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Amphiphilic, lauric acid-coupled pluronic-based nano-micellar system for efficient glipizide delivery

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ABSTRACT

Glipizide; an insulin secretagogue belonging to the sulfonylurea class, is a widely used antidiabetic drug for managing type 2 diabetes. However, the need for life-long administration and repeated doses poses challenges in maintaining optimal blood glucose levels. In this regard, orally active sustained-release nano-formulations can be a better alternative to traditional antidiabetic formulations. The present study explored an innovative approach by formulating orally active sustained-release nano-micelles using the amphiphilic lauric acid-conjugated-F127 (LAF127) block copolymer. LAF127 block copolymer was synthesized through esterification and thoroughly characterized before being employed to develop glipizide-loaded nano-micelles (GNM) *via* the thin-film hydration technique. The optimized formulation exhibited mean particle size of 341.40 ± 3.21 nm and depicted homogeneous particle size distribution with a polydispersity index (PDI) < 0.2. The formulation revealed a surface charge of −17.11 ± 6.23 mV. The *in vitro* release studies of glipizide from developed formulation depicted a sustained release profile. Drug loaded micelles exhibited a substantial reduction in blood glucose levels in diabetic rats for a duration of up to 24 h. Notably, neither the blank nano-micelles of LAF127 nor the drug loaded micelles manifested any indications of toxicity in healthy rats. This study provides an insight on suitability of synthesized LAF127 block copolymer for development of effective oral drug delivery systems for anti-diabetic activity without any significant adverse effects.

1. Introduction

Type 2 diabetes mellitus is a global health challenge, characterized by insulin resistance, impaired insulin secretion, and chronic hyperglycemia (Popoviciu et al., 2023). As one of the leading causes of morbidity and mortality worldwide, this disease presents substantial burden on public health systems and individual well-being (Ong et al., 2023). The rising prevalence of Type 2 diabetes is often attributed to genetic predispositions, lifestyle factors and dietary habits (Misra, et al., 2023). Present clinical regimens including oral hypoglycemic agents and insulin, pose significant challenges due to their adverse effects, limited target specificity, and challenges in administration, impacting patient compliance and overall treatment efficacy (Demirbilek, et al., 2023).

Glipizide is a lipophilic drug that belongs to Biopharmaceutical Classification System II, commonly prescribed to type 2 diabetes patients. In type 2 diabetes, lifelong administration of drugs is necessary to manage elevated blood glucose levels (El-Dakroury 2023). A higher dose or frequent administration of medicine is needed to achieve therapeutic concentration, which may cause severe side effects, including

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constipation, diarrhea, gas; drowsiness, and sometimes seizure occurs due to hypoglycemia (Jha et al., 2023, Yuan, 2023, Simos, et al., 2021). Administration of glipizide leads to higher insulin production, resulting in lowering blood sugar levels and managing diabetes. However, poor oral bioavailability is always troublesome due to its poor water solubility (Okur et al., 2017). To overcome this issue, various drug delivery approaches, including microspheres (Patel, et al., 2005), cyclodextrin complex (Nie et al., 2011), Eudragit nanoparticles (Naha et al., 2013), solid self-nanoemulsifying drug delivery systems (Dash et al., 2015), poly(lactic-co-glycolic acid), chitosan-xanthan beads (Kulkarni et al., 2015), nanosuspension (Raja and Venkataramana et al., 2020), chitosan nanoparticles (Demirbilek, et al., 2023), and nanocochleates (Jain et al., 2023) to improve the absorption and bioavailability of glipizide have been investigated. However, there has been no investigation related to development of amphiphilic lauric acid-conjugated-F127 (LAF127) block copolymer based Nano-micelles of glipizide. The development of amphiphilic block copolymer-based nano-micelles could offer a distinctive advantage over other drug delivery systems designed to enhance Glipizide bioavailability. Nano-micelles offer enhanced solubilization of drugs due to their amphiphilic nature, enabling encapsulation of hydrophobic drugs within their core structure. The ease of preparation, often through simple self-assembly processes, distinguishes polymeric micelles from more complex nanoparticle formulations (Negut and Bita, 2023). Furthermore, their capacity for sustained and controlled drug release contributes to improved therapeutic effects (Jiao, et al., 2018, Ramezani and Shamsara, 2016).

Poloxamers, also known as Pluronics, represent ideal biodegradable polymers for diverse routes of administration owing to their remarkable compatibility with a wide array of active compounds and excipients used in pharmaceutical formulations and are listed as excipients in the United States and British Pharmacopoeias (Batrakova and Elena, 2008, Gao, et al., 2011, He, et al., 2017). These are non-ionic triblock poly (ethylene oxide)-poly(propylene-oxide)-poly (ethylene oxide) (PEO-PPO-PEO) copolymers with an A-B-A arrangement. Pluronic F127 has a critical micelle concentration (CMC) of 0.26–0.8 wt%. The stability and degradation dynamics of nanoparticles are highly influenced by the CMC of amphiphilic molecules employed for their development. Specifically, a higher CMC value implies higher chances of early dissociation of nanocarriers before reaching their target site leading to the premature release of encapsulated drugs. To address this issue, modified block copolymers have been investigated such as stearic acid-conjugated Pluronic F127 for effective doxorubicin delivery (Gao, et al., 2011), Pluronic/poly(lactic acid) vesicles for the administration of insulin (Nguyen, et al., 2021), and chitosan-Pluronic P123-biotin micelles for paclitaxel delivery (Xiong, et al., 2007) etc. These findings motivated us to investigate the application of Pluronic F127-based conjugated copolymer in treating diabetes. The novel lauric acid coupled Pluronic F127 (LAF127) pentablock copolymer was synthesized and glipizide was loaded within prepared nano-micelles. These nanocarriers were evaluated for particle size and size distribution, surface charge, drug entrapment, loading efficiency etc. The *in vivo* toxicological and antidiabetic effects in Wistar albino rats were studied on oral administration.

2. Materials and methods

Pluronic F127 [$(EO)_{101}$-$(PO)_{46}$-$(EO)_{101}$ (molecular weight = 12,600 g/mol), lauric acid, pyrene, streptozotocin, and nicotinamide were purchased from Sigma-Aldrich. Dialysis bag (12–14 kDa cut-off) and glucose were procured from HiMedia, India. Glipizide was bought from Swapnroop Drugs and Pharmaceuticals, Aurangabad, India. All synthetic-grade solvents were obtained from Molychem, India, and employed as such without distillation.

2.1. Synthesis of LAF127 copolymer

The synthesis of LAF127 copolymer was carried out according to the previously reported methods (Kamboj and Verma, 2019; Bae, et al., 2005). The process involved an esterification reaction between the hydroxyl group of Pluronic F127 and the carboxyl group of lauric acid at the melting phase. Briefly, Pluronic F127 (5.0 g) and lauric acid (5.0 g) were melted at 155 °C in 100 mL round bottom flask ensuring a homogeneous molten phase through steady stirring. Nitrogen gas was purged to maintain an inert environment in the flask. The LAF127 block copolymer was purified by adding molted mass into the mixture of ethyl acetate and petroleum ether in equal ratios for the separation of undissolved lauric acid. The above-mixed solution was filtered to remove un-dissolved lauric acid, and the organic solvent was vaporized at 25 °C and then desiccated under vacuum for 24 h to obtain LAF127 copolymer. The structure of the synthesized product was confirmed by proton nuclear magnetic resonance (^{1}H NMR), and Fourier transform infrared (FTIR) spectroscopy.

