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Formulation and Evaluation of Furosemide Nanosuspensions by High-Speed Homogenization: An Approach to Enhance Dissolution Rate

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Abstract

This research focused on developing and assessing a furosemide nanosuspension to improve its dissolution rate, a key obstacle to its oral bioavailability, using a high-speed homogenization method. As a Biopharmaceutics Classification System (BCS) Class IV drug, furosemide's poor solubility in water and highly inconsistent absorption create major challenges for reliable therapeutic delivery. We prepared nanosuspensions through high-speed homogenization, a top-down particle size reduction method, using Poloxamer 407 as a steric stabilizer. To systematically refine the formulation, we applied a 3^2 full factorial design, examining how stabilizer concentration (2%, 3.5%, and 5% w/v) and homogenization speed (5,000, 10,000, and 15,000 rpm) affected particle characteristics and drug release. The resulting nanosuspensions underwent comprehensive characterization, including particle size, polydispersity index (PDI), zeta potential, Scanning Electron Microscopy (SEM) for surface morphology, and solid-state analysis via Differential Scanning Calorimetry (DSC) and Fourier-Transform Infrared (FTIR) spectroscopy. The optimized formulation (Fs6), created with 5% w/v Poloxamer 407 at 10,000 rpm, showed a mean particle size of 384 nm, a very low PDI of 0.132, and a zeta potential of -30.7 mV, which suggests good physical stability. It also contained a high drug content of 95.1%. This formulation achieved a significantly faster dissolution rate, releasing over 95% of the drug within 10 minutes in simulated gastric fluid (pH 1.2). The release kinetics were best fitted to the Zero-order model, with Fickian diffusion identified as the underlying release mechanism. Our study confirms that high-speed homogenization is a viable and scalable technique for creating stable furosemide nanosuspensions. The remarkable improvement in dissolution points to a promising path for boosting the oral bioavailability of furosemide and other difficult BCS Class IV drugs.

Keywords: Furosemide; Nanosuspension; High-Speed Homogenization; Poloxamer 407; Dissolution Enhancement; BCS Class IV.

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Introduction

The Challenge of Poorly Soluble Drugs in Pharmaceutical Development

One of the most significant and persistent hurdles in contemporary pharmaceutical science is the poor aqueous solubility of active pharmaceutical ingredients (APIs). It is estimated that this problem affects roughly 40% of newly discovered drug candidates and as many as 60% of compounds currently in the development pipeline, presenting a formidable obstacle to their clinical application. For a drug taken orally, sufficient dissolution in bodily fluids is a fundamental requirement for absorption and, ultimately, for achieving its therapeutic effect. The Biopharmaceutics Classification System (BCS) offers a valuable scientific framework that classifies drugs based on their water solubility and intestinal permeability, the two main factors governing oral drug absorption. Drugs in BCS Class II (low solubility, high permeability) and Class IV (low

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solubility, low permeability) are especially difficult to formulate, as their absorption is typically constrained by how quickly and completely they can dissolve(1).

To tackle this issue, formulators have developed several traditional techniques, such as creating salts, using co-solvents, preparing solid dispersions, and forming complexes with cyclodextrins. While these strategies have worked for some drugs, they are not universally effective and often have drawbacks. These can include the need for large amounts of excipients, potential toxicity, intricate and expensive manufacturing, or a reduction in the final product's stability. Consequently, there is a pressing need for dependable, adaptable, and scalable technologies that can effectively enhance the dissolution and bioavailability of the ever-increasing number of poorly soluble drugs emerging from discovery research(1).

Furosemide: A BCS Class IV Case Study

Furosemide is a powerful loop diuretic that has been essential in treating edema related to congestive heart failure, liver cirrhosis, and kidney disease, as well as in managing hypertension, since its introduction in 1964. Despite its long history of clinical use, furosemide poses major formulation difficulties due to its challenging physicochemical properties. As a weakly acidic drug with a pKa around 3.8, it is considered practically insoluble in water, with a solubility below 0.1 mg/mL, especially in the acidic environment of the stomach (pH ~2). Because of its low solubility combined with low intestinal permeability, furosemide is officially classified as a BCS Class IV drug, the most difficult category for oral administration(2).

