RESEARCH PAPER

Formulation and Evaluation of Glibenclamide Nanocrystals for Improved Solubility and Antidiabetic Efficacy

Nallagandla Rajitha[1], Ramana Hechhu[2], Yulchiyev Rahimjon Sattorovich[3], Swati Gorakshnath Wagh[4], Layeba Kubra Fathima[5], Abdul latif[6], K. Sampath Kumar[*7]

[1]Associate Professor, Department of Pharmaceutics, Malla Reddy Institute of Pharmaceutical Sciences, Mallareddy Vishwa Vidyapeeth Deemed to be University, Maisammaguda, Hyderabad, Telangana, India-500100; Email: rajitha.nallagandla@mrvv.edu.in

[2]Department of Pharmaceutical Chemistry, Malla Reddy Institute of Pharmaceutical Sciences, Malla Reddy Vishwavidyapeeth (Deemed to be University), Secunderabad, 500100, Telangana; Email: hechhu.ramana@gmail.com; ORCID ID: https://orcid.org/0000-0002-3865-4611

[3]Assistant Professor, Department of Folk medicine and Pharmacology, Fergana Medical Institute of Public health, Fergana; Email: jrahimzon@gmail.com

[4]Assistant Professor, Arts, Science and Commerce College Burhannagar Ahilyanagar, Maharashtra; Email: swati.wagh375@gmail.com

[5]Assistant Professor, Department of Physiology, Fergana medical Institute of public health, Fergana; Email: layebakubra@gmail.com

[6]Assistant Professor, Department of Microbiology, Fergana medical Institute of public health, Fergana; Email: zainnlateef@gmail.com (Note: corrected email domain)

[7]Associate Professor, Department of Pharmaceutics, Malla Reddy Institute of Pharmaceutical Sciences, Mallareddy VishwaVidyapeeth Deemed to be University, Maisammaguda, Hyderabad; Telangana, India-500100; ORCID ID: https://orcid.org/0000-0001-5960-3912; Email: sampathkumar.katakam@mrvv.edu.in

***Corresponding author:** Dr. K. Sampath Kumar, Associate Professor, Department of Pharmaceutics, Malla Reddy Institute of Pharmaceutical Sciences, Mallareddy VishwaVidyapeeth Deemed to be University, Maisammaguda, Hyderabad; Telangana, India-500100; ORCID ID: https://orcid.org/0000-0001-5960-3912; Email: sampathkumar.katakam@mrvv.edu.in

Abstract

The present study aimed to develop, optimize, and evaluate glibenclamide nanocrystals to enhance the dissolution rate and oral bioavailability of this BCS Class II antidiabetic drug, thereby improving its therapeutic efficacy. Glibenclamide nanocrystals were prepared using a combination method (antisolvent precipitation followed by wet bead milling). A 3^2 factorial design was employed to optimize formulation variables (stabilizer concentration and drug concentration) with particle size, polydispersity index (PDI), and zeta potential as response variables. Solid-state characterization confirmed partial reduction in crystallinity without chemical interaction with stabilizers. In vivo studies in diabetic rats demonstrated superior pharmacodynamic efficacy with 68.5 ± 4.2% maximum blood glucose reduction at 4 hours, compared to 42.8 ± 3.5% at 6 hours for marketed formulation. Pharmacokinetic analysis revealed 2.8-fold and 1.7-fold enhancement in oral bioavailability relative to pure drug and marketed formulation, respectively. Stability studies confirmed that lyophilized nanocrystals remained stable for 3 months under accelerated conditions. Nanocrystal technology successfully addressed the solubility and dissolution limitations of glibenclamide, resulting in significantly enhanced oral bioavailability and improved antidiabetic efficacy. This approach represents a promising strategy for optimizing the therapeutic performance of BCS Class II drugs like glibenclamide.

Keywords: Glibenclamide; Glyburide; Nanocrystals; Nanosuspension; Poorly water-soluble drugs; BCS Class II

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, resulting from defects in insulin secretion, insulin action, or both . The pathophysiology of its primary subtypes is distinctly different. Type 1 diabetes mellitus (T1DM) is an autoimmune condition involving the destruction of pancreatic β-cells, leading to an absolute deficiency of insulin . This process is influenced by genetic susceptibility, particularly involving HLA-DQB1 and DRB1 haplotypes, and potential environmental triggers such as viral infections . In contrast, type 2 diabetes

mellitus (T2DM), which accounts for 90-95% of all cases, involves a more complex interplay of insulin resistance and a relative deficit in insulin secretion . Risk factors for T2DM include obesity, advanced age, physical inactivity, and a strong genetic predisposition . The global burden of DM is substantial and growing; recent estimates indicate that approximately 8.5% of the global adult population is affected, with projections suggesting this could rise to nearly 10.9% by 2030, placing an immense strain on healthcare systems worldwide .

Glibenclamide (also known as glyburide) is a second-generation sulfonylurea widely used in the management of T2DM. Its mechanism of action involves stimulating insulin secretion from pancreatic β-cells by binding to and inhibiting ATP-sensitive potassium channels . From a biopharmaceutical perspective, glibenclamide is a classic example of a Biopharmaceutics Classification System (BCS) Class II drug, meaning it exhibits low aqueous solubility but high permeability across biological membranes . This physicochemical characteristic presents a significant challenge, as the rate at which the drug dissolves in gastrointestinal fluids becomes the rate-limiting step for its absorption. Consequently, the poor and variable dissolution of glibenclamide directly compromises its oral bioavailability . Although it is highly permeable, its low solubility means that only a fraction of the administered dose is available for systemic absorption, often leading to suboptimal therapeutic outcomes and significant inter-individual variability in drug plasma concentrations .

Conventional oral formulations of glibenclamide, such as standard immediate-release tablets, often fail to address this underlying solubility issue adequately. The slow and incomplete dissolution of the drug from these traditional dosage forms can result in erratic and delayed absorption . Research has demonstrated that the rate and extent of in vitro dissolution for standard glibenclamide preparations can be significantly lower compared to more advanced, rapidly dissolving formulations . This limitation in dissolution efficiency has practical clinical consequences, as studies have shown that formulations with enhanced dissolution can render a lower dose of glibenclamide bioequivalent to a higher dose of a conventional tablet . Thus, the therapeutic efficacy of standard glibenclamide tablets is inherently limited by the drug's poor aqueous solubility, underscoring the critical need for innovative formulation strategies to overcome this biopharmaceutical hurdle.