2.1.1. Proton nuclear magnetic resonance

The ^{1}H NMR spectrum of starting materials (lauric acid and Pluronic F127) and synthesized LAF127 copolymer was taken using deuterated chloroform ($CDCl_3$) as a solvent on a Bruker Avance II 400 NMR spectrometer and are given in parts per million (ppm) downfield from tetramethylsilane as internal standard.

2.1.2. FTIR analysis

The FTIR analysis was carried out to confirm the structure of the synthesized copolymer and drug excipient study. For this, FTIR spectrum of LAF127 copolymer, glipizide (pure drug), and physical mixture of LAF127 with glipizide was taken using Bruker Alpha (1–206-0280), Germany, in the range 400–4000 cm^{-1}. The potassium bromide (KBr) disc technique was used to prepare pellet for investigation.

2.2. Determination of critical micelle concentration (CMC)

The CMC value of pentablock LAF127 copolymer was evaluated by the previously reported fluorescence spectroscopic technique (Moghimi and Hunter, 2000). Acetone was used to solubilize pyrene, and 6.0×10^{-7} M concentration was achieved by adjusting concentration with deionized water. The acetone present in the pyrene solution was evaporated under reduced pressure. LAF127 and Pluronic F127 were mixed separately in dry pyrene to achieve a series of concentrations. A uniform amount of pyrene (6.0×10^{-7} M) was added to each polymer concentration. All the concentrations of polymers with pyrene were incubated in the dark for 6 h at 25 °C. After an incubation period, a spectrofluorometer was used to estimate the concentration of pyrene present in soluble form in the micelles phase. The 341 nm (excitation) and 360 to 450 nm (emission) wavelengths were selected for this estimation. The whole experiment was executed in triplicate. The fluorescence intensity ratio (I_{383}/I_{373}) versus logarithmic concentration (log c) was plotted to calculate the CMC value.

2.3. Preparation of drug-loaded LAF127 nano-micelles

The LAF127 copolymer was used to prepare glipizide-loaded nanomicelles (GNM) by the earlier reported thin-film hydration technique with slight modification (Gao, et al., 2011). Briefly, the LAF127 block copolymer was solubilized in 15 mL of methylene chloride, and glipizide was mixed in the 15 mL of methanol. Glipizide solution was added dropwise in the solution containing LAF127 copolymer from syringe under high-speed homogenization (IKA T25 ultra homogenizer, manufactured by IKA, Germany). The obtained emulsion was vacuum dried at 25 °C overnight for the evaporation of the solvent. The resultant film was hydrated with deionized water (15 mL) and stirred for 45 min at 750 rpm to obtain micellar dispersion. Further, the dispersion was

lyophilized to obtain the polymeric micelles. The lyophilized nano-micelles were stored in an amber-colored airtight container at 2 °C until further experimentation. The blank nano-micelles were also prepared with a similar procedure without adding glipizide.

2.4. Characterization of GNM

2.4.1. Determination of particle size and size distribution

The particle size and PDI of micelles were estimated using Malvern zeta sizer at 25 °C in disposable cuvettes. The value of PDI represents the distribution of particle size i.e., a greater PDI value implies a distribution of a wide range of particles which can lead to the development of aggregates and lower particle suspension stability and homogeneity **(**Zhao, et al., 2009**)**. For all samples, the viscosity, refractive index, and dielectric constant values were fixed at 10 cps, 1 and −0.8 mV, respectively. Three replicates were measured for each sample, and instrument performance was checked using polystyrene beads as a standard **(**Gao, et al., 2011**)**.

2.4.2. Zeta potential measurement

The zeta potential is a surface charge indicator that governs particle stability in a dispersion. It uses the dynamic light scattering principle to find the net surface charge of developed polymeric micelles. To analyze the electrophoretic mobility of polymeric micelles, samples were kept in an electrophoretic cell and the zeta potential was measured in triplicate.

2.4.3. Estimation of glipizide content in polymeric micelles

The amount of free glipizide was determined by the previously reported method **(**Gao, et al., 2011**)**. Briefly, glipizide nano-micelles dispersion was transferred to the centrifugal filter tubes; centrifuged at 12,000 x g, and the supernatant was analyzed for the quantity of glipizide using UV–Visible spectrophotometer (Lab India 3000^{+}) at 274 nm. The percent drug entrapment and loading efficiency were computed using equations (1) and (2), respectively.

$$\textbf{Entrapment efficiency} = \frac{\textbf{Amout of glipizide entrapped}}{\textbf{Amout of glipizide added}} \times 100 \quad (1)$$

$$\textbf{Loading efficiency} = \frac{\textbf{Amout of glipizide added} - \textbf{Amout of unentrapped glipizide}}{\textbf{Total amount of nanomicelles}} \times 100 \quad (2)$$

2.4.4. Morphological examination of polymeric micelles

The surface morphology of optimized nano-micelles was examined by Transmission Electron Microscope (TEM; Technai, FEI Co., The Netherland). One drop of polymeric micelles was kept on a copper grid for TEM imaging, and one drop of a 2 percent (w/v) aqueous solution of phosphotungstic acid was applied to enhance contrast. The additional staining liquid was removed by tissue paper, and the dried sample was examined under TEM microscope.

2.4.5. Drug release analysis

The *in vitro* drug release study pattern from GNM was evaluated by an earlier reported method **(**Rani, et al., 2017**)**. Precisely weighed nano-dispersion and drug suspension (glipizide equal to 4 mg) was kept in dialysis bags. The dialysis bags were dipped in 0.1 M phosphate buffer solution (150 mL; pH 7.4) in an Erlenmeyer flask. The temperature of the buffer solution was kept at 37.0 ± 0.5 °C and swirled at 100 rpm. At fixed intervals, a 1 mL aliquot was taken from the dissolution medium and was replaced with phosphate buffer to keep sink conditions. The quantity of glipizide present in withdrawn samples was estimated by UV–Visible spectrophotometer.

2.4.6. Stability studies

The stability of the optimized formulation (GNM1) was assessed for 90 days. The purpose of the stability studies was to assess the effect of storage on particle size, PDI, and drug content. The optimized polymeric micelles were kept in amber-colored glass bottles at 5.0 ± 3 °C and also at 25.0 ± 1 °C away from direct light for 90 days. The impact of storage on particle size, PDI, and drug content (%) of drug-loaded optimized formulation was determined by Malvern zeta-sizer and UV–Visible spectrophotometer, respectively.