A particularly challenging aspect of furosemide's behavior is its narrow "absorption window." The drug is absorbed most effectively from the stomach and the upper parts of the small intestine, where its permeability is greatest. This is an example of segmental-dependent permeability, where a drug's transport characteristics vary along the gastrointestinal tract. This leads to a critical paradox: the very acidic regions that are ideal for permeability are also where furosemide's solubility is lowest. Conversely, in the lower intestine, where the pH is higher and solubility improves, its permeability is much weaker. This mismatch—being in the right place for absorption but in the wrong physical state (undissolved)—is a major reason for its erratic and widely fluctuating oral bioavailability, reported to be anywhere from 10% to 90%.[7] This high degree of variability can result in unpredictable treatment outcomes, make dosing difficult, and require close patient monitoring. Therefore, any strategy to improve furosemide's clinical effectiveness must aim to dramatically accelerate its dissolution rate in the stomach, thereby maximizing the amount of dissolved drug ready for absorption in this crucial proximal window(2).

Nanosuspension Technology for Bioavailability Enhancement

In recent times, nanosuspension technology has risen as a potent and adaptable platform for tackling the complex issues presented by poorly soluble drugs. A nanosuspension is a two-phase, carrier-free system composed of pure drug particles sized in the sub-micron range (usually under 1000 nm), dispersed in a water-based medium and stabilized by a small quantity of suitable surfactants or polymers. The core principle behind the effectiveness of nanosuspensions is the dramatic increase in the drug's dissolution speed, a concept explained by the Noyes-Whitney equation. By shrinking particles to the nanoscale, the drug's surface-area-to-volume ratio is vastly expanded, which directly accelerates the rate of dissolution. Additionally, the Ostwald-Freundlich equation suggests that reducing particle size to the sub-micron level also boosts the drug's saturation solubility, which widens the concentration gradient (Cs−Cb) that drives absorption(4).

This technology offers numerous compelling benefits, including the potential for better oral bioavailability, faster onset of action, less pharmacokinetic variability between individuals, reduced food-related effects on absorption, high drug-loading capability, and suitability for various administration routes like oral, parenteral, pulmonary, and topical. Nanosuspension technology is an excellent match for BCS Class II and IV drugs such as furosemide because it directly addresses the dissolution-rate-limited absorption that characterizes these challenging compounds(5).

The Top-Down Method: High-Speed Homogenization

Nanosuspension production generally follows one of two paths: "bottom-up" methods, which build nanoparticles from a solution through controlled precipitation or crystallization, and "top-down" methods, which use mechanical force to break down larger drug particles. Top-down techniques like high-pressure homogenization (HPH) and media milling are favored in the pharmaceutical industry for their proven scalability, reliability, and consistency. These methods apply intense mechanical energy to a coarse drug suspension, fracturing the drug crystals into nanoparticles(6).

This research utilizes high-speed homogenization (HSH), a top-down method that is simpler, more accessible, and more economical than HPH, making it ideal for laboratory-scale development and optimization. In HSH, a rotor-stator assembly spins at high velocity, creating powerful shear forces, turbulence, and cavitation in the liquid. These forces work together to efficiently break down the suspended drug particles to the desired nanometer scale. While HSH might not produce particles as small as those from HPH, it can reliably generate particles in the sub-micron range (e.g., up to 600 nm) and is a superb tool for systematically exploring how formulation and process factors affect nanosuspension characteristics.(7).

The Critical Role of Stabilizers: Poloxamer 407

A key challenge in any nanosuspension formulation is managing the inherent thermodynamic instability of nanoparticles. The huge surface area created during size reduction leads to high surface free energy, which creates a

strong thermodynamic push for particles to aggregate or for crystals to grow through Ostwald ripening—a phenomenon where larger, more stable particles grow as smaller, more soluble ones dissolve. To prevent this and ensure the long-term physical stability of the colloid, stabilizers are crucial. These molecules attach to the surface of the newly created nanoparticles and stop them from clumping together by creating repulsive forces, either through electric charges (ionic stabilization) or by forming a protective physical shield (steric stabilization(3).

