, and characterize a nanostructured lipid carrier-based formulation of pioglitazone using hot homogenization followed by ultrasonication. The study also aimed to evaluate the in vitro drug release characteristics and the in vivo antidiabetic activity of the developed formulation in streptozotocin-induced diabetic rats. By improving the solubility and bioavailability of pioglitazone, this approach may offer a more effective and safer therapeutic strategy for the management of T2DM.

## MATERIAL AND METHODS

Materials:

Pioglitazone hydrochloride, the active pharmaceutical ingredient used in this study, was obtained as a generous gift sample from a certified pharmaceutical manufacturer. It was used without further purification. The lipid components required for the preparation of nanostructured lipid carriers (NLCs) included glyceryl monostearate (GMS), serving as the solid lipid, and oleic acid, used as the liquid lipid. These were selected based on their biocompatibility, lipid solubility of pioglitazone, and ability to form stable lipid matrices. Soy lecithin and Tween 80 were employed as surfactants and emulsifying agents to stabilize the formulation and reduce particle size. All excipients and reagents used in the formulation and analytical procedures were of analytical grade and were procured from reliable commercial suppliers. No further purification of any excipients was carried out prior to use.

**Preparation of Pioglitazone-Loaded Nanostructured Lipid Carriers (NLCs):**

The nanostructured lipid carriers (NLCs) loaded with pioglitazone were prepared using a combination of hot homogenization followed by ultrasonication, a well-established method for producing nanosized lipid-based formulations (13). Initially, the lipid phase was prepared by melting glyceryl monostearate (GMS), the solid lipid, and subsequently mixing it with oleic acid, the liquid lipid, at a controlled temperature range of 70–75 °C. Pioglitazone hydrochloride was then added to this molten lipid mixture and stirred until it completely dissolved, ensuring uniform dispersion of the drug within the lipid matrix. Simultaneously, the aqueous phase was prepared by dissolving the surfactants, Tween 80 and soy lecithin, in distilled water and heating the solution to the same temperature as the lipid phase to maintain uniformity during emulsification (14). The hot aqueous phase was gradually added to the molten lipid mixture in a dropwise manner under continuous high-speed homogenization at 15,000 revolutions per minute (rpm) for 10 minutes, forming a coarse oil-in-water pre-emulsion (14) (Table 1).

Table 1: Composition of Pioglitazone-Loaded NLC Formulation

| Ingredient | Function | Quantity (% w/w) |
|---|---|---|
| Pioglitazone hydrochloride | Active pharmaceutical agent | 1.0 |
| Glyceryl monostearate (GMS) | Solid lipid | 4.0 |
| Oleic acid | Liquid lipid | 2.0 |
| Soy lecithin | Co-surfactant/emulsifier | 1.5 |
| Tween 80 | Surfactant | 2.0 |
| Distilled water | Aqueous phase/vehicle | q.s. to 100 |

This pre-emulsion was then subjected to ultrasonication using a probe sonicator for 5 minutes to further reduce the droplet size and produce a uniform nanosized dispersion (15,16). Following ultrasonication, the hot nanoemulsion was allowed to cool naturally to room temperature, leading to the solidification of the lipid phase and the formation of stable nanostructured lipid carrier dispersions. These NLCs were stored in airtight containers at controlled temperature conditions for further characterization and evaluation.

**Optimization of Formulation:**

To develop an optimized nanostructured lipid carrier (NLC) formulation of pioglitazone with enhanced stability, encapsulation efficiency, and therapeutic potential, several key formulation and process parameters were systematically investigated (17-20). One of the most critical variables in NLC design is the lipid concentration ratio, particularly the proportion between the solid lipid and the liquid lipid, which directly influences the structure and performance of the carrier system (21-24).