2.5. In vivo studies

2.5.1. Animals

Female Wistar albino rats weighing 250 to 300 g were taken for *in vivo* investigations. The rats were purchased from the Lala Lajpat Rai University of Veterinary and Animal Sciences breeding facility in Hisar, India. The animals were housed in polypropylene cages in Maharshi Dayanand University's central animal house in Rohtak under controlled environmental conditions (23.0 ± 1 °C, 12 h/12 h dark/light cycle, and 55.0 ± 5 % humidity) for *in vivo* studies. The animals had full access to conventional animal feed and water. The Institutional Animal Ethical Committee (IAEC 151/57) approved the animal study protocols, and the experiments were accomplished as per CPCSEA norms.

2.5.2. Acute oral toxicity studies

The animals were starved for 12 h before receiving the first dose. The randomly selected six animals were allotted into three goups. Group I animals received normal saline, Group II animals received optimized formulation (GNM1 = 1.5 mg/kg), and Group III animals received blank nano-micelles, respectively. The polymeric nano-micelles were given orally in a daily dose for 21 days **(**Dhana-lekshmi, et al., 2010; Mutalik et al., 2006**)**. The duration of survival and mortality were used to measure acute toxicity. Possible adverse reactions, such as neuromuscular reactions, variation in body, nose and eye condition, the color of skin and motor activity were monitoried in the animal under observation.

2.5.3. Streptozotocin-induced antidiabetic studies

The previously reported method was selected to induce diabetes in Wistar albino rats using streptozotocin **(**Rani, et al., 2017, Kataoka, et al., 2012**)**. In Wistar albino rats, type 2 diabetes was developed by injecting nicotinamide (110 mg/kg b.w.) solution prepared in saline and streptozotocin (55 mg/kg b.w.) solubilized within 0.1 M citrate buffer (pH 4.5) *via* intraperitoneal route. To avoid the hypoglycemic effect of streptozotocin, nicotinamide was given 15 min before the drug was given. After 6 h of receiving the nicotinamide-streptozotocin injection, animals were allowed to drink 5 % glucose solution for the next 24 h to control the hypoglycemia.

The fasting blood sugar level was measured on the seventh day of nicotinamide-streptozotocin injection to evaluate type-II diabetes. Antidiabetic studies were performed on rodents with glucose levels ≥ 350 mg/dl. The rats were divided into four groups, with six animals in each group. Group (1) rats acted as a control group wherein normal saline was administered through an oral route for 21 successive days. Group (2) was selected from nicotinamide-streptozotocin-administered diabetic rats as a diabetic control group. In group 3, animals with confirmed type-II diabetes were treated with standard glipizide suspension (1.5 mg/kg b.w.). Group 4 animals with diabetes received GNM1 (equivalent to 1.5 mg/kg b.w.). Blood was collected from the tail veins of overnight starved rodents, and glucose levels were monitored at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h. The blood glucose levels were monitored with a calibrated digital glucose meter (Accu-Chek®).

2.6. Statistical analysis

One-way ANOVA was used to examine the *in vivo* antidiabetic results, followed by a Dunnet Multi-comparison test. Results are cited as significant values ($p < 0.01$ and $p < 0.05$).

3. Results

3.1. Characterization of LAF127 pentablock copolymer

The esterification reaction occurs between the carboxylic acid and the hydroxyl group of two chemical moieties with the elimination of a water molecule (Gao, et al., 2011). The hydroxyl group of the Pluronic F127 was esterified with the carboxylic group of lauric acid (Scheme 1). The reaction between lauric acid and Pluronic F127 was confirmed by the FTIR spectra of prepared copolymer's peak due to ester bond (C = O vibrational stretching) at 1733.09 cm^{-1}. Major features of FTIR spectra of LAF127 are depicted in Fig. 1. The ^{1}H NMR spectra of starting materials (Lauric acid and Pluronic F127) and synthesized LAF127 in $CDCl_3$ were taken, and the ppm value of different groups were determined (Fig. 2). The ^{1}H NMR spectra of lauric acid at 0.86–0.88 ppm indicate the protons of the CH_3 group, while the peaks at 1.26–1.35 ppm correspond to the protons of the CH_2 group of the acyl chain. The characteristic ^{1}H NMR peaks of lauric acid and Pluronic F127 were present in LAF127, and the values are tabulated in Table 1.

3.2. CMC analysis of block copolymers

Block copolymer having amphiphilic nature carries hydrophobic and hydrophilic chains. In an aqueous medium, the amphiphilic polymer chains self-assembled themselves to form polymeric micelles on or above CMC (Kataoka, et al., 2012). The CMC value of any polymer, including Pluronic F127 is affected by variations in temperature. Previous reports state that a lower CMC value of Pluronic F127 is observed at a higher temperature (0.11 wt% at 37 °C) whereas, at a lower temperature, a higher CMC value is obtained (0.25 wt% at 25 °C) (Perry, et al., 2011). Considering this fact, room temperature (25 °C) was selected for this study.

The ratio of emission characteristics (I_{383}) and vibrational peak (I_{373}) was taken to measure the polarity and CMC value of amphiphilic polymer. The low pyrene concentration was detected at lower polymers concentrations, and after a particular polymer concentration, the elevation in pyrene intensity was observed. This elevated intensity was responsible for two linear sections having different slopes. The fluorescence intensity ratio of I_{383}/I_{373} versus logarithm of block copolymers was plotted (Fig. 3), where the intersection of two lines corresponded to the CMC value. From obtained results, the CMC value of LAF127 block copolymer was calculated as 3.35×10^{-5} wt%. The CMC value of Pluronic F127 is reported as 0.383 wt% (Bahadori, et al., 2014), which is higher than CMC value of novel LAF127 block copolymer. Due to incorporation of lauric acid side chains on both terminals of Pluronic F127, LAF127 copolymer's CMC value was reduced. The lower CMC value is favorable for structural integrity of nano-micelles and stability.