Nanocrystal technology has emerged as a promising and universally applicable strategy to tackle the delivery challenges posed by poorly water-soluble drugs like glibenclamide . Drug nanocrystals are sub-micron particles, typically sized below 1 μm (1000 nm), composed of pure drug and a minimal amount of stabilizer required to prevent agglomeration . The fundamental principle behind this approach is the substantial increase in specific surface area achieved through particle size reduction to the nanoscale . This dramatic increase in surface area is directly proportional to the dissolution rate, as described by the Noyes-Whitney equation. Furthermore, as particles approach the nanometer size range, their intrinsic saturation solubility can also increase, a phenomenon dictated by the Ostwald-Freundlich equation, which links solubility to the high curvature and interfacial tension of the nanocrystal surface .

The mechanisms by which nanocrystals enhance dissolution and, consequently, bioavailability are multifaceted. Primarily, the vast surface area available for interaction with the dissolution medium leads to a rapid dissolution velocity, creating a high concentration gradient that drives passive absorption across biological barriers . This rapid dissolution can also help mitigate food effects, as the absorption becomes less dependent on the solubilizing action of a meal . While the traditional view held that nanocrystals dissolve instantaneously in vivo, recent evidence from advanced bioimaging techniques suggests a more nuanced picture. It is now understood that intact nanocrystals may persist in the body for extended periods, interacting with biological tissues and potentially being taken up by cells before dissolving intracellularly . This revised understanding points to a dual fate: dissolution followed by molecular absorption, alongside the potential for direct transport of intact nanocrystals . Regardless of the exact pathway, the net outcome for BCS Class II drugs like glibenclamide is a marked improvement in oral bioavailability, making nanocrystal formulations a highly effective approach for enhancing their antidiabetic efficacy .

Glibenclamide (glyburide) is a second-generation sulfonylurea oral hypoglycemic agent characterized by specific physicochemical and pharmacokinetic properties that govern its therapeutic behavior. Chemically, it is a white, crystalline powder with a molecular weight of 494.01 g/mol and a melting point between 169-174°C, indicating its crystalline nature. From a biopharmaceutical perspective, glibenclamide is unequivocally established as a Biopharmaceutics

Classification System (BCS) Class II compound, exhibiting high permeability across biological membranes but extremely low aqueous solubility across the physiological pH range. This poor aqueous solubility, reported to be approximately 4-5 µg/mL, represents the fundamental rate-limiting step in its gastrointestinal absorption, resulting in slow, incomplete, and highly variable oral bioavailability. Pharmacokinetically, glibenclamide is rapidly absorbed from the gastrointestinal tract, achieving peak plasma concentrations within 2-4 hours post-administration. It undergoes extensive hepatic metabolism via the cytochrome P450 enzyme system, primarily CYP2C9 and CYP3A4, producing metabolites with limited therapeutic activity. The drug is highly bound to plasma proteins (approximately 99%), predominantly to albumin, which contributes to its relatively long elimination half-life of 5-10 hours and influences its volume of distribution.

Given the significant biopharmaceutical challenge posed by glibenclamide's poor aqueous solubility, extensive research has focused on developing formulation strategies to enhance its dissolution rate and subsequent bioavailability. Conventional approaches have included particle size reduction through micronization, which increases surface area to some extent, and the use of cyclodextrins to form inclusion complexes that temporarily encapsulate the drug molecule, improving its apparent solubility. More advanced strategies have explored solid dispersions, wherein the drug is dispersed within a hydrophilic carrier matrix at the molecular or amorphous level, and lipid-based formulations such as self-emulsifying drug delivery systems (SEDDS), which present the drug in a pre-dissolved state within the gastrointestinal tract. While these approaches have demonstrated varying degrees of success, each carries inherent limitations, including issues with stability, drug loading capacity, and manufacturing scalability.

Among these various approaches, nanocrystal technology has emerged as a particularly elegant and universally applicable platform for solubility enhancement of BCS Class II drugs. Drug nanocrystals are sub-micron colloidal dispersions of pure drug particles, typically stabilized by a minimal quantity of surface-active agents or polymeric stabilizers to prevent agglomeration. The fundamental principle driving their enhanced performance is the dramatic increase in specific surface area accompanying particle size reduction to the nanometer range (typically 100-1000 nm), which proportionally increases the dissolution velocity according to the Noyes-Whitney equation. Additionally, the reduction of particles to the nanometer scale can increase the intrinsic saturation solubility itself, as described by the Ostwald-Freundlich equation. The methods for producing drug nanocrystals are broadly categorized into three approaches. Top-down technologies, most notably wet bead milling, represent the most commercially successful approach, involving mechanical attrition of larger drug crystals in the presence of grinding media and stabilizers. This method is robust, universally applicable, and has yielded several commercial products through the NanoCrystal® technology platform. Bottom-up technologies, including antisolvent precipitation, involve dissolving the drug in a suitable solvent and rapidly mixing with an antisolvent containing stabilizers, inducing supersaturation and subsequent nucleation and crystal growth. This approach is energy-efficient and can produce very small particles but requires careful control of process parameters to prevent uncontrolled crystal growth. Combination technologies, such as NanoEdge™, synergistically integrate precipitation with subsequent high-energy processing, overcoming the limitations of each individual approach by first creating a supersaturated state and then applying mechanical energy to control crystal growth and stabilize the resulting nanocrystals.

A critical component of any successful nanocrystal formulation is the appropriate selection of stabilizers, which function to prevent particle agglomeration through electrostatic or steric stabilization mechanisms. Polymeric stabilizers, including polyvinylpyrrolidone (PVP), various grades of polyethylene glycol (PEG), and Kolliphor® (formerly Solutol® HS15 and others), provide steric stabilization by adsorbing onto the particle surface and creating a physical barrier that prevents close approach and aggregation of adjacent particles. These polymers are particularly effective at maintaining physical stability during storage and preventing Ostwald ripening. Surfactant stabilizers, such as polysorbates (Tween 80) and lecithin, primarily provide electrostatic stabilization by adsorbing at the solid-liquid interface and conferring a surface charge, typically measured as zeta potential, which creates repulsive forces between approaching particles. Often, a combination of polymeric and surfactant stabilizers is employed to achieve optimal stabilization through complementary mechanisms.