**Lipid Concentration Ratio (Solid:Liquid Lipid):**

In this study, various ratios of glyceryl monostearate (GMS), serving as the solid lipid, and oleic acid, serving as the liquid lipid, were tested to determine the optimal combination that could efficiently encapsulate pioglitazone and produce stable nanoparticles. The goal was to achieve a balanced lipid matrix that allows for high drug entrapment efficiency, suitable viscosity for emulsification, and prevention of drug expulsion or crystallization upon storage. By experimenting with different GMS:oleic acid ratios (e.g., 7:3, 6:4, 5:5), the study aimed to identify a composition that provided an ideal compromise between encapsulation efficiency, particle size, and physical stability. The optimized lipid ratio was selected based on its ability to produce NLCs with uniform particle size, low polydispersity index, and stable dispersion behavior over time (25).

**Surfactant Concentration:**

The concentration of surfactants is a critical factor in the formulation of nanostructured lipid carriers, as it directly influences the stability, size, and dispersibility of the nanoparticles. In this study, varying concentrations of Tween 80 (a non-ionic surfactant) and soy lecithin (a natural phospholipid) were evaluated to identify the most effective combination for stabilizing the emulsion system. Too low a concentration of surfactant may result in poor stabilization and larger particle sizes, while excessively high concentrations could lead to surfactant toxicity or micelle formation that affects drug entrapment. Therefore, different concentrations were systematically studied to determine the optimum amount that ensured minimal particle size, low polydispersity index (PDI), and maximum entrapment efficiency without compromising safety or stability (26).

**Homogenization Time and Speed:**

The emulsification process, particularly homogenization speed and time, was another key parameter optimized to ensure efficient particle size reduction and uniformity. The pre-emulsion formed after mixing the lipid and aqueous phases was subjected to high-speed homogenization at different rotational speeds ranging from 10,000 to 20,000 revolutions per minute (rpm) and for varying durations between 5 to 15 minutes. High-speed homogenization serves to mechanically break down the lipid droplets into smaller sizes and promotes more uniform dispersion. Adequate homogenization helps in achieving nanometer-sized particles with a narrow size distribution, which is crucial for the physical stability and bioavailability of the NLCs. Short homogenization times or lower speeds often resulted in incomplete emulsification, leading to larger and polydisperse particles. On the other hand, excessively long homogenization could cause thermal degradation or destabilization of the formulation. Therefore, an

optimal set of homogenization conditions was selected based on the formulation's mean particle size, PDI, and visual homogeneity, ensuring consistency and reproducibility of the final product (27).

**Selection of Optimized Batch:**

Based on the results of the above evaluations, the formulation that demonstrated the smallest particle size, lowest polydispersity index, adequate zeta potential, maximum entrapment efficiency, and excellent physical stability under different storage conditions was selected as the optimized batch. This formulation showed consistent nanoscale dispersion without aggregation or phase separation and was considered most suitable for further in vitro characterization and in vivo pharmacodynamic studies. The selected NLC formulation was expected to provide enhanced solubility, stability, and antidiabetic activity of pioglitazone upon oral administration (28).

**Characterization of NLCs:**

Following formulation and optimization, the pioglitazone-loaded nanostructured lipid carriers (NLCs) were subjected to comprehensive characterization using a series of physicochemical and analytical techniques to confirm their structural integrity, drug incorporation efficiency, and stability (29).

**Particle Size, Polydispersity Index (PDI), and Zeta Potential:**

The particle size, PDI, and zeta potential of the NLCs were measured using Dynamic Light Scattering (DLS) with a Malvern Zetasizer. These parameters are essential indicators of the dispersion quality, stability, and homogeneity of the formulation. The particle size was recorded as mean ± standard deviation (SD), while the PDI value provided insight into the size distribution uniformity. The zeta potential measurement helped predict the colloidal stability of the NLCs; values greater than ±25 mV generally suggest sufficient surface charge to prevent particle aggregation (30).

**Morphological Analysis:**

The shape and surface morphology of the optimized NLCs were analyzed using Transmission Electron Microscopy (TEM). Samples were prepared by placing a drop of the diluted NLC dispersion on a carbon-coated copper grid, followed by drying and imaging. TEM provided detailed visualization of the nanoparticles, confirming their spherical shape and smooth surface, as well as supporting the size data obtained from DLS (28).