3.3. Compatibility studies

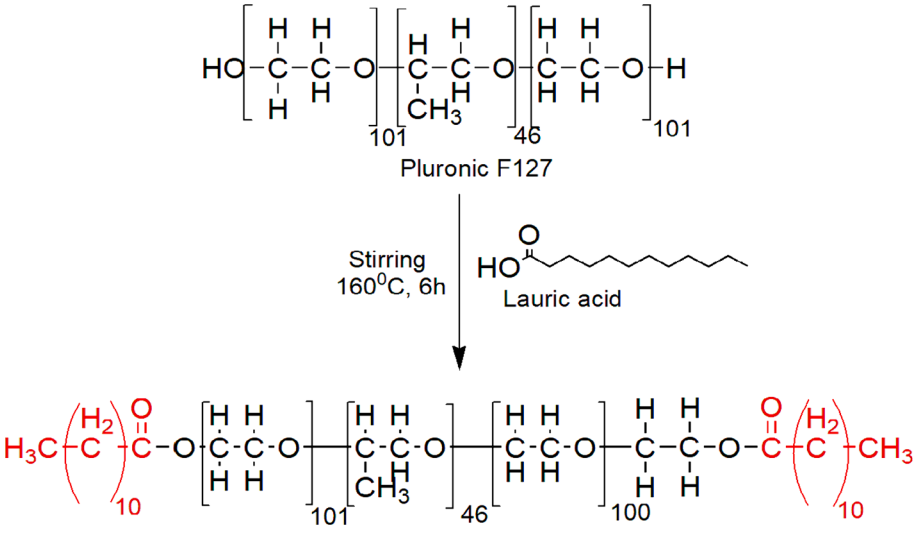
FTIR spectra give a clear idea regarding the interactions between functional groups present in the drug and polymeric excipients. The interactions between LAF127 and glipizide were studied by comparing the FTIR spectra of LAF127 copolymer, drug, physical mixture, and GNM1 (Fig. 1). The FTIR spectra of glipizide displayed peaks due to –NH stretching at 3250.04 cm^{-1}, C–H stretching at 2939.13 cm^{-1}, C=O stretching at 1691.10 cm^{-1}, –CONH- stretching at 1650.03 cm^{-1}, C=C aromatic stretching at 1590.24 cm^{-1}, C–H aromatic bending at 1443.0 cm^{-1}, O=S=O peak at 1334.2, 1161.0 cm^{-1}. The peaks observed in pure glipizide were also present in the physical mixture of (LAF127 and glipizide; 1:1), and GNM1. The FTIR spectra of LAF127, pure glipizide, and GNM1 were compared, and no significant shift in peaks was observed. A slight reduction in peak intensities was observed in the physical mixture and GNM1, which might be due to the presence of hydrogen bonding. FTIR analysis comparison revealed that the LAF127 and glipizide were compatible for development of nano-formulation in the current investigation.

3.4. Polymeric micelles preparation and characterization

The polymeric nano-micelles of glipizide were developed by thin-film hydration method and glipizide to LAF127 copolymer quantity was altered to find the best-optimized batch. The particle size, PDI, surface charge, drug loading, and entrapment efficiency of the GNM were tested, and results are presented in Table 2.

The effect of ratio of glipizide and copolymer was analyzed on different formulation parameters. To find an optimized formulation, all the batches were compared, and best GNM batch was selected. The increase in polymer concentration from 1:1 to 1:5 lead to increase in the size and PDI of nano-micelles, whereas zeta potential, drug loading, and entrapment efficiency were reduced. The small particle size and high entrapment efficiency were observed by using drug and copolymer in equal ratios (1:1).

Among eight developed formulations, the GNM1 batch showed the lowest size (341.40 ± 3.21 nm) and highest negative value of zeta potential (-17.11 ± 6.23), which supports the stability of the nano-formulation during the storage phase and was employed for further investigation. The Fig. 4 represents particle size distribution and zeta



Scheme 1. Synthesis of LAF127 copolymer.

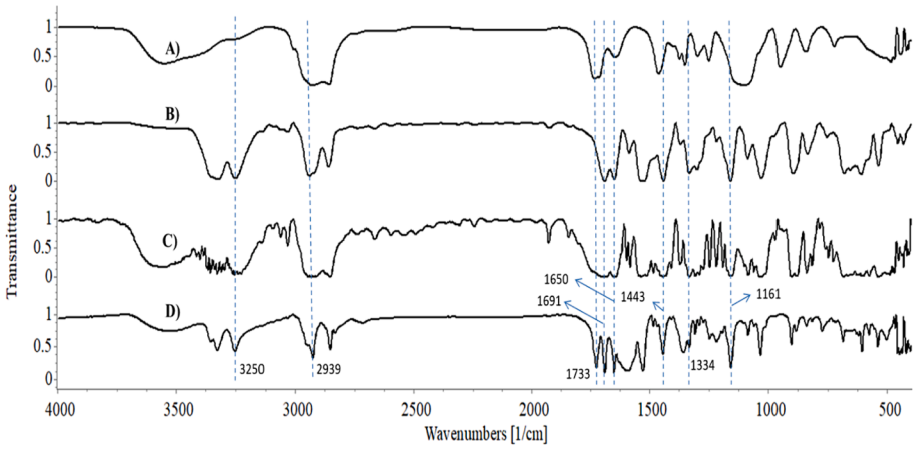


Fig. 1. FTIR spectra of (A) LAF127 copolymer, (B) Glipizide, (C) Physical mixture of LAF127 and glipizide, and (D) GNM1.