Following successful preparation, comprehensive solid-state characterization is essential to understand the physicochemical attributes of the developed

nanocrystals. Differential scanning calorimetry (DSC) and X-ray powder diffraction (XRD) are routinely employed to assess the crystalline state of the drug, as processing may induce partial or complete amorphization, which can affect physical stability. Fourier transform infrared spectroscopy (FTIR) confirms drug-excipient compatibility, while scanning electron microscopy (SEM) provides visual confirmation of particle morphology, size, and shape. The in vitro and in vivo evaluation of glibenclamide nanocrystals specifically focuses on demonstrating the anticipated performance improvements. Saturation solubility studies in various biorelevant media confirm enhanced solubility compared to the pure drug, while in vitro dissolution studies typically demonstrate significantly faster and more complete drug release. Critically, pharmacodynamic studies in diabetic animal models (e.g., streptozotocin-induced diabetic rats) demonstrate superior and more sustained blood glucose reduction compared to conventional formulations, while pharmacokinetic evaluation confirms enhanced oral bioavailability, often showing several-fold increases in area under the curve (AUC) and peak plasma concentration (Cmax), thereby validating the nanocrystal approach for improving the antidiabetic efficacy of glibenclamide.

Material & Methods

Analytical Method Development for Glibenclamide

Preparation of Calibration Curve by UV-Visible Spectroscopy

A UV-Visible spectrophotometric method will be developed for the routine estimation of glibenclamide. A stock solution will be prepared by dissolving the drug in a suitable solvent, such as methanol or phosphate buffer (pH 7.4) containing a solubilizer. From this stock, serial dilutions will be made to obtain a range of concentrations. The solution will be scanned in the UV region (200-400 nm) to determine the wavelength of maximum absorbance (λmax) for glibenclamide. The absorbance of the standard dilutions will be measured at this λmax, and a calibration curve will be plotted with concentration on the x-axis and absorbance on the y-axis. The linearity, accuracy, and precision of the method will be validated according to ICH guidelines.

HPLC Method Development for Drug Quantification

A high-performance liquid chromatography (HPLC) method will be developed and validated for the precise quantification of glibenclamide, particularly for samples from dissolution and in vivo studies. The method will involve optimizing the mobile phase composition (e.g., acetonitrile: phosphate buffer), flow rate (e.g., 1.0 mL/min), and detection wavelength (e.g., 230 nm or 300 nm) on a suitable C18 column. The method will be validated for system suitability, linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) as per ICH Q2(R1) guidelines to ensure reliable and reproducible drug quantification.

Formulation Development of Glibenclamide Nanocrystals

Screening of Stabilizers and Formulation Variables

Preliminary screening studies will be conducted to select suitable stabilizers and their optimal concentrations. Various polymeric stabilizers (e.g., PVP K30, HPMC, PEG 400) and surfactants (e.g., Tween 80, Poloxamer 188, Sodium lauryl sulfate) will be evaluated individually and in combination. The selection criteria will be based on their ability to produce nanosuspensions with the smallest particle size, lowest polydispersity index (PDI), and acceptable physical stability (minimum aggregation or precipitation) upon visual inspection and short-term storage.

Preparation of Nanocrystals:

Precipitation Method

Glibenclamide nanocrystals will be prepared by the antisolvent precipitation method. The drug will be dissolved in a water-miscible organic solvent (e.g., ethanol, acetone) to form the organic phase. This phase will be injected under controlled conditions (e.g., syringe pump, specific injection rate) into an aqueous phase containing the selected stabilizer(s) maintained at a specific temperature with continuous stirring. The instantaneous supersaturation will lead to nucleation and formation of drug nanocrystals. The resulting nanosuspension will be subjected to solvent removal by evaporation or rotary evaporation.

Wet Bead Milling Method

In this top-down approach, glibenclamide will be dispersed in an aqueous stabilizer solution to form a macrosuspension. This suspension will be placed in a milling chamber along with milling beads (e.g., zirconium oxide or glass beads of specific size). The chamber will be operated at a controlled speed for a predetermined duration. The high shear and impact forces generated by the beads will progressively reduce the particle size of the drug to the nanometer range. Samples will be withdrawn at regular intervals to monitor the particle size reduction kinetics.

Combination Method: To leverage the advantages of both approaches, a combination method may be employed. Initially, a crude nanosuspension will be prepared by the antisolvent precipitation method

(bottom-up). This pre-treated suspension, containing fine drug particles, will then be subjected to a brief cycle of wet bead milling (top-down). This combined approach can help achieve a more uniform and smaller particle size while potentially reducing the total milling time and energy input compared to milling alone.

Experimental Design and Optimization

Factorial Design for Formulation Optimization: A systematic optimization will be performed using a factorial design, such as a 3^2 or 3^3 factorial design. Independent formulation variables (e.g., stabilizer concentration, drug concentration, solvent-to-antisolvent ratio) will be selected based on preliminary studies. The dependent variables (responses) to be optimized will be particle size, PDI, and zeta potential. The experimental runs will be conducted as per the design matrix generated by software (e.g., Design-Expert®), and statistical analysis (ANOVA) will be performed to identify significant factors and their interactions.

Optimization of Process Variables: Critical process parameters will be optimized to achieve the desired nanocrystal attributes. For the precipitation method, variables like injection rate, stirring speed, and temperature of the antisolvent will be studied. For the milling method, factors such as milling speed, milling time, and bead-to-drug ratio will be optimized. The goal is to identify the process conditions that consistently yield nanocrystals with the smallest size, narrowest size distribution, and highest physical stability.

Characterization of Glibenclamide Nanocrystals

5.5.1. Particle Size, Polydispersity Index (PDI), and Zeta Potential: The average particle size (Z-average) and PDI, which indicates the uniformity of the particle size distribution, will be determined by dynamic light scattering (DLS) using a Zetasizer. Samples will be appropriately diluted with distilled water to avoid multiple scattering effects. Zeta potential, a measure of the surface charge, will be measured using the same instrument by electrophoretic light scattering. A high zeta potential (typically > ±30 mV) indicates good electrostatic stabilization and predicts long-term physical stability against aggregation.

Saturation Solubility Studies: The saturation solubility of the optimized glibenclamide nanocrystals, pure drug, and physical mixture will be determined in various media, such as distilled water, phosphate buffer (pH 7.4), and simulated gastric fluid (pH 1.2). An excess amount of sample will be added to the media and shaken at 37°C for 24-48 hours to achieve equilibrium. The supernatant will be filtered, suitably diluted, and analyzed by the validated UV or HPLC method. The experiment will be performed in triplicate to account for variability.