For this study, Poloxamer 407 (also sold as Pluronic® F127) was chosen as the stabilizer. Poloxamer 407 is a non-ionic, triblock copolymer with a central hydrophobic polyoxypropylene (PPO) block surrounded by two hydrophilic polyoxyethylene (PEO) blocks. Its stabilizing effect is mainly steric. The hydrophobic PPO center attaches to the surface of the hydrophobic furosemide particles through hydrophobic interactions, while the long, flexible, and water-attracting PEO chains extend into the aqueous medium. This forms a thick hydrophilic layer around each particle, physically blocking them from getting close enough for attractive van der Waals forces to cause them to stick together permanently. Poloxamer 407 is frequently used in pharmaceutical products, is considered Generally Regarded as Safe (GRAS), and has been demonstrated to enhance the stability and dissolution of many drugs, making it a safe and effective option for an oral delivery system.(9)

Rationale and Objectives of the Study

Given the significant clinical challenge posed by furosemide's BCS Class IV properties and its critical gastric absorption window, a formulation strategy that can rapidly enhance its dissolution in acidic media is paramount for improving its therapeutic predictability and overall bioavailability. The preparation of a nanosuspension via high-speed homogenization offers a practical, scalable, and effective approach to achieve this goal, with Poloxamer 407 serving as a well-established and safe stabilizer.

Therefore, the specific objectives of this investigation were:

Considering the major clinical hurdle presented by furosemide's BCS Class IV status and its vital gastric absorption window, a formulation that can quickly and significantly boost its dissolution in acidic conditions is essential for improving its therapeutic reliability and bioavailability. Creating a nanosuspension with high-speed homogenization is a practical, scalable, and efficient way to meet this need, with Poloxamer 407 acting as a proven and safe stabilizer. Thus, the specific goals of this research were:

1. To create furosemide nanosuspensions via high-speed homogenization using Poloxamer 407 as the stabilizer.
2. To methodically optimize the formulation with a 3^2 full factorial design, examining how stabilizer concentration and homogenization speed affect key quality metrics like particle size and in vitro dissolution.
3. To conduct a thorough characterization of the optimized nanosuspension's physicochemical properties, such as particle size distribution, zeta potential, surface morphology, and solid-state features.
4. To ascertain the drug release kinetics and clarify the release mechanism of the optimized formulation.

Materials and Methods

Materials

Furosemide (purity >99%) was provided as a gift sample from Aventis Pharma (Ankleshwar, Gujarat, India). Poloxamer 407 (Pluronic® F127) was acquired from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade acetone, ethanol, isopropyl alcohol (IPA), and methanol were sourced from local suppliers. All other chemicals and solvents were of analytical grade. High-purity deionized water, produced on-site, was used in all experiments.

Pre-formulation Studies

Solubility Determination

The equilibrium solubility of furosemide was measured in several solvents (deionized water, ethanol, acetone, IPA, and methanol) via the shake-flask method. An excess quantity of furosemide powder was placed in 5 mL of each solvent within sealed glass vials. These suspensions were continuously agitated on a magnetic stirrer (Remi, 2MLH, India) at 80 rpm for 24 hours at room temperature (25 ± 2 °C) to ensure equilibrium was attained. Afterward, the samples were passed through a 0.45 µm PTFE syringe filter to clear any undissolved drug. The resulting clear filtrate was appropriately diluted with the corresponding solvent, and the concentration of dissolved furosemide was measured spectrophotometrically at its determined maximum wavelength (λmax) with a UV-Visible spectrophotometer(10).

Analytical Method Development (UV-Visible Spectrophotometry)

An analytical method for quantifying furosemide was established and validated. A 1000 µg/mL primary stock solution of furosemide was made by dissolving 10 mg of the drug in 10 mL of methanol. This stock was then diluted with simulated gastric fluid (SGF, pH 1.2, prepared according to USP standards without enzymes) to generate a set of standard solutions ranging from 5 to 200 µg/mL. The absorbance of each standard was recorded on a double-beam UV-Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) from 200–400 nm to find the wavelength of maximum absorbance (λmax). A calibration curve was plotted (absorbance vs. concentration), and the method's linearity was confirmed by calculating the coefficient of determination (R2) from the linear regression(10).