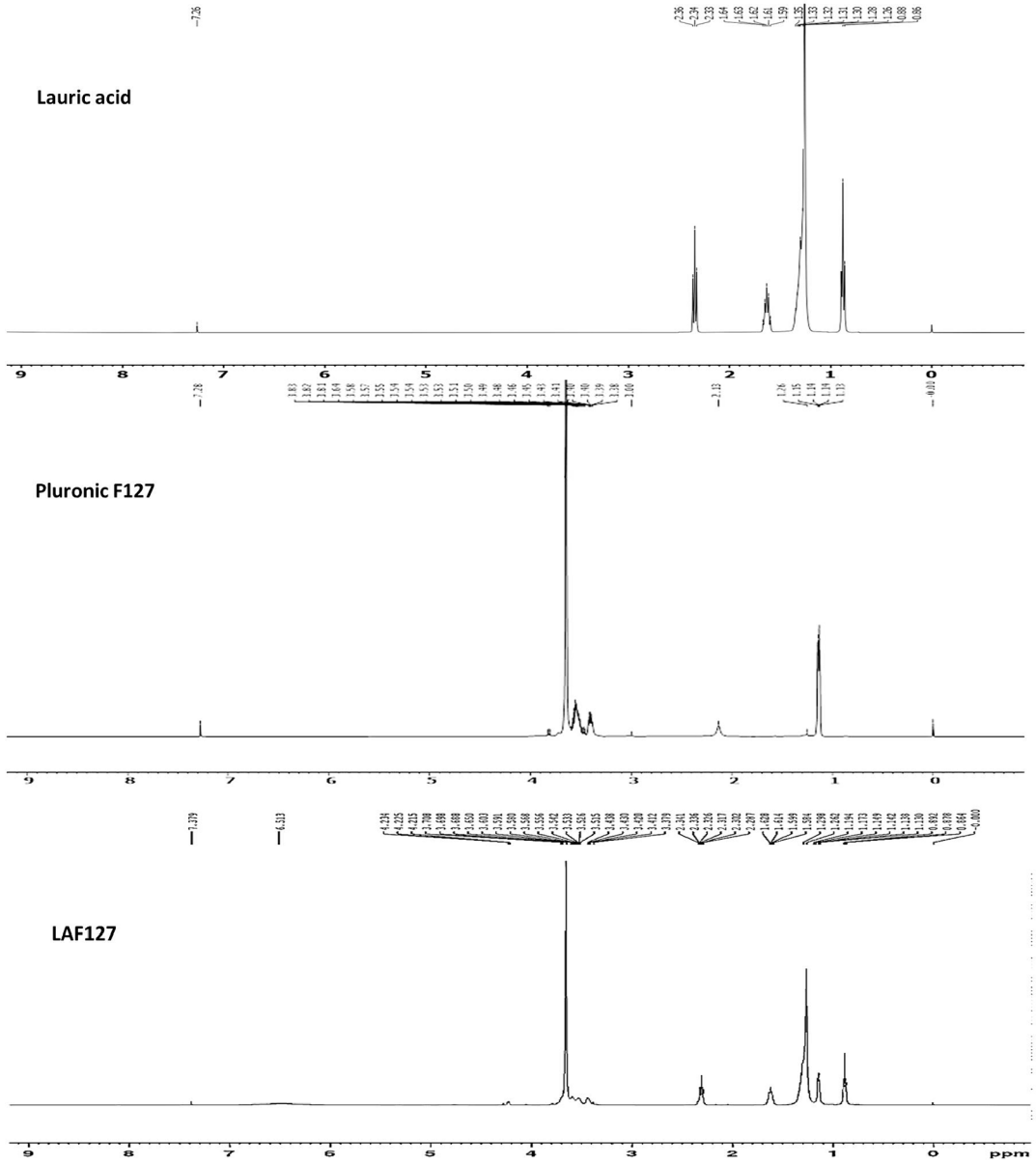


Fig. 2. Comparative ^{1}H NMR spectra of lauric acid, PF127 and LAF127 block copolymer.

Table 1
Main characteristics of ^{1}H NMR spectra of LAF127 copolymer in deuterated chloroform.

δ (PPM)	Assign values in ^{1}H NMR Spectra
CH_2-O in PEO	3.708–3.688
CH_2-CH_2-O in PEO	2.341–2.287
CH_2-**CH**(CH_3)-O in PEO	1.628–1.584
CH_2CH(**CH_3**)-O in PEO	1.298–1.130
CH_2 in LA	0.892–0.864

PEO: Polyethylene oxide; LA: Lauric acid.

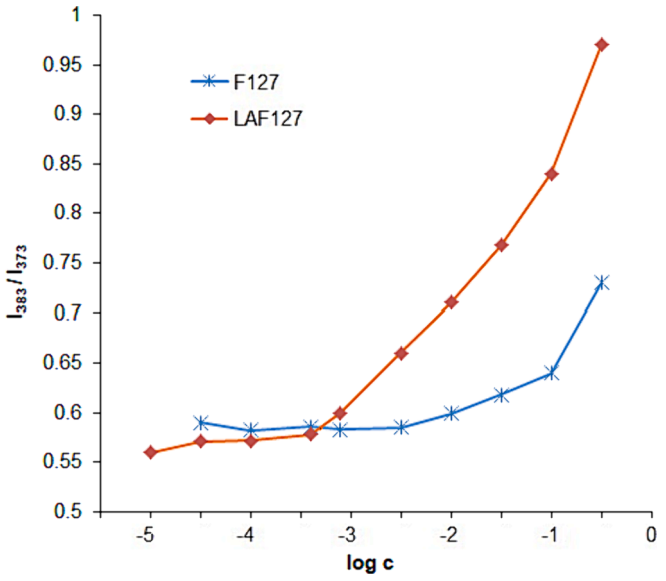


Fig. 3. Plot of florescent intensity of I_{383}/I_{373} of pyrene against concentrations (log c) of polymers.

potential graphs of GNM1. Drug entrapment and loading efficiency of GNM1 were observed as 69.24 ± 4.23 and 37.11 ± 3.32 %, respectively.

3.5. *In vitro drug release study*

A previously reported *in vitro* technique was adopted to analyze the pattern of glipizide release from micelles performed at physiological conditions (pH 7.4) (Dash, et al., 2015). The cumulative release (%) of glipizide versus time graph is shown in Fig. 5. At first 1 h, 10.1 ± 2.6 % drug was released from pure glipizide suspension, and 71.1 ± 3.5 % and 77.2 ± 2.3 % drug was released at 12 h and 18 h, sequentially. The burst release of glipizide was observed from GNM1 followed by sustained release. Within the first 1 h, 8.4 ± 2.1 % of the drug was released, and up to 12 h, 52.3 ± 3.4 % was released from the dialysis bag. The release profile was observed for 24 h and 88.2 ± 2.6 % glipizide was released from GNM1 depicting sustained release. The preliminary burst release of drug from GNM1 may be occurred because of osmotic pressure difference outside and inside the block copolymer (Kamboj and Verma, 2019; Choi. et al., 2008). The burst release of a drug just after administration of dosage form can quickly and effectively control the elevated blood glucose level in diabetic patients. The comparison of cumulative glipizide release from pure drug suspension (particle size 121 ± 43.68 μm) and GNM1 bags *via* determining similarity (f2) and dissimilarity (f1) factors revealed 18.04 and 92.13 values, respectively. It indicates that developed formulation demonstrated different release behavior (sustained release) compared to drug suspension. The sustained release pattern from GNM1 is due to the encapsulation potential of the LAF127 block copolymer to encapsulate the glipizide effectively.

3.6. *Morphological studies by TEM*

TEM is a specialized electron microscopic technique employed for imaging samples by scanning their surfaces, thereby generating multiple signals that convey information about the topography of the sample. The optimized nano-micelles were subjected to TEM analysis and they exhibited a spherical morphology (Fig. 6). The particle size seen under TEM was almost comparable to the size obtained by the zeta sizer.

3.7. *Stability studies*

The stability studies were done to evaluate the storage conditions for the developed formulation. GNM1 stability experiments were carried out for three months at 5.0 ± 3 °C, and 25.0 ± 1 °C and findings are displayed in Table 3. The size of nano-micelles suspension stored at both temperatures was found to be less than 350 nm. A slight change in GNM1 size, surface charge, PDI, and entrapment efficiency was observed. During 1st month of stability study, fewer changes in these parameters were observed compared to 3rd month. The color change was inspected at every fifteen days intervals, and no precipitation, visual aggregation, or color change was observed. TEM images after three months of storage revealed that there was no change in the nano-micelle's structure at both temperatures. Stability studies indicate that prepared LAF127 block copolymer can maintain morphological properties of micelles without losing loaded drug on storage for three months.

The semi-amorphous structure of the LAF127 copolymer in GNM1 may be responsible for a slight decline in entrapment efficiency and an elevation in micelles size. The semi-amorphous form of the lipophilic portion of the copolymer converts into the more stable amorphous form when exposed to heat or light (kinetic energy), allowing particle size to expand and the entrapped drug to be released from the matrix phase (Elbahwy et al., 2017). The stability study's findings were statistically insignificant. The nanoparticles kept at 5.0 ± 3 °C displayed a lesser variation in the considered parameters compared to 25.0 ± 1 °C, which indicates that the 5.0 ± 3 °C temperature was the optimum storage temperature.

Table 2
Results of screened parameters of prepared nano-micelles.