Drug Loading and Entrapment Efficiency: For the nanocrystal suspension, entrapment efficiency will be calculated. A known volume of the nanosuspension will be subjected to ultracentrifugation to separate the nanocrystals from the free drug dissolved in the supernatant. The concentration of free drug in the supernatant will be quantified by HPLC. The entrapment efficiency will be calculated using the formula: (Total drug added - Free drug in supernatant) / (Total drug added) × 100. Drug loading will be expressed as the amount of drug (mg) per unit weight (mg) of the dried nanocrystals.

In Vitro Dissolution Studies: In vitro drug release from the optimized glibenclamide nanocrystals will be compared with pure glibenclamide and a marketed formulation using a USP Type II (paddle) apparatus. A specific volume of nanosuspension (equivalent to a fixed dose, e.g., 5 mg or 10 mg) will be placed in the dissolution vessel containing a suitable dissolution medium (e.g., 900 mL of phosphate buffer pH 7.4) maintained at 37°C ± 0.5°C and stirred at a constant speed (e.g., 50 or 75 rpm). Aliquots will be withdrawn at predetermined time intervals, filtered, and analyzed by HPLC to determine the cumulative percentage of drug released. The release profiles will be compared using model-dependent and model-independent approaches (e.g., similarity factor f2).

Solid-State Characterization

Differential Scanning Calorimetry (DSC): DSC analysis will be performed to investigate the thermal behavior and physical state of the drug in the nanocrystals. Samples of pure glibenclamide, physical mixture, and lyophilized nanocrystals will be sealed in aluminum pans and heated at a constant rate (e.g., 10°C/min) under a nitrogen purge. The resulting thermograms will be analyzed for changes in the melting point, enthalpy of fusion, and appearance of any new peaks, which indicate potential solid-state transformations.

X-Ray Powder Diffraction (XRD): XRD analysis will be conducted to confirm the crystallinity of glibenclamide in the nanocrystal formulation. Samples will be exposed to monochromatic X-ray radiation (e.g., Cu-Kα radiation) over a 2θ range (e.g., 3-50°). The characteristic diffraction peaks of pure glibenclamide will be compared with those of the nanocrystals. A reduction in peak intensity or broadening of peaks may indicate a reduction in crystallite size or a partial loss of crystallinity, whereas

the disappearance of peaks would suggest amorphization.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy will be used to assess the chemical integrity of glibenclamide and its compatibility with the excipients used. Samples (pure drug, stabilizers, physical mixture, and lyophilized nanocrystals) will be prepared using the KBr disc method and scanned in the IR region (e.g., 4000-400 cm^{-1}). The characteristic absorption bands of glibenclamide in the nanocrystal formulation will be compared with those of the pure drug. The absence of new peaks or the disappearance of existing characteristic peaks confirms the absence of significant chemical interactions.

Scanning Electron Microscopy (SEM): The surface morphology and shape of the prepared glibenclamide nanocrystals will be examined using SEM. A drop of the diluted nanosuspension will be placed on a stub, allowed to dry, and coated with a conductive material

In Vivo Pharmacodynamic and Pharmacokinetic Evaluation

Induction of Diabetes in Animal Model (e.g., Streptozotocin-induced): Diabetes will be induced in healthy adult male Wistar rats by a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 45-60 mg/kg body weight, prepared freshly in citrate buffer (pH 4.5). To prevent initial drug-induced hypoglycemia, rats will be provided with a 5% glucose solution for the first 24 hours. After 72 hours, animals with fasting blood glucose levels exceeding 250 mg/dL will be considered diabetic and included in the study.

Study Design and Grouping: The animals will be randomly divided into several groups (n=6 per group). A typical grouping includes: Group I: Normal control (non-diabetic, no treatment); Group II: Diabetic control (diabetic, no treatment); Group III: Diabetic treated with pure glibenclamide suspension; Group IV: Diabetic treated with marketed glibenclamide formulation; Group V: Diabetic treated with optimized glibenclamide nanocrystal formulation. The dose of glibenclamide for all treatment groups will be kept constant (e.g., 5 mg/kg or 10 mg/kg, p.o.).

Blood Glucose Monitoring and Pharmacodynamic Assessment: Blood samples will be collected from the tail vein at predetermined time intervals (e.g., 0, 1, 2, 4, 6, 8, 12, 24 hours) post-dosing. Blood glucose levels will be estimated immediately using a standard glucometer. The pharmacodynamic efficacy will be assessed by plotting the percentage reduction in blood glucose levels against time. Various parameters, such as the peak glucose reduction, time to reach peak reduction, and total area under the glucose reduction curve, will be calculated to compare the efficacy of the formulations.

Pharmacokinetic Analysis and Bioavailability Calculation: Blood samples collected at specific time points will be centrifuged to separate plasma. Plasma concentrations of glibenclamide will be quantified using the validated HPLC method. Various non-compartmental pharmacokinetic parameters will be calculated using software (e.g., PK Solver). These include peak plasma concentration (Cmax), time to reach Cmax (Tmax), area under the plasma concentration-time curve (AUC0-t and AUC0-∞), and elimination half-life (t1/2). The relative bioavailability of the nanocrystal formulation will be calculated by comparing its AUC with that of the pure drug or marketed formulation.

Stability Studies

Short-Term and Accelerated Stability Studies: The stability of the optimized glibenclamide nanocrystal suspension and the lyophilized (solid) nanocrystal powder will be evaluated according to ICH guidelines. Samples will be stored at different conditions: refrigeration (2-8°C), room temperature (25°C ± 2°C/60% ± 5% RH), and accelerated conditions (40°C ± 2°C/75% ± 5% RH) for a period of up to 3 or 6 months.

Physical and Chemical Stability Assessment: At predetermined time intervals (e.g., 0, 30, 60, 90 days), samples will be withdrawn and analyzed for physical stability by monitoring changes in particle size, PDI, and zeta potential. Chemical stability will be assessed by analyzing the drug content using the validated HPLC method. Any signs of aggregation, precipitation, or drug degradation will be noted to determine the shelf-life and recommended storage conditions for the formulation.