Drug-Excipient Compatibility Studies

Fourier-Transform Infrared (FTIR) Spectroscopy

To check for potential chemical reactions between furosemide and the stabilizer, FTIR spectra were recorded for pure furosemide, pure Poloxamer 407, and their physical mixture (made by gently grinding a 1:1 w/w ratio in a mortar). Each sample was made into a potassium bromide (KBr) pellet by compressing 1-2 mg of the sample with about 100 mg of FTIR-grade KBr. The pellets were scanned from 4000 to 400 cm^{-1}. The spectra were then overlaid and checked for new peaks or the disappearance or major shifts of furosemide's characteristic peaks, which would signal a chemical interaction(11).

Differential Scanning Calorimetry (DSC)

The thermal properties of the components and the final product were evaluated using a DSC instrument. Thermograms were recorded for pure furosemide, Poloxamer 407, their 1:1 w/w physical mixture, and the freeze-dried powder of the optimized nanosuspension. Samples (3-5 mg) were sealed in standard aluminum pans and heated from 25 °C to 300 °C at 10 °C/min under a 50 mL/min nitrogen flow. An empty pan served as a reference. The thermograms were examined for any changes in melting points, peak shapes, or new thermal events that could indicate an interaction or a change in the drug's physical state(11).

Preparation of Furosemide Nanosuspensions by High-Speed Homogenization

Furosemide nanosuspensions were created using a top-down method with a high-speed homogenizer. Typically, a specific concentration of Poloxamer 407 was dissolved in 20 mL of deionized water with a magnetic stirrer. Then, 60 mg of furosemide powder was added to form a coarse pre-suspension. To help wet the hydrophobic drug and create a more even dispersion, a small amount of acetone (2 mL) was added as a co-solvent. This pre-suspension was immediately processed with a high-speed homogenizer (IKA T25 digital Ultra-Turrax, Germany) at a set speed for 15 minutes. To avoid overheating, the beaker was kept in an ice bath. After homogenization, the nanosuspension was stirred gently in an open beaker for 1 hour at room temperature to let any remaining acetone evaporate(7).

Experimental Design for Optimization

We used a 3^2 full factorial design, a statistical method aligned with Quality by Design (QbD) principles, for systematic optimization of the nanosuspension. This design allowed for an efficient study of how key process and formulation variables affect product quality. The two independent variables were Poloxamer 407 concentration (X1) and homogenization speed (X2), identified as a Critical Material Attribute (CMA) and a Critical Process Parameter (CPP), respectively. Each variable was tested at three levels: low (-1), medium (0), and high (+1). The levels for X1 were 2%, 3.5%, and 5% w/v, and for X2 were 5000, 10000, and 15000 rpm. This resulted in nine experimental runs (formulations Fs1 to Fs9). The dependent variables (responses), or Critical Quality Attributes (CQAs), were mean particle size (nm), polydispersity index (PDI), and the cumulative drug percentage released in 10 minutes (Q10) (12).

Physicochemical Characterization of Nanosuspensions

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

The mean hydrodynamic particle size (Z-average), PDI, and zeta potential were measured using Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Before measurement, each nanosuspension was diluted with deionized water to get a suitable scattering intensity and prevent multiple scattering effects. Zeta potential was measured to evaluate the surface charge and predict the physical stability of the colloid against aggregation. All measurements were done in triplicate at 25 °C(13).

Surface Morphology Analysis (Scanning Electron Microscopy - SEM)

The surface structure of the optimized nanosuspension was examined using an SEM (JEOL JSM-6380 LV, Japan). For imaging, a drop of the optimized nanosuspension was placed on an aluminum stub with double-sided carbon tape and air-dried. The dried sample was then coated with a thin layer of gold-palladium in a sputter coater to make it conductive and prevent charging. The sample was then scanned and imaged at an acceleration voltage of 15 kV.