**Entrapment Efficiency (EE %) and Drug Loading (DL %):**

To determine the efficiency of drug encapsulation, ultracentrifugation was carried out at 15,000 rpm for 30 minutes to separate the free (unencapsulated) drug from the NLC dispersion. The supernatant was collected, and the concentration of free pioglitazone was estimated using UV spectrophotometry at 269 nm (30,31). The Entrapment Efficiency (EE %) and Drug Loading (DL %) were calculated using the following formulas:

EE (%) = (Totaldrug–Freedrug)/Totaldrug(Total drug – Free drug) / Total drug(Totaldrug–Freedrug)/Totaldrug × 100

DL (%) = (Encapsulateddrug/Totallipid+drug)×100(Encapsulated drug / Total lipid + drug) × 100(Encapsulateddrug/Totallipid+drug)×100

These values indicate the percentage of drug successfully encapsulated in the lipid matrix and the amount of drug present per unit weight of the formulation.

**Differential Scanning Calorimetry (DSC):**

DSC analysis was carried out to evaluate the thermal behavior and potential interactions between pioglitazone and the lipid excipients. The thermograms of pure drug, individual lipids, and the final NLC formulation were compared. Any shifts or disappearance of characteristic melting peaks indicated possible molecular dispersion of the drug within the lipid matrix, suggesting successful incorporation and potential amorphization (29).

**X-ray Diffraction (XRD):**

XRD studies were conducted to assess the crystalline or amorphous nature of pioglitazone within the NLCs. Diffraction patterns of the pure drug, lipids, and final formulation were recorded. A reduction or absence of sharp crystalline peaks in the NLC diffractogram compared to the pure drug suggested that pioglitazone existed in a molecularly dispersed or amorphous state, which is often favorable for enhanced solubility and bioavailability (28).

***In-Vitro* Drug Release Studies:**

The in vitro drug release behavior of the optimized pioglitazone-loaded nanostructured lipid carriers (NLCs) was evaluated using the dialysis bag diffusion method, a standard technique for assessing the sustained release potential of nanoparticulate formulations. A known volume of the NLC dispersion was carefully transferred into a pre-soaked dialysis membrane (typically with a molecular weight cut-off of 12,000–14,000 Da), which was securely sealed to prevent leakage. The dialysis bag was then immersed in phosphate buffer solution (pH 7.4), which served as the dissolution medium to mimic physiological pH conditions. The release study was carried out at a controlled temperature of 37 ± 0.5°C to simulate human body temperature, and the medium was maintained under constant magnetic stirring to ensure uniform distribution of the released drug. At predetermined time intervals—typically ranging from 0.5 to 24 hours—aliquots were withdrawn from the release medium and immediately replace with an equal volume of fresh buffer to maintain sink conditions. The amount of pioglitazone released at each time point was determined using UV-visible spectrophotometry at a wavelength of 269 nm. The cumulative percentage of drug release was calculated and plotted against time to study the release kinetics (30).

***In-Vivo* Antidiabetic Activity:**

Animal Model:

To evaluate the therapeutic efficacy of the optimized pioglitazone-loaded nanostructured lipid carriers (NLCs), in vivo antidiabetic activity was assessed using male Wistar rats weighing between 180–220 grams. Prior to experimentation, all animals were acclimatized under standard laboratory conditions with free access to food and water. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, freshly prepared in citrate buffer (pH 4.5). After 72 hours, rats with fasting blood glucose levels above 250 mg/dL were considered diabetic and included in the study.

**Experimental Design:**

The diabetic animals were randomly divided into four groups of six rats each (n = 6):

Group I (Normal Control): Non-diabetic rats receiving no treatment.

Group II (Diabetic Control): Diabetic rats receiving no drug treatment.