Batch Code	Glipizide: LAF127 (w/w)	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Drug loading (%)
GNM1	1:1	341.4 ± 3.21	0.135 ± 0.02	−17.11 ± 6.23	69.24 ± 4.23	37.11 ± 3.32
GNM2	1:2	785.5 ± 4.71*	0.281 ± 0.02*	−12.31 ± 5.41*	55.47 ± 4.34*	30.31 ± 3.54 ns
GNM3	1:3	795.2 ± 5.45*	0.542 ± 0.01*	−10.47 ± 5.51*	48.4 ± 3.51*	23.42 ± 2.05*
GNM4	1:4	812.62 ± 5.34*	0.817 ± 0.02*	−8.72 ± 4.25*	31.87 ± 3.38*	17.31 ± 3.32*
GNM5	1:5	845.54 ± 5.81*	0.637 ± 0.03*	−4.36 ± 4.61*	37.15 ± 3.53*	9.24 ± 2.08*
GNM6	1.5:1	889.04 ± 6.51*	0.704 ± 0.05*	−2.67 ± 5.34*	41.63 ± 3.92*	31.42 ± 2.73 ns
GNM7	2:1	788.5 ± 4.51*	0.531 ± 0.03*	−10.91 ± 4.52*	21.62 ± 3.62*	7.54 ± 3.42*
GNM8	3:1	735.2 ± 5.34*	0.582 ± 0.04*	−8.52 ± 4.36*	16.35 ± 3.81*	5.72 ± 3.44*
Blank Nano- micelles	0:1	301.51 ± 4.35*	0.114 ± 0.03*	−16.89 ± 5.34*	–	–

* The parameters of nano-micelles were analyzed using a one-way ANOVA, followed by a Dunnett multiple comparison test. Results are cited as significant values ($p < 0.05$) as compared to GNM1.

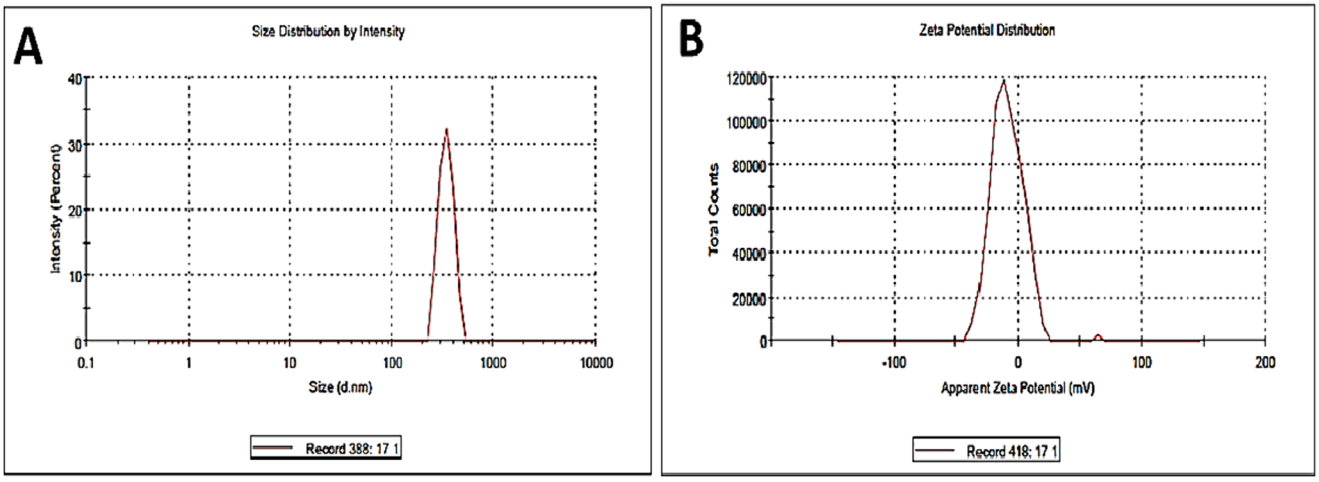


Fig. 4. Particle size distribution (A) and zeta potential (B) of GNM1.

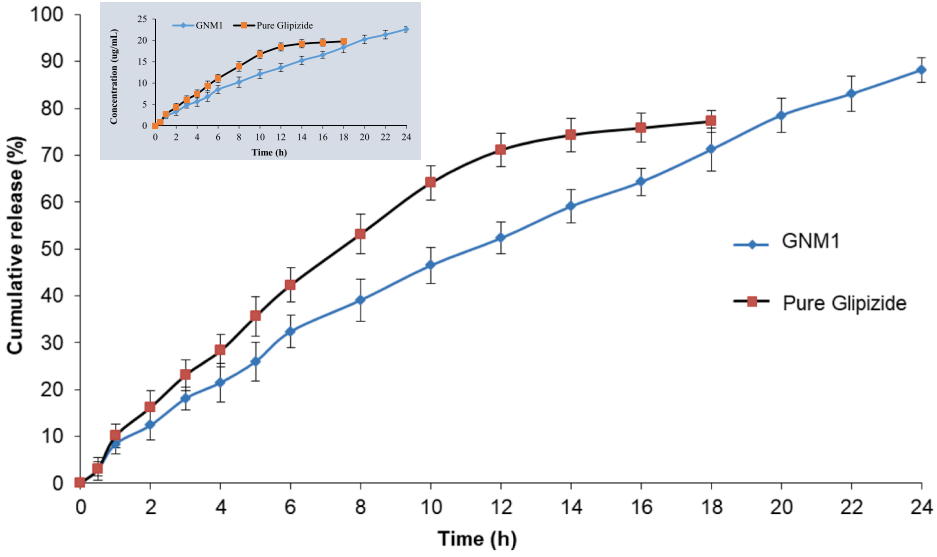


Fig. 5. Comparison of percentage cumulative release of glipizide from pure drug and GNM1. Inset shows concentration (microgram/mL) versus time graph.

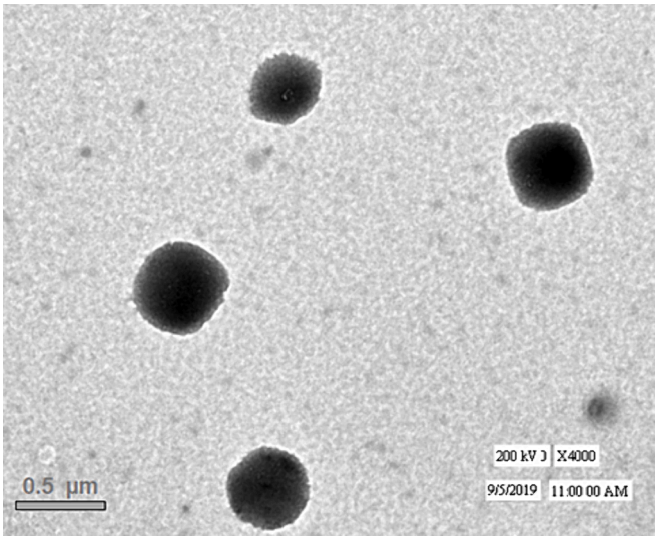


Fig. 6. TEM images of GNM1.

Table 3
Results of short-term stability studies of GNM1.