Drug Content Determination

The drug content of the nanosuspensions was measured to find out how much furosemide was successfully included in the final product. The redispersed suspension was centrifuged at 15,000 rpm for 40 minutes at 25 °C, separating the solid drug nanoparticles from the supernatant containing any free drug. The furosemide concentration in the supernatant was measured spectrophotometrically at its λmax of 272 nm after dilution with methanol. The drug content was calculated by subtracting the amount of free drug from the total initial amount of drug used.

In Vitro Dissolution Testing

In vitro dissolution tests were performed using the USP Type II (Paddle) apparatus (Electrolab, TDT-08L, India). The dissolution medium was 250 mL of SGF (pH 1.2) with 0.5% w/v Tween 80, added to maintain sink conditions. The medium's temperature was kept at 37 ± 0.5 °C, and the paddle speed was 100 rpm. At set times (2, 4, 6, 8, and 10 minutes), 5 mL aliquots were taken, and an equal volume of fresh,

pre-warmed medium was immediately added back. The samples were filtered through a 0.1 µm PTFE syringe filter, and the concentration of dissolved furosemide was measured spectrophotometrically at its λmax(14).

Kinetic Modeling of Drug Release

To understand the drug release mechanism from the optimized nanosuspension, the in vitro dissolution data were fitted to several mathematical models:

- Zero-order model: Qt=Q0+K0t
- First-order model: logC=logC0−K1t/2.303
- Higuchi model: Qt=KHt
- Korsmeyer-Peppas model: Mt/M∞=KKPtn

Here, Qt is the cumulative drug amount released at time t, Q0 is the initial drug amount, C0 is the initial drug concentration, C is the drug concentration at time t, and Mt/M∞ is the fraction of drug released at time t. K0, K1, KH , and KKP are the release rate constants. The coefficient of determination (R2) was used to find the best-fit model. The release exponent (n) from the Korsmeyer-Peppas model helped characterize the release mechanism. For a spherical shape, an n value ≤ 0.43-0.5 suggests Fickian diffusion(15).

Stability Studies

The physical and chemical stability of the best formulation (Fs6) was tested over 30 days. The formulation was stored in sealed glass vials under two conditions: refrigerated (2–8 °C) and room temperature (25 ± 2 °C / 60 ± 5% RH). Samples were taken at 0, 15, and 30 days. At each point, they were checked for aggregation, caking, or sedimentation and analyzed for changes in mean particle size, PDI, zeta potential, and drug content to evaluate their stability.

Statistical Analysis

All tests were done in triplicate (n=3), with results shown as mean ± standard deviation (SD). Data from the 3^2 factorial design were analyzed with Design-Expert® software (Version 12, Stat-Ease Inc., Minneapolis, MN, USA). One-way analysis of variance (ANOVA) was used to check the statistical significance of the independent variables' effects on the responses. A p-value under 0.05 was considered statistically significant.

Results

Pre-formulation and Drug-Excipient Compatibility

The initial studies confirmed furosemide's known physicochemical properties and its compatibility with Poloxamer 407, a crucial step for successful formulation.

The solubility results in Table 1 showed that furosemide is practically insoluble in water, which aligns with its classification and was the main reason for this study. It was much more soluble in the tested organic solvents, with acetone showing the highest solubility, followed by isopropyl alcohol, ethanol, and methanol. This finding supported the use of a small amount of acetone as a co-solvent to help wet and disperse the drug during formulation.

Table 1: Equilibrium Solubility of Furosemide in Various Solvents at 25 ± 2 °C

Sr. No.	*Solvent*	*Solubility (M)*
1	Water	0.000
2	Ethanol	0.041
3	Acetone	0.120
4	IPA	0.050
5	Methanol	0.038

The UV scan of furosemide in SGF (pH 1.2) showed a maximum absorbance wavelength (λmax) at 274 nm. The calibration curve for quantification was linear from 5-200 µg/mL, with a regression equation of y=0.005x+0.012 and a coefficient of determination (R2) of 0.998. This high R2 value confirmed the method's linearity and suitability for accurately measuring furosemide in later studies.