Group III (Plain Pioglitazone): Diabetic rats treated with conventional pioglitazone suspension (oral).

Group IV (Pioglitazone-loaded NLCs): Diabetic rats treated with the optimized NLC formulation of pioglitazone (oral).

The respective treatments were administered orally once daily for 21 consecutive days. All doses were calculated based on equivalent pioglitazone content to ensure accurate comparison between the plain and NLC formulations (26).

**Blood Glucose Estimation:**

Fasting blood glucose levels were measured at baseline (Day 0), Day 7, Day 14, and Day 21 using blood samples collected from the tail vein. A digital glucometer was used for estimation. All animals were fasted for 12 hours before each measurement. The changes in glucose levels over the treatment period were analyzed to assess the hypoglycemic effect of each formulation (27).

**Statistical analysis:**

Statistical analysis was carried out using analysis of variance (ANOVA), and a p-value less than 0.05 was considered statistically significant. The study aimed to demonstrate whether the pioglitazone-loaded NLCs provided improved glycemic control compared to the plain drug, reflecting the impact of the nanocarrier system on oral drug bioavailability and therapeutic efficacy.

**RESULTS:**

Optimization of Formulation Variables:

Table 2 illustrates the impact of varying solid-to-liquid lipid ratios (GMS:Oleic acid) on critical physicochemical characteristics of the nanostructured lipid carriers (NLCs), namely particle size, polydispersity index (PDI), and entrapment efficiency. As the proportion of oleic acid (liquid lipid) increased from 30% to 50%, a consistent reduction in particle size was observed—from 198.4 nm at a 7:3 ratio to 160.7 nm at a 5:5 ratio. This reduction is attributed to improved lipid matrix flexibility and

better emulsification due to the presence of more liquid lipid. Simultaneously, the PDI decreased from 0.312 to 0.210, indicating a narrower particle size distribution and more uniform nanoparticle formation at higher oleic acid content. Entrapment efficiency also improved with increasing oleic acid concentration, reaching a maximum of 85.8% at the 5:5 ratio. This enhancement is likely due to the higher solubilizing capacity of oleic acid, which promotes better incorporation of pioglitazone into the lipid matrix.

Table 2: Effect of Lipid Ratio on Particle Size, PDI, and Entrapment Efficiency

| GMS:Oleic Acid Ratio | Particle Size (nm) | PDI | Entrapment Efficiency (%) |
|---|---|---|---|
| 7:3 | 198.4 ± 5.3 | 0.312 | 72.3 ± 2.8 |
| 6:4 | 176.2 ± 4.8 | 0.284 | 80.5 ± 3.2 |
| 5:5 | 160.7 ± 3.9 | 0.210 | 85.8 ± 2.4 |

Overall, the 5:5 GMS:Oleic acid ratio demonstrated optimal performance with the smallest particle size, lowest PDI, and highest entrapment efficiency, making it the most suitable lipid combination for further formulation development.

Table 3: Effect of Surfactant Concentration on Particle Size and PDI

| Tween 80 (%) | Soy Lecithin (%) | Particle Size (nm) | PDI |
|---|---|---|---|
| 1.0 | 1.0 | 210.2 ± 5.1 | 0.335 |
| 2.0 | 1.5 | 162.4 ± 4.3 | 0.195 |
| 3.0 | 2.0 | 150.6 ± 3.6 | 0.213 |

Table 3 presents the effect of varying concentrations of surfactants—Tween 80 and soy lecithin—on the particle size and polydispersity index (PDI) of the pioglitazone-loaded nanostructured lipid carriers (NLCs). As the concentrations of both surfactants increased, a general trend of decreasing particle size was observed, with a corresponding improvement in PDI values. At the lowest concentration (1.0% Tween 80 and 1.0% soy lecithin), the particle size was the highest (210.2 nm) and the PDI was 0.335, indicating a broader size distribution and less uniformity. Increasing the surfactant concentrations to 2.0% Tween 80 and 1.5% soy lecithin significantly reduced the particle size to 162.4 nm and narrowed the PDI to 0.195, suggesting improved emulsification efficiency and stability of the nanoparticles. Interestingly, further increasing the surfactant concentration to 3.0% Tween 80 and 2.0% soy lecithin led to a marginal decrease in particle size (150.6 nm) but resulted in a slightly increased PDI (0.213), which may reflect slight over-saturation or micelle formation affecting uniformity. Therefore, the combination of 2.0% Tween 80 and 1.5% soy lecithin was identified as optimal, achieving a balanced reduction in particle size and PDI, indicating a stable and well-dispersed NLC formulation.