Storage temperature	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Visible changes
0 day (5.0 ± 3 °C; 25.0 ± 1 °C)	341.40 ± 3.21	0.135 ± 0.02	−17.11 ± 1.23	69.24 ± 4.23	Clear
1 month (5.0 ± 3 °C)	343.10 ± 4.52	0.138 ± 0.02	−17.02 ± 2.34	69.03 ± 3.43	Clear
3 month (5.0 ± 3 °C)	345.00 ± 4.33	0.140 ± 0.02	−16.98 ± 1.13	68.95 ± 4.62	Clear
1 month (25.0 ± 1 °C)	348.22 ± 4.84	0.141 ± 0.02	−16.89 ± 1.42	68.98 ± 3.21	Clear
3 month (25.0 ± 1 °C)	350.20 ± 5.62	0.145 ± 0.03	−16.53 ± 0.85	68.89 ± 3.82	Clear

n = 3, mean values ± SD.

3.8. Acute oral toxicity studies

The GNM1 (1.5 mg/kg b.w.) was chosen for acute oral toxicity studies in Wistar albino rats. The nano-sized glipizide formulation (GNM1), blank nano-micelles, and the control group did not demonstrate any mortality nor abnormality or behavioral changes during the observation period in toxicity studies (Table 4). A non-significant change in body weight growth was seen between the control and test groups. The 1.5 mg/kg daily oral dose of GNM1 and blank nano-micelles did not seem to retard the growth as well as food and water consumption. Generally, retard gain in body weight is considered as a sign of toxicity after the administration of a toxic substance (Dhana-Lekshmi, et al., 2011). The non-toxic behavior might be due to metabolically biodegradation of used copolymer and leaving metabolites that are not harmful to the biological system.

3.9. In-vivo antidiabetic activity

The streptozotocin was used to induce diabetes in Wistar albino rats, which were confirmed on the 7th day after administration of streptozotocin. The animals having blood glucose levels $\geq$ 350 dl/mL were selected for this study. The efficacy of the GNM1 was evaluated at oral doses of 1.5 mg/kg b.w. Glucose levels were checked in the blood of animals of different groups. The GNM1-administered group significantly lower glucose levels ($p < 0.05$) compared to diabetes control group. The blood glucose reduced significantly ($p < 0.05$) at 1, 2, 4, 6, 8, and 12 h compared to the normal saline group. GNM1 has sustained the blood glucose level up to 24 h, and results were better at 4, 6, 8, 12, and 24 h compared to standard glipizide (Fig. 7). The GNM1 has sustained the blood glucose level up to 24 h whereas on 4 h onward blood glucose level starts increasing in the standard drug-treated group. The above finding clearly states that the encapsulated glipizide can control blood glucose better than the standard glipizide for longer duration.

4. Discussion

Oral drug delivery based on nanotechnology is among the interesting fields of research and is explored by formulation scientists worldwide for many active molecules, including antidiabetic drugs. Still, to date, no official antidiabetic drug based on nanotechnology is available in the market. Nano-sized drug preparations enhance the bioavailability, provide controlled release, and have better therapeutic effects (Ghezzi, et al., 2021). The nano-micelles are commonly prepared by block copolymers to deliver lipophilic drugs. These polymeric excipients are excellent nano-carrier, but the stability of nano-micelles is always limited to their applications (Adav, et al., 2021). The polymers with amphiphilic nature are identified for various micellization properties. Micelles formation depends upon CMC value, and a polymer's appropriate CMC value also increases the stability (Awfik, et al., 2021). Polymeric micelles developed using amphiphilic block copolymers are commonly used to encapsulate Biopharmaceutics Classification System class II (low solubility with higher permeability) drugs, improving medication solubility and stability and can provide sustained-release pattern (Ying, et al., 2014). Because of their nano-size, polymeric micelles can cross the biological membrane and be absorbed into the systemic circulation with more ease and consequently enhancing the pharmacological action and reducing toxic effects (Gong, et al., 2012, Perumal, et al., 2022). Above CMC level, amphiphilic copolymers form micellar nanoparticles with unique core–shell structures that act as a repository for a lipophilic therapeutic agent (Abedanzadeh, et al., 2020).

In the present study, pentablock LAF127 copolymer was synthesized by reacting Pluronic F127 triblock copolymer with lauric acid. The resultant product was characterized by FTIR and ^{1}H NMR spectroscopy. The presence of hydrocarbon signals in the ^{1}H NMR spectra of polyethylene oxide chain and lauric acid-derived chains support the structure of the LAF127 copolymer. The hydroxyl group of Pluronic F127 esterified with a carboxylic group of lauric acid, and FTIR spectra peak at 1733.09 cm^{-1} confirmed the successful ester bond formation.

The pyrene fluorescence test was used to screen the micellization property of block copolymers (Jiao, et al., 2018). Pyrene is a polycyclic aromatic hydrocarbon that acts as a fluorescent probe and efficiently detects any change in polarity during micelles formation. The fluorescence intensity ratio of I_{383}/I_{373} versus logarithm of block copolymers was plotted, and the intersection of the intensity plot of F127 with the intensity plot of LAF127 was considered as a micellization point. Due to the incorporation of hydrophobic monomers of lauric acid, the CMC value of the LAF127 copolymer was found to be lesser than reported for Pluronic F127. The stability of micelles during storage and optimum release of loaded drug from core-shell is controlled by CMC value. The length of the link chain associated with block copolymer is an important factor in deciding the CMC value (Bahadori, et al., 2014). In the present investigation, hydrocarbon chains associated with terminals of LAF127 copolymer are responsible for low CMC value. The low CMC value of LAF127 copolymer justifies the structural integrity and stability of micelles on dilution in the biological system as well as during storage. The low CMC leads to lower degradation of drug; as a result, better bioavailability of the drug in *in vivo* system.

The compatibility between polymeric excipients and drug molecules is identified as one of the essential key features to finding the efficiency of polymer-based formulation systems. Every drug interacts with polymers uniquely because of its diverse physical and chemical properties. The compatibility of polymers with a drug directly influences various properties of a delivery vehicle, such as drug-loading capacity, drug stability, and release kinetics. Due to this, compatibility or degree of interaction must be evaluated to design a desired drug delivery system (Dash, et al., 2015). The FTIR spectra of LAF127 and glipizide were compared with the spectra of physical mixture and GNM1. All the important peaks of pure drug and LAF127 copolymer were detected in the physical mixture as well as in GNM1. As the LAF127 and glipizide are compatible with each other, their nano-micelles were fabricated by the thin-film hydration method (Gao, et al., 2011). Among all the ratios of glipizide and LAF127, 1:1 (GNM1) showed the lowest particle size, negative surface charge, and best PDI value. The entrapment and loading efficiency of GNM1 was also better than other ratios. Increasing the amount of copolymer (GNM1 to GNM51) resulted in a significant increase ($p < 0.05$) in particle size as reported in Table 2. This can be attributed to a rise in the number of assembled block copolymers, accompanied by an increase in the size of the insoluble core. Consequently, there is an enhancement in overall particle size (Cabral, et al., 2018). Surprisingly, drug loading and entrapment efficiency were also found to be significantly reduced ($p < 0.05$) with increase in amount of polymer. The possible reason could be that with higher polymer concentrations, there's a greater likelihood of head-to-head interactions among the amphiphiles, which can trap drug particles on the micelle surface rather than allowing them to penetrate deeper into the core (Woodhead, and Hall, 2011). Furthermore, increasing the amount of drug while keeping the polymer amount constant (from GNM6 to GNM8) resulted in a reduction in the entrapment efficiency and drug loading ($p < 0.05$). This phenomenon can be attributed to the fact that, after achieving maximum loading capacity, the drug tends to precipitate (Ahmad et al., 2014). The increase or decrease in drug to copolymer

Table 4
Behavioral parameters of GNM1 and blank nano-micelles in rats.