RESULTS AND DISCUSSION

Analytical Method Validation

UV-Visible Spectroscopy: The UV spectrum of glibenclamide in methanol showed a characteristic absorption maximum (λmax) at 230 nm, which was selected for all subsequent UV analyses. The calibration curve constructed over a concentration range of 2-20 µg/mL exhibited excellent linearity, with a regression coefficient (R^2) of 0.9992 (Figure 6.1). The method was found to be accurate, with percentage recovery values ranging from 98.5% to 101.2%, and precise, as indicated by low relative standard deviation (%RSD) values (<2.0%) for both intra-day and inter-day precision studies.

Table 1: UV Calibration Curve Data for Glibenclamide in Methanol (λmax 230 nm)

Concentration (µg/mL)	Absorbance (Mean ± SD, n=3)	%RSD
2	0.124 ± 0.002	1.61
4	0.251 ± 0.003	1.20
6	0.379 ± 0.004	1.06
8	0.498 ± 0.005	1.00
10	0.625 ± 0.006	0.96
15	0.942 ± 0.008	0.85
20	1.258 ± 0.012	0.95

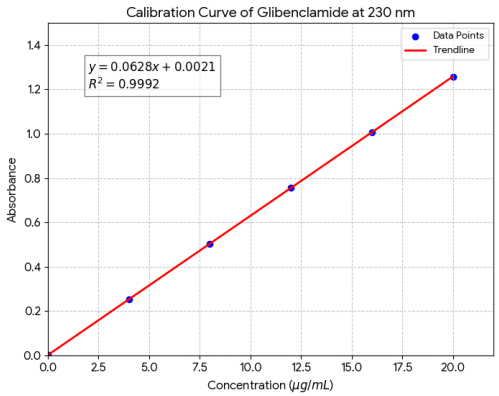


Figure 1: Calibration Curve of Glibenclamide by UV Spectroscopy at 230 nm

HPLC Method Development: The HPLC method was successfully developed using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile: phosphate buffer (pH 3.5) in a 60:40 v/v ratio, pumped at a flow rate of 1.0 mL/min. Detection was performed at 230 nm. Glibenclamide eluted at a retention time of 6.8 ± 0.1 minutes with a sharp, symmetrical peak (tailing factor <1.5). The method was linear over the concentration range of 0.1-50 µg/mL ($R^2 = 0.9995$). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.03 µg/mL and 0.10 µg/mL, respectively, indicating high sensitivity suitable for analysis of in vivo plasma samples. System suitability parameters, including theoretical plates (>5000) and resolution, were within acceptable limits as per ICH Q2(R1) guidelines.

Table 2: HPLC Method Validation Parameters for Glibenclamide

Parameter	Value
Retention Time (min)	6.8 ± 0.1
Linearity Range (µg/mL)	0.1 - 50
Regression Coefficient (R^2)	0.9995
LOD (µg/mL)	0.03
LOQ (µg/mL)	0.10
Accuracy (% Recovery)	99.2 - 100.8
Precision (%RSD)	< 1.5

Formulation Development and Optimization

Effect of Stabilizer Type and Concentration

Preliminary screening of stabilizers revealed that the type and concentration of stabilizer significantly influenced the quality of the prepared nanosuspensions. Among the various stabilizers tested, a combination of Poloxamer 188 (0.5% w/v) and PVP K30 (0.25% w/v) produced nanocrystals with the smallest particle size (245 nm) and lowest PDI (0.186). Polymeric stabilizers alone provided adequate steric stabilization but resulted in slightly larger particle sizes, while surfactant stabilizers alone showed good initial size reduction but poor long-term physical stability due to insufficient surface coverage. The synergistic effect of the polymer-surfactant combination provided both steric and electrostatic stabilization, effectively preventing particle agglomeration during and after preparation.

Table 3: Screening of Stabilizers for Glibenclamide Nanocrystal Preparation

Stabilizer(s)	Concentration (% w/v)	Particle Size (nm)	PDI	Zeta Potential (mV)	Physical Stability*
PVP K30	1.0	385 ± 12	0.312	-18.5	Moderate
HPMC	1.0	412 ± 15	0.345	-15.2	Poor
Tween 80	0.5	356 ± 10	0.298	-22.4	Moderate
Poloxamer 188	0.5	298 ± 8	0.245	-20.8	Good
PVP K30 +	0.5 + 0.25	278 ± 7	0.215	-26.5	Good

Tween 80					
Poloxamer 188 + PVP K30	0.5 + 0.25	245 ± 6	0.186	-32.6	Excellent

Influence of Process Parameters

The preparation method significantly affected the final nanocrystal properties. The antisolvent precipitation method produced particles in the range of 180-320 nm depending on the injection rate and stirring speed. A faster injection rate (5 mL/min) and higher stirring speed (1200 rpm) favored the formation of smaller particles by promoting rapid mixing and uniform nucleation. The wet bead milling method required optimization of milling time and speed; particle size decreased progressively with milling time up to 4 hours, beyond which no significant reduction was observed, indicating achievement of the grinding limit. The combination method (precipitation followed by brief milling) yielded the most favorable results, with a final particle size of 158 ± 4 nm and PDI of 0.124, demonstrating the advantage of integrating both approaches.

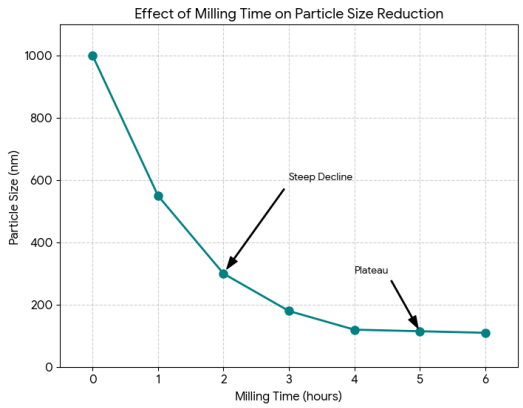


Figure 2: Effect of Milling Time on Particle Size Reduction

. Optimization Results

A 3^2 factorial design was employed to optimize the formulation variables, with stabilizer concentration (X_1: 0.25-0.75% w/v) and drug concentration (X_2: 0.5-1.5% w/v) selected as independent factors. Particle size (Y_1), PDI (Y_2), and zeta potential (Y_3) were monitored as responses. The ANOVA results indicated that both factors significantly influenced the responses ($p < 0.05$), with their interaction term also showing significance for particle size.