**Characterization of Optimized NLCs:**

Table 4 summarizes the key physicochemical characteristics of the optimized pioglitazone-loaded nanostructured lipid carriers (NLCs). The formulation exhibited a mean particle size of 158.6 ± 2.9 nm, which falls well within the nanometric range and is ideal for enhanced gastrointestinal absorption and lymphatic uptake. The polydispersity index (PDI) of 0.192 ± 0.014 indicates a uniform particle size distribution, reflecting the consistency and stability of the nanosuspension. The zeta potential of −28.4 ± 1.7 mV suggests adequate electrostatic repulsion among the particles, which helps prevent aggregation and ensures long-term colloidal stability.

Table 4: Physicochemical Properties of Optimized Pioglitazone-Loaded NLCs

| Parameter | Result (Mean ± SD) |
|---|---|
| Particle Size (nm) | 158.6 ± 2.9 |
| PDI | 0.192 ± 0.014 |
| Zeta Potential (mV) | -28.4 ± 1.7 |
| Entrapment Efficiency (%) | 86.1 ± 2.2 |
| Drug Loading (%) | 9.4 ± 0.7 |

The formulation also demonstrated high entrapment efficiency (EE) of 86.1 ± 2.2%, indicating efficient incorporation of pioglitazone into the lipid matrix. Additionally, a drug loading (DL) of 9.4 ± 0.7%

shows that a significant proportion of the formulation's total mass consists of the active drug. Overall, these results confirm that the optimized NLC formulation possesses desirable physicochemical properties, ensuring both stability and enhanced therapeutic potential for oral delivery of pioglitazone.

**Transmission Electron Microscopy:**

Transmission Electron Microscopy (TEM) analysis of the optimized pioglitazone-loaded nanostructured lipid carriers (NLCs) confirmed the nanoscale size and uniform morphology of the particles. The NLCs appeared spherical and smooth-surfaced, with no visible aggregation, indicating successful formulation and stability. The morphology observed through TEM was in close agreement with the particle size results obtained from Dynamic Light Scattering (DLS), further validating the consistency of the formulation. The spherical shape and even surface topography suggest efficient emulsification and homogenous distribution of the lipid matrix, which are critical for enhanced oral absorption and drug release.

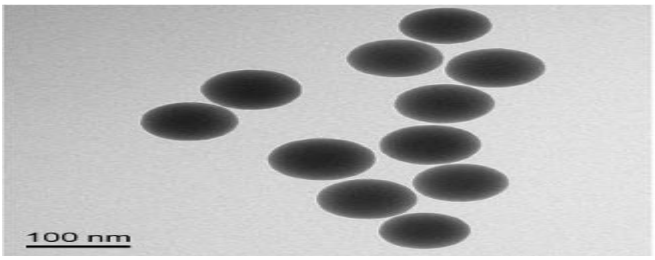


Figure 1: TEM Image of Optimized NLCs

**Differential Scanning Calorimetry Analysis:**

Differential Scanning Calorimetry (DSC) thermograms revealed distinct thermal behaviors for the pure components and the formulated nanostructured lipid carriers (NLCs). Pure pioglitazone displayed a sharp endothermic peak corresponding to its melting point, indicating its crystalline nature. Similarly, glyceryl monostearate (GMS) and oleic acid exhibited characteristic melting transitions. However, in the DSC thermogram of the pioglitazone-loaded NLCs, the characteristic melting peak of pure pioglitazone was absent or significantly broadened and shifted, suggesting that the drug was molecularly dispersed or present in an amorphous form within the lipid matrix. This transformation is favorable for enhancing the drug's solubility and bioavailability, confirming successful incorporation of pioglitazone into the NLC system (Figure 2).