FTIR spectroscopy was used to check for chemical interactions between furosemide and Poloxamer 407. Furosemide's spectrum showed its typical absorption peaks, including those for the N-H stretch of the secondary amine, the S=O stretch of the sulfonamide, and the C=O stretch of the carboxylic acid. Importantly, all these main peaks were present in the 1:1 physical mixture's spectrum without significant shifts or new peaks, confirming no chemical interaction between the drug and polymer.

DSC analysis further supported this compatibility. The thermogram of pure furosemide had a sharp endothermic peak at 221 °C, its melting point. Poloxamer 407 had its melting peak at a much lower 56 °C. The physical mixture's thermogram showed both distinct peaks at their respective temperatures, indicating no significant solid-state interaction that would change their thermal properties.

Optimization of Furosemide Nanosuspensions using Factorial Design

A 3^2 full factorial design was used to create nine formulations (Fs1-Fs9) and systematically study the effects of Poloxamer 407 concentration (X1) and homogenization speed (X2) on the nanosuspensions' key qualities. The results for particle size, PDI, zeta potential, and drug content for all nine batches are in Table 2.

The data showed clear trends. Homogenization speed had a strong and expected effect on particle size; increasing the speed from 5000 to 15000 rpm generally led to smaller particles, as higher energy input causes more efficient particle breakdown. The smallest particle size (236.8 nm) was in batch Fs8 (3.5% stabilizer, 15000 rpm). The stabilizer's effect was less straightforward and seemed to interact with the speed. The PDI, which measures the particle size distribution's width,

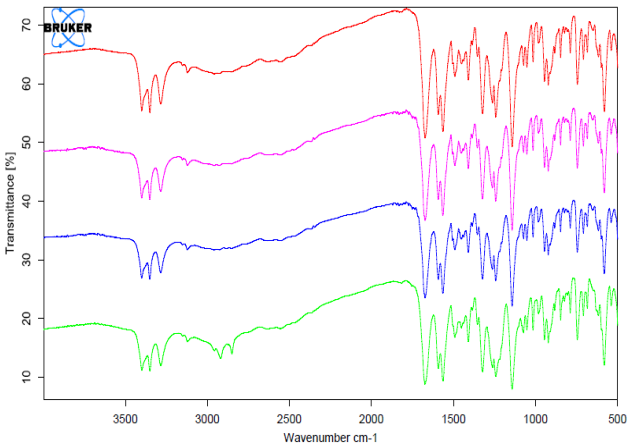

Figure 1: Overlay of FTIR Spectra of Pure Furosemide, Poloxamer 407, and their Physical

varied greatly. Notably, formulations Fs4 and Fs6, both made at 10,000 rpm, had very low PDI values (0.089 and 0.132), indicating a very uniform particle population. In contrast, many other formulations, especially at the lowest and highest speeds, had high PDI values (>0.7), suggesting a wide or mixed distribution. The zeta potential generally became more negative as both speed and stabilizer concentration increased, with the most negative values (below -37 mV) in the batches made at 15,000 rpm.

A key finding was the effect on drug content. The batches at the highest speed of 15,000 rpm (Fs7, Fs8, Fs9) had the lowest drug content, from 65-75%, suggesting that the intense energy might have caused some drug degradation or loss. In contrast, the highest drug recovery (95%) was in batch Fs6.

This led to a critical decision. Although batch Fs8 had the smallest particles, it was considered suboptimal due to its low drug content (67%). The goal is not just to get