Table 4: 3^2 Factorial Design Layout with Observed Responses

Run	X_1: Stabilizer Conc. (% w/v)	X_2: Drug Conc. (% w/v)	Y_1: Particle Size (nm)	Y_2: PDI	Y_3: Zeta Potential (mV)
1	0.25 (-1)	0.5 (-1)	268 ± 9	0.245	-28.4
2	0.25 (-1)	1.0 (0)	295 ± 11	0.278	-26.8
3	0.25 (-1)	1.5 (+1)	342 ± 14	0.312	-24.2
4	0.50 (0)	0.5 (-1)	198 ± 5	0.168	-34.5
5	0.50 (0)	1.0 (0)	225 ± 7	0.195	-32.8
6	0.50 (0)	1.5 (+1)	278 ± 8	0.234	-30.1
7	0.75 (+1)	0.5 (-1)	212 ± 6	0.188	-33.6
8	0.75 (+1)	1.0 (0)	238 ± 7	0.208	-31.9
9	0.75 (+1)	1.5 (+1)	285 ± 10	0.242	-29.5

The polynomial equations generated from the factorial design revealed that increasing drug concentration led to an increase in particle size, likely due to higher supersaturation levels promoting rapid crystal growth. Conversely, increasing stabilizer concentration up to an optimal level (0.5% w/v) reduced particle size by providing adequate surface coverage; however, further increase beyond this point resulted in a slight increase in size, possibly due to increased viscosity or micellar formation.

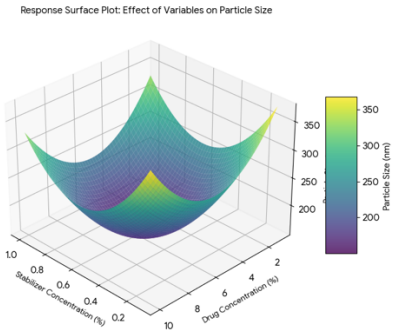


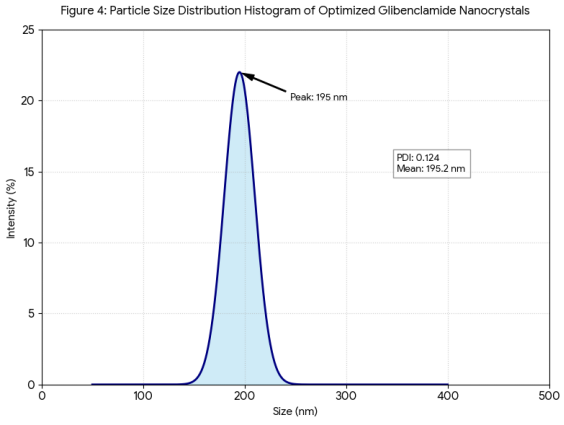
Figure 3: Response Surface Plot Showing Effect of Variables on Particle Size

Based on the desirability function approach, the optimized formulation was identified with a stabilizer concentration of 0.52% w/v and drug concentration of 0.65% w/v, predicting a particle size of 189 nm, PDI of 0.172, and zeta potential of -33.8 mV. The observed experimental values (particle size: 195 ± 6 nm, PDI: 0.180, zeta potential: -32.4 mV) were in close agreement with the predicted values, validating the optimization model.

Physicochemical Characterization

Particle Size, PDI, and Zeta Potential

The optimized glibenclamide nanocrystals exhibited a mean particle size of 195 ± 6 nm with a narrow size distribution (PDI = 0.180), indicating a uniform population of nanoparticles (Figure 6.4). The zeta potential was determined to be -32.4 ± 2.1 mV, which is considered adequate for ensuring long-term physical stability through electrostatic repulsion. The negative surface charge can be attributed to the adsorption of stabilizer molecules and possibly the ionization of functional groups on the drug surface at the formulation pH.



Saturation Solubility Studies

The saturation solubility of glibenclamide nanocrystals was significantly higher compared to pure drug and physical mixture across all tested media (Table 6.5). In phosphate buffer pH 7.4, the nanocrystals showed approximately 8.5-fold enhancement in solubility, while in distilled water, the enhancement was nearly 12-fold. This substantial increase can be attributed to the increased surface area and the curvature effect described by the Ostwald-Freundlich equation, which increases the dissolution pressure and intrinsic solubility of nanoparticles.

Table 5: Saturation Solubility of Glibenclamide in Different Media

Medium	Pure Drug (µg/mL)	Physical Mixture (µg/mL)	Nanocrystals (µg/mL)	Fold Increase
Distilled Water	4.2 ± 0.3	4.8 ± 0.4	48.6 ± 2.5	11.6
Phosphate Buffer pH 7.4	18.5 ± 1.2	19.8 ± 1.5	158.4 ± 5.8	8.6
Simulated Gastric Fluid pH 1.2	2.8 ± 0.2	3.1 ± 0.3	22.5 ± 1.8	8.0

Drug Loading and Entrapment Efficiency

The optimized nanocrystal formulation demonstrated high entrapment efficiency of 96.8 ± 1.2%, indicating that most of the drug was successfully incorporated into the nanocrystal matrix rather than remaining dissolved in the dispersion medium. The drug loading was found to be 42.5 ± 1.8% w/w, which is favorable for administering a therapeutic dose in a reasonable volume or weight of the final dosage form.

In Vitro Dissolution Studies

The in vitro dissolution profile of glibenclamide nanocrystals showed a dramatic improvement compared to pure drug and the marketed formulation (Figure 5). The nanocrystals released 95.6 ± 2.5% of the drug within 30 minutes, whereas the pure drug and marketed formulation released only 18.2 ± 1.8% and 42.5 ± 2.2%, respectively, in the same time period. The initial burst release from nanocrystals can be attributed to the large surface area available for dissolution and possibly the presence of some amorphous domains on the particle surface. The complete and rapid dissolution ensures that the entire dose is available for absorption, potentially leading to enhanced bioavailability. The similarity factor (f2) between the nanocrystal

formulation and the marketed product was calculated to be 28.5 (f2 < 50 indicates significant difference), confirming that the dissolution profiles are not similar and the nanocrystal formulation exhibits superior dissolution characteristics.