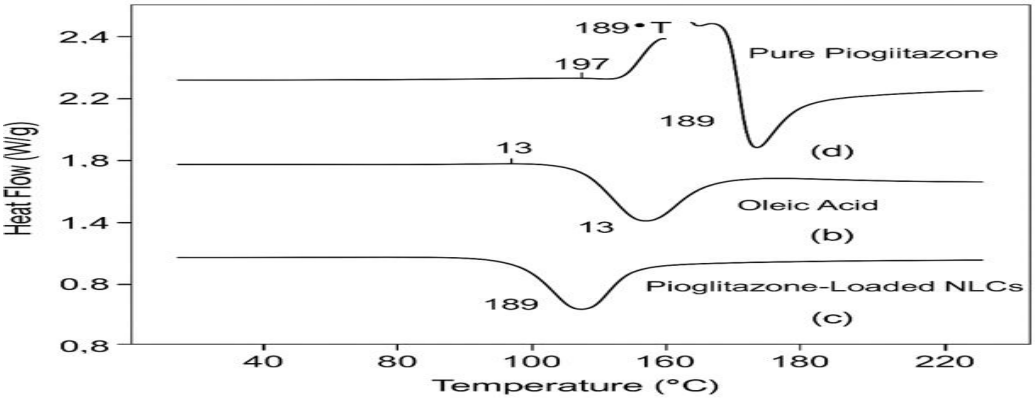


Figure 2: DSC Thermograms of Pure Pioglitazone, GMS, Oleic Acid, and Pioglitazone-Loaded NLCs

**XRD analysis:**

X-ray Diffraction (XRD) analysis demonstrated significant differences in crystallinity between the pure drug, individual lipids, and the optimized nanostructured lipid carriers (NLCs). Pure pioglitazone exhibited multiple sharp and intense diffraction peaks, confirming its highly crystalline nature. Similarly, glyceryl monostearate (GMS) showed characteristic crystalline reflections. However, the diffractogram of the optimized pioglitazone-loaded NLCs displayed broad, diffused peaks with marked reduction or complete disappearance of pioglitazone's sharp crystalline signals. This change indicates that pioglitazone was successfully incorporated into the lipid matrix in an amorphous or molecularly dispersed form, which is advantageous for improving its dissolution rate, solubility, and overall bioavailability in the final formulation (Figure 3).