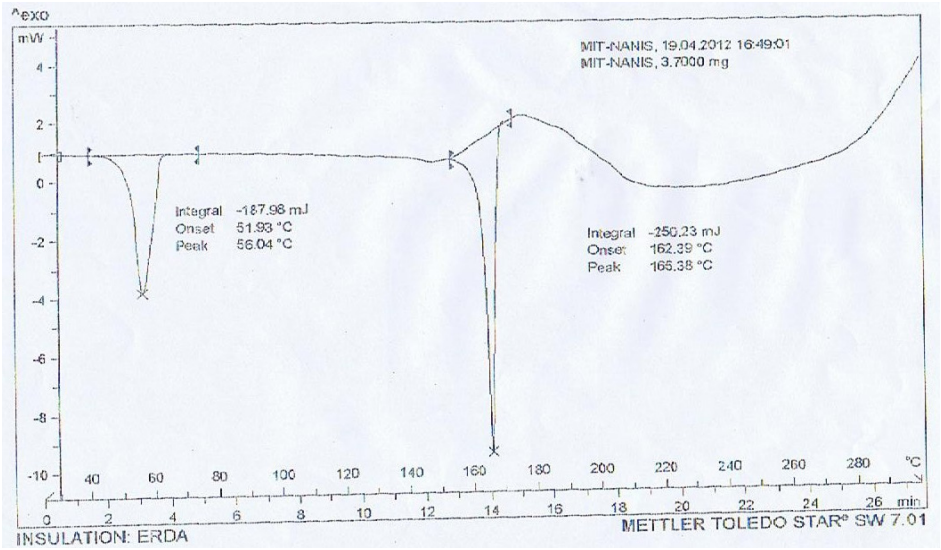

Figure 2: DSC graph of optimized formulation

Table 2: 3^2 Factorial Design Layout and Measured Responses for Furosemide Nanosuspensions

Batch No.	Stabilizer Conc. (X1, %)	Speed (X2, rpm)	Particle Size (nm)	PDI	Zeta Potential (mV)	Drug Content (%)
Fs1	2.0	5000	424	0.728	-17.1	88
Fs2	3.5	5000	488	0.738	-27.1	76
Fs3	5.0	5000	315	0.935	-25.6	82
Fs4	2.0	10000	374.6	0.089	-25.3	69
Fs5	3.5	10000	408.5	0.441	-35.3	79
Fs6	5.0	10000	384	0.132	-30.7	95
Fs7	2.0	15000	272	0.872	-37.3	75
Fs8	3.5	15000	236.8	0.918	-37.9	67
Fs9	5.0	15000	240.8	0.902	-38.4	65

the smallest particles but to create the most effective and stable product. Thus, batch Fs6 (5% Poloxamer 407, 10,000 rpm) was chosen as the optimized formulation. This choice was based on a comprehensive look at its properties: it had a good particle size (384 nm), an excellent low PDI (0.132) showing uniformity, a high enough zeta potential (-30.7 mV) for stability, and most importantly, the highest drug content (95%). This combination represents a robust "sweet spot," where the conditions were strong enough for significant size reduction and fast dissolution but gentle enough to keep the drug intact.

Characterization of the Optimized Nanosuspension (Fs6)

The optimized formulation, Fs6, was further characterized to confirm its quality. Its main physicochemical properties are in Table 3. The formulation was a homogeneous, milky white dispersion with no visible aggregation or settling.

The SEM image of the dried Fs6 formulation showed that the particles were in the nanometer range with a fairly uniform, somewhat spherical to cuboidal shape. The image clearly shows well-dispersed particles without much clumping, which supports the low PDI value from the Zetasizer analysis.

The DSC thermogram of the freeze-dried Fs6 formulation gave insight into the drug's solid state after processing. The typical melting peak of furosemide was still there, confirming its crystalline nature. However, the peak was slightly shifted to a lower temperature and was broader than the sharp peak of the pure drug. This change suggests a partial reduction in the drug's crystallinity, likely on the nanoparticle surfaces, a common result of high-energy processes like homogenization. This slight amorphization can also help enhance the drug's dissolution rate and saturation solubility.

In Vitro Performance

The in vitro dissolution tests showed a dramatic and significant improvement in the dissolution rate for all nine nanosuspension formulations compared to raw furosemide powder. In most cases, over 90% of the drug dissolved within the first 10 minutes. The optimized formulation, Fs6, was one of the best, with 95.16% drug release in 10 minutes. This rapid and almost complete dissolution in a simulated gastric environment is a direct result of the vastly increased surface area of the nanosized particles, achieving the study's main goal.