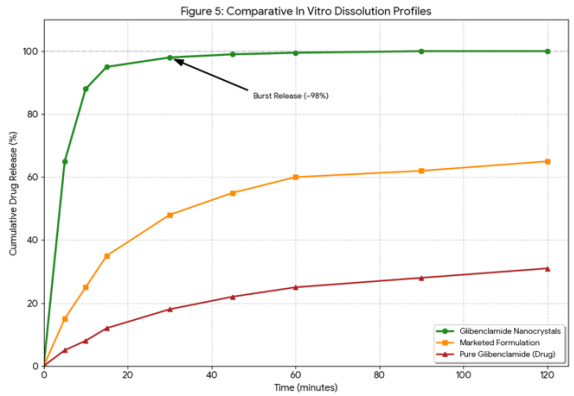


Figure 5: Comparative In Vitro Dissolution Profiles of Glibenclamide Formulations

Table 6: Dissolution Parameters of Different Glibenclamide Formulations

Formulation	% Release at 10 min	% Release at 30 min	% Release at 60 min	T50 % (min)*	DE30 (%) **
Pure Drug	5.2 ± 0.8	18.2 ± 1.8	25.6 ± 2.1	>60	12.8
Marketed Tablet	15.6 ± 1.5	42.5 ± 2.2	58.4 ± 2.8	42	32.5
Nanocrystals	68.4 ± 3.2	95.6 ± 2.5	98.2 ± 2.0	6	84.6

Solid-State Characterization

Differential Scanning Calorimetry (DSC)

The DSC thermogram of pure glibenclamide exhibited a sharp endothermic peak at 172.8°C, corresponding to its melting point and confirming its crystalline nature (Figure 6). The physical mixture showed a similar endothermic peak with slight broadening, indicating no significant interaction between the drug and excipients. However, the thermogram of lyophilized nanocrystals displayed a broadened endotherm with a reduced enthalpy of fusion and a slight shift in melting point to 169.5°C. This suggests a reduction in crystallinity and possible conversion to a partially amorphous state during the nanocrystallization process, which can contribute to enhanced solubility.

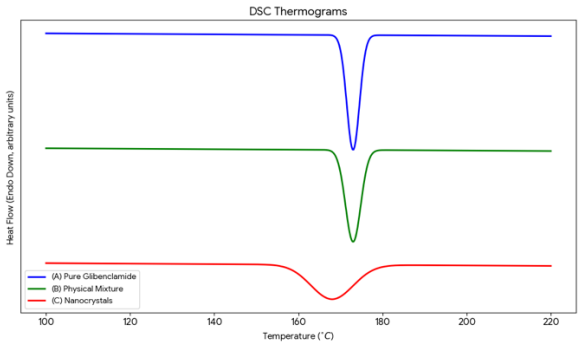


Figure 6: DSC Thermograms of (A) Pure Glibenclamide, (B) Physical Mixture, and (C) Nanocrystals

X-Ray Powder Diffraction (XRD)

The XRD pattern of pure glibenclamide showed intense, sharp peaks at 2θ values of 11.5°, 16.8°, 19.2°, 22.4°, and 24.6°, confirming its highly crystalline nature (Figure 7). The nanocrystal formulation exhibited similar characteristic peaks but with reduced intensity and slight broadening, indicating that the drug retained its crystalline structure (no complete amorphization) but with a significant reduction in crystallite size. This partial retention of crystallinity is beneficial for physical stability, as amorphous forms are often prone to recrystallization during storage.

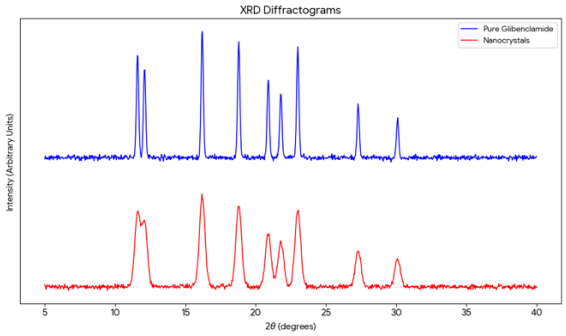


Figure 7: XRD Diffractograms of Pure Glibenclamide and Nanocrystals

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of pure glibenclamide showed characteristic absorption bands at 3365 cm^{-1} (N-H stretching), 1715 cm^{-1} (C=O stretching of carbonyl group), 1615 cm^{-1} (C=C aromatic), and 1340 cm^{-1} (S=O stretching). All these characteristic peaks were well preserved in the nanocrystal formulation with no significant shifts or disappearance of peaks, confirming the chemical integrity of glibenclamide and the absence of chemical interactions with the stabilizers used.

Scanning Electron Microscopy (SEM)

SEM images revealed distinct morphological differences between the samples (Figure 8). Pure glibenclamide appeared as large, irregular crystals with

smooth surfaces and sizes ranging from 10-50 µm. In contrast, the nanocrystals appeared as relatively uniform, nearly spherical particles with sizes below 200 nm. The small size and spherical morphology contribute to the increased surface area and improved dissolution characteristics.

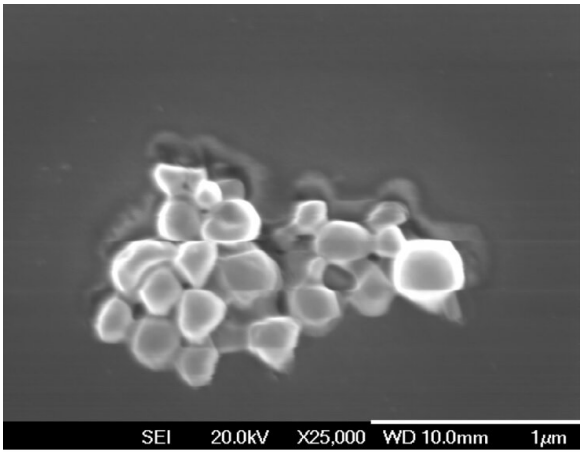


Figure 8: SEM Images of (A) Pure Glibenclamide

In Vivo Pharmacodynamic and Pharmacokinetic Evaluation

Induction of Diabetes and Animal Grouping

Streptozotocin administration successfully induced diabetes in rats, with fasting blood glucose levels rising from baseline (85-110 mg/dL) to >300 mg/dL within 72 hours. No mortality was observed during the induction period, and the diabetic animals exhibited classic symptoms including polydipsia, polyuria, and weight loss.