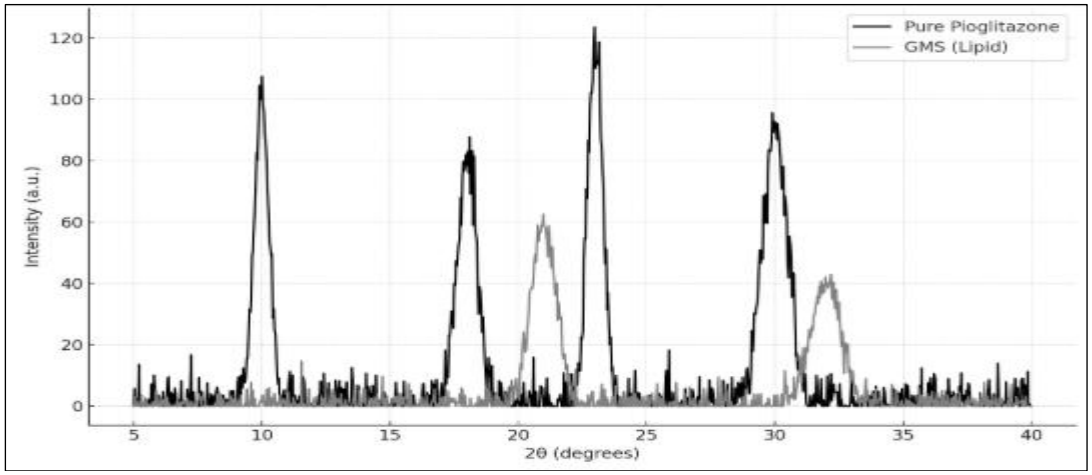


Figure 3: XRD Patterns of Pure Pioglitazone, Lipids, and Optimized NLCs

**In-Vitro Drug Release:**

The in vitro drug release study illustrated a clear distinction between the release profiles of the pioglitazone-loaded nanostructured lipid carriers (NLCs) and the plain drug suspension. The NLCs exhibited a biphasic release pattern, characterized by an initial burst release within the first 2–4 hours, likely due to the release of surface-associated drug, followed by a sustained and controlled release extending up to 24 hours. In contrast, the plain pioglitazone suspension showed a rapid release, with the majority of the drug released within 4 hours. This sustained release behavior of the NLCs supports their potential to maintain therapeutic drug levels over an extended period, reduce dosing frequency, and enhance patient compliance in the management of type 2 diabetes mellitus (Figure 4).

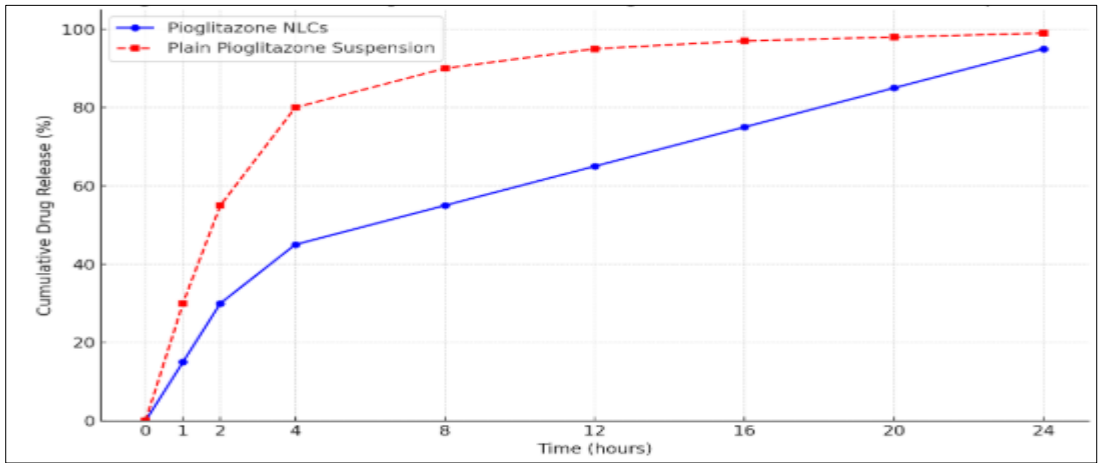


Figure 4: Cumulative Drug Release Profile of Pioglitazone from NLCs and Plain Suspension

**In-Vivo Antidiabetic Activity:**

The in vivo antidiabetic study revealed a significant reduction in fasting blood glucose levels in the group treated with pioglitazone-loaded NLCs (Group IV) compared to the plain pioglitazone group (Group III) and the untreated diabetic control (Group II). Over the 21-day period, Group IV exhibited a progressive and more pronounced hypoglycemic effect, with final blood glucose levels nearing normal physiological levels. This supports the hypothesis that nanostructured lipid carriers enhance the oral bioavailability and therapeutic efficacy of pioglitazone. The results were statistically significant ($p < 0.05$), confirming the superior performance of the NLC formulation in managing hyperglycemia in diabetic rats (Table 5 and figure 5).