Behavioral parameters	GNM1	Blank Nano-micelles
Motor activity	Normal	Normal
Salivation and Lacrimation	Normal	Normal
Respiration	Normal	Normal
Body weight	No significant change	No significant change
Righting reflex	Positive	Positive
Convulsions	Negative	Negative
Skin color	Normal	Normal

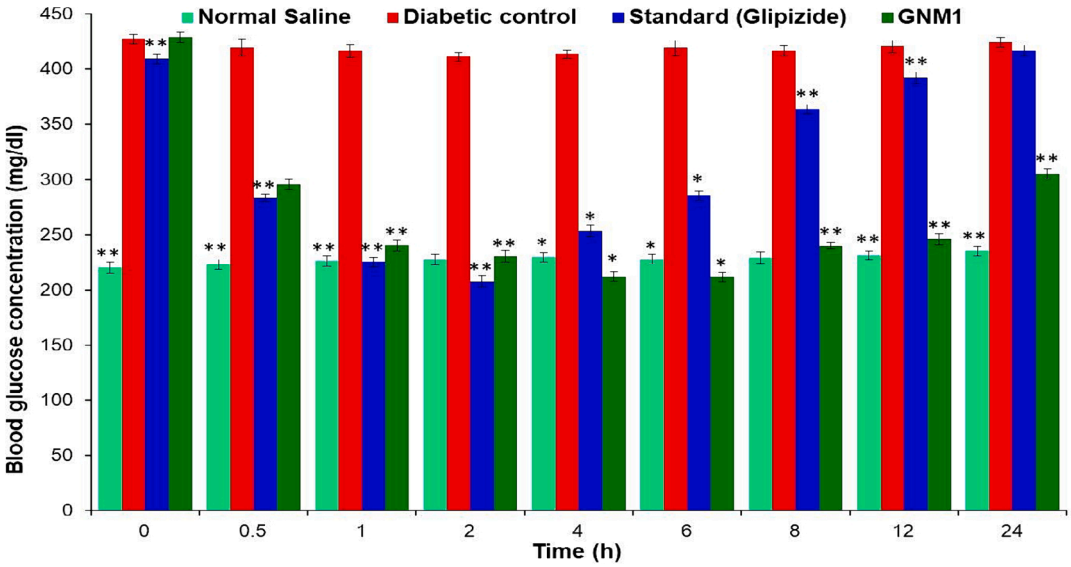


Fig. 7. Comparison of results of blood glucose levels in different animal groups receiving various treatments using streptozotocin-induced diabetic rat model (mean ± SD; n = 6). Values are represented as mean ± S.D; @.* = $p < 0.05$; ** = $p < 0.01$; compared to diabetic control.

ratio leads to unfavorable results; hence, GNM1 (1:1) was considered an optimized batch. In the current investigation, the solubility of the free drug was determined to be 0.176 ± 0.002 mg/mL. Following encapsulation in polymeric micelles, a substantial improvement was observed, with the solubility increasing to 35.466 ± 2.063 mg/mL. The surface morphological examination of GNM1 revieled development of sperical shaped micelles that the glipizide is encapsulated inside the lipophilic central part of LAF127 block copolymer (GNM1).

Before proceeding to *in vivo* antidiabetic activity and acute toxicity studies, drug release from pure drug and GNM1 was estimated by the dialysis bag method. From the dialysis bag of GNM1, nearly 88.2 ± 2.6 % of the drug was released in a sustained manner up to 24 h. The extended-release time of glipizide from GNM1 is highly acceptable for management of diabetes.

The optimized batch (GNM1) and blank nano-micelles of LAF127 do not show any toxicity or change in the behavior of animals. The blood glucose level in diabetic animals was controlled up to 24 h due to the sustained drug release from GNM1. The conventional oral dose form does not have sustained-release properties. In various cases, fast release of drugs from these formulations can lead to a sharp elevation of drug concentration at a toxic level (Adepu, et al., 2021). Drug concentration fluctuation can be controlled by using sustained-release nano-formulations that can control blood glucose levels better than conventional dosage forms.

For the preparation of a sustained-release delivery vehicle in the nano-range, selecting a suitable polymeric excipient is always needed to attain desired properties in nano-formulation. In the current investigation, synthesized block copolymer was found to be a suitable carrier for the nano-micelles formulation and released the drug sustainably via the oral route. The reported copolymer can be used as a nano-carrier to design and formulate oral nano-formulations of the antidiabetic drug.

5. Conclusion

In the present work, it was observed that it is possible to encapsulate glipizide into polymeric nano-micelles fabricated from the LAF127 block copolymer. The presence of terminal side chains in LAF127 copolymer reduces the CMC value. The LAF127 copolymer can efficiently encapsulate glipizide in an equal amount with good stability in the form of nano-micelles. The altered ratio of drug and copolymer affects the particle size, surface charge, encapsulation, and drug loading efficiency. These polymeric nano-micelles possess a sustained release profile and can regulate the blood glucose level of diabetic animals up to 24 h when administered orally. The acute oral toxicity study revealed that the polymeric nano-micelles are safe in animals and biocompatible and justified their application in the preparation of nano-micelles for oral delivery of the drug. Continuous efforts to advance our understanding of the LAF127 block copolymer-based nano-micelles may open avenues for innovative and efficient oral delivery strategies specifically tailored for lipophilic drugs. Furthermore, the integration of advanced imaging and tracking techniques may provide valuable insights into the *in vivo* behavior of these nano-micelles, facilitating a more comprehensive understanding of their pharmacokinetics and biodistribution.

CRediT authorship contribution statement

Vipan Kumar: Conceptualization, Formal analysis, Investigation, Methodology. **Neelam Poonia:** Data curation, Formal analysis, Resources. **Pradeep Kumar:** Methodology, Project administration, Software. **Prabhakar Kumar Verma:** Resources, Writing – original draft, Writing – review & editing. **Abdulrahman Alshammari:** Funding acquisition, Methodology, Writing – review & editing. **Norah A. Albekairi:** Funding acquisition, Writing – original draft, Writing – review & editing. **Atul Kabra:** Validation, Visualization, Writing – original draft, Writing – review & editing. **Neera Yadav:** Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics Statement

The animal protocol was approved by Institution Animals Ethics Committee (IAEC) (No. IAEC 151/57) of Maharshi Dayanand University, Rohtak, India. All the experiments and animal care were in accordance with the regulations set forth by the Committee for the Control

and Supervision of Experiments on Animals (CCSEA), the Government of India, and ARRIVE guidelines were followed.

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