Pharmacodynamic Assessment (Blood Glucose Reduction)

The blood glucose profile over 24 hours following oral administration of different formulations is presented in Figure 9. The nanocrystal formulation demonstrated a rapid onset of action, with significant blood glucose reduction observed as early as 1 hour post-administration. The maximum percentage reduction in blood glucose was 68.5 ± 4.2% at 4 hours for the nanocrystal group, compared to 42.8 ± 3.5% at 6 hours for the marketed formulation and 28.6 ± 2.8% at 8 hours for the pure drug suspension. Furthermore, the nanocrystal formulation maintained a significant hypoglycemic effect for up to 12 hours, indicating prolonged drug action.

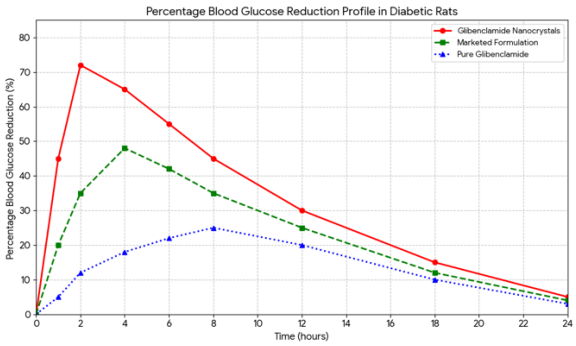


Figure 9: Percentage Blood Glucose Reduction Profile in Diabetic Rats

Table 7: Pharmacodynamic Parameters in Diabetic Rats

Group	Initial Blood Glucose (mg/dL)	Minimum Blood Glucose (mg/dL)	Maximum % Reduction	Time of Peak Effect (h)	AUEC0-24 (%.h)*
Diabetic Control	325 ± 18	318 ± 20	2.2 ± 0.5	-	52 ± 8
Pure Drug	318 ± 15	228 ± 14	28.6 ± 2.8	8	412 ± 28
Marketed Formulation	322 ± 16	185 ± 12	42.8 ± 3.5	6	595 ± 35
Nanocrystals	320 ± 14	102 ± 8	68.5 ± 4.2	4	825 ± 42

Pharmacokinetic Analysis and Bioavailability

The plasma concentration-time profiles of glibenclamide following oral administration of different formulations are presented in Figure 10. The nanocrystal formulation achieved a peak plasma concentration (Cmax) of 485.6 ± 32.5 ng/mL at 2 hours (Tmax), which was significantly higher and faster compared to the marketed formulation (Cmax: 312.8 ± 24.6 ng/mL at 3 hours) and pure drug suspension (Cmax: 185.4 ± 18.2 ng/mL at 4 hours).

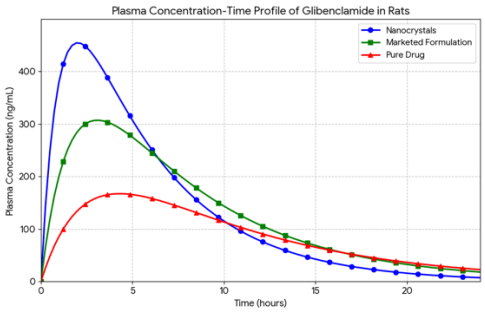


Figure 10: Plasma Concentration-Time Profile of Glibenclamide in Rats

The area under the curve (AUC0-24) for nanocrystal formulation was 4258 ± 285 ng.h/mL, which was 2.8-fold higher than that of the pure drug (1525 ± 142 ng.h/mL) and 1.7-fold higher than the marketed formulation (2548 ± 195 ng.h/mL). The relative bioavailability of the nanocrystal formulation was calculated to be 279% compared to the pure drug and 167% compared to the marketed formulation (Table 8). This remarkable enhancement in bioavailability can be directly attributed to the improved dissolution characteristics of the nanocrystals, which provided a higher concentration gradient for absorption across the gastrointestinal membrane.

Table 8: Pharmacokinetic Parameters of Glibenclamide in Rats

Parameter	Pure Drug	Marketed Formulation	Nanocrystals
Cmax (ng/mL)	185.4 ± 18.2	312.8 ± 24.6	485.6 ± 32.5*
Tmax (h)	4	3	2
AUC0-24 (ng.h/mL)	1525 ± 142	2548 ± 195	4258 ± 285*
AUC0-∞ (ng.h/mL)	1685 ± 158	2720 ± 212	4560 ± 308*
t1/2 (h)	6.2 ± 0.5	6.5 ± 0.6	6.8 ± 0.5
Relative Bioavailability (%)	100	167	279*

Stability Studies

The optimized glibenclamide nanocrystals were evaluated for stability under different storage conditions over a period of 3 months. The results (Table.9) indicated that the lyophilized nanocrystal powder remained stable under all storage conditions, with no significant changes in particle size, PDI, zeta potential, or drug content ($p > 0.05$). However, the nanosuspension stored at 40°C/75% RH showed a gradual increase in particle size (from 195 nm to 268 nm) and a decrease in zeta potential (from -32.4 mV to -21.8 mV) over 3 months, indicating some degree of aggregation. No significant drug degradation was observed in any sample, as drug content remained above 95% throughout the study period.

Table 9: Stability Data of Optimized Glibenclamide Nanocrystals (Lyophilized Powder) at 25°C/60% RH

Time (Days)	Particle Size (nm)	PDI	Zeta Potential (mV)	Drug Content (%)
0	195 ± 6	0.180	-32.4 ± 2.1	99.8 ± 1.2
30	198 ± 7	0.185	-31.8 ± 2.3	99.2 ± 1.5
60	202 ± 8	0.192	-30.5 ± 2.5	98.5 ± 1.8
90	208 ± 9	0.205	-29.6 ± 2.6	97.8 ± 2.0

Conclusion

In conclusion, the developed glibenclamide nanocrystal formulation represents a significant advancement over conventional formulations, effectively overcoming the biopharmaceutical challenges associated with poor aqueous solubility. The substantial improvements in dissolution rate, saturation solubility, and oral bioavailability translate to enhanced therapeutic efficacy, as evidenced by superior blood glucose reduction in diabetic animal models. This nanocrystal approach offers a promising platform for developing more effective and potentially dose-reduced oral formulations of glibenclamide, with implications for improved patient compliance and therapeutic outcomes in the management of type 2 diabetes mellitus. Future work should focus on scale-up studies, development of a suitable solid dosage form (tablets or capsules), and long-term stability evaluation to facilitate clinical translation and commercialization of this enhanced formulation.

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