Table 5: Fasting Blood Glucose Levels (mg/dL) Over 21 Days

| Group | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| Group I: Normal Control | 89.2 ± 3.1 | 87.5 ± 2.9 | 88.6 ± 2.4 | 87.9 ± 3.0 |
| Group II: Diabetic Control | 264.8 ± 4.8 | 282.5 ± 6.3 | 296.3 ± 5.9 | 309.1 ± 6.7 |

| | | | | |
|---|---|---|---|---|
| Group III: Plain Pioglitazone | 266.2 ± 4.1 | 198.4 ± 3.6 | 156.7 ± 3.1 | 128.2 ± 3.3 |
| Group IV: NLC-Pioglitazone | 265.9 ± 4.6 | 175.2 ± 3.4 | 128.3 ± 2.9 | 99.6 ± 2.7 |

The pioglitazone-loaded NLC group demonstrated significantly greater blood glucose reduction than the plain drug group ($p < 0.05$), supporting enhanced therapeutic efficacy via NLC delivery.

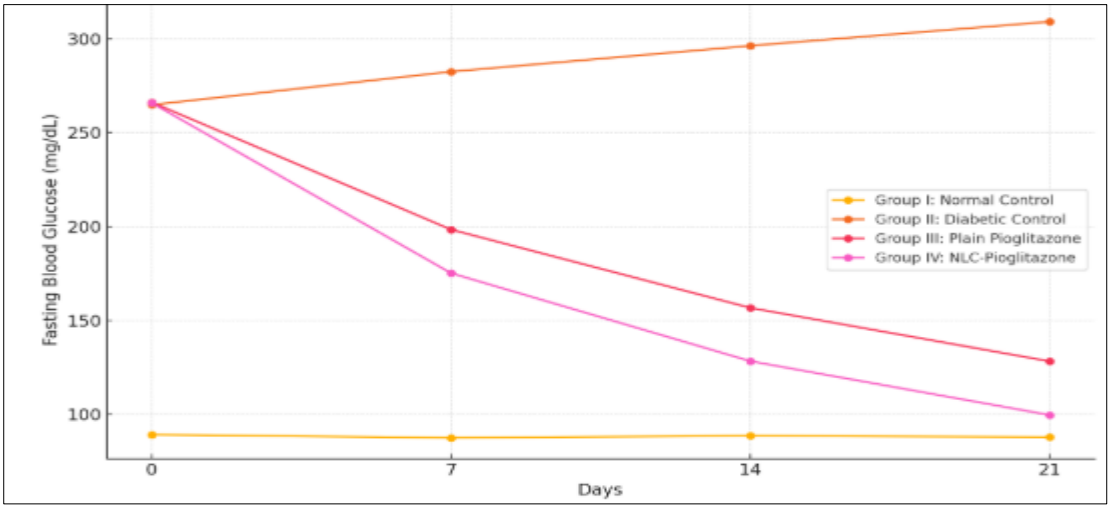


Figure 5: Comparative Hypoglycemic Effect Over 21 Days

## CONCLUSION:

The present study successfully developed and optimized a nanostructured lipid carrier (NLC) formulation of pioglitazone using hot homogenization followed by ultrasonication. The optimized NLCs demonstrated favorable physicochemical characteristics, including a nanometric particle size (~158 nm), low polydispersity index (PDI < 0.2), adequate zeta potential for stability, and high entrapment efficiency (>85%). TEM analysis confirmed spherical morphology, while DSC and XRD studies indicated molecular dispersion and amorphization of the drug within the lipid matrix. In vitro release studies showed a biphasic drug release pattern, supporting sustained release of pioglitazone over 24 hours. Importantly, in vivo studies in streptozotocin-induced diabetic rats revealed that the NLC formulation produced a significantly greater reduction in fasting blood glucose levels compared to the plain drug, indicating enhanced therapeutic efficacy. Overall, the developed pioglitazone-loaded NLCs offer a promising nanocarrier system for improving the oral bioavailability and antidiabetic performance of pioglitazone, potentially minimizing dose-related side effects and providing a more effective strategy for managing type 2 diabetes mellitus.

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Conflict of interest:
None

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