Table 3: Physicochemical Characterization of Optimized Formulation (Fs6)

Parameter	*Result*
Mean Particle Size (nm)	384
Polydispersity Index (PDI)	0.132
Zeta Potential (mV)	-30.7
Drug Content (%)	95.1
Appearance	Homogeneous, milky white dispersion

Drug Release Kinetics and Stability

To understand the mechanism of this rapid dissolution, the release data from the optimized batch (Fs6) were fitted to various kinetic models. The results are in Table 4. The dissolution profile of Fs6 had the highest coefficient of determination (R2=0.989) for the Zero-order model, suggesting a constant drug release rate over the study period. To further investigate the physical mechanism, the data were also fitted to the Korsmeyer-Peppas model. The calculated release exponent (n) was 0.496. For a spherical matrix, this value indicates a Fickian diffusion-controlled release mechanism.

The stability of the optimized nanosuspension (Fs6) was checked over 30 days. The formulation stored in the refrigerator (2–8 °C) showed no significant changes in particle size, PDI, zeta potential, or drug content, indicating excellent stability. In contrast, the sample stored at room temperature (25 °C) had a slight but noticeable increase in mean particle size and a small decrease in drug content after

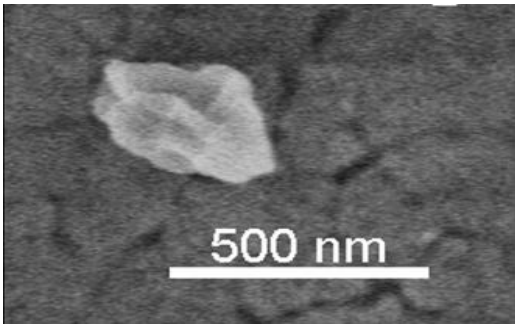

Figure 3: Scanning Electron Micrograph (SEM) of the Optimized Furosemide Nanosuspension (Fs6)

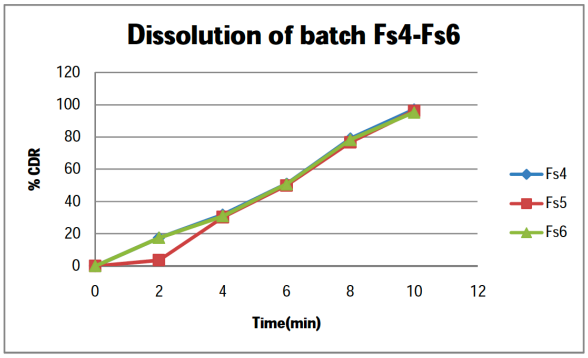

Figure 4: Comparative In Vitro Dissolution Profiles of Furosemide Nanosuspensions (Fs4-Fs6) in SGF (pH 1.2)

30 days. These results suggest that while the formulation is stable, refrigerated storage is best for its long-term stability and performance.

Discussion

This research successfully created a furosemide nanosuspension with greatly improved dissolution properties using high-speed homogenization. Analyzing these results offers key insights into the formulation strategy, the effects of process variables, and the basic principles behind the nanosystem's performance.

Nanosuspension as a Clinically Relevant Strategy for Furosemide's Bioavailability Paradox

Choosing a nanosuspension for furosemide is strongly justified by its difficult biopharmaceutical properties. As a BCS Class IV drug, its absorption is limited by both low solubility and low permeability. The biggest issue, however, is its narrow absorption window in the stomach and upper GI tract, where its solubility is lowest due to the acidic pH. This creates a classic dissolution rate-limited absorption problem, where the drug can't dissolve fast enough at its best absorption site to be effectively absorbed.

This study's results directly solve this problem. The optimized nanosuspension (Fs6) released over 95% of its drug in just 10 minutes in a simulated gastric environment (pH 1.2). This extremely fast dissolution is clinically important. It should maximize the concentration of dissolved, absorbable furosemide right at its main absorption site. By delivering the drug in a readily soluble, high-surface-area form at the right place and time, this method could overcome the main barrier to its bioavailability. This could lead to more stable plasma concentrations, less pharmacokinetic variability, and more predictable therapeutic effects compared to conventional solid forms, which have slow and erratic dissolution. This aligns with other research showing that nanosizing is a very effective way to improve furosemide's dissolution and bioavailability.

Deconstructing the Influence of Process and Formulation Variables

The 3^2 factorial design gave a systematic and quantitative view of how process and formulation variables affect the final product. The effect of homogenization speed was clear and followed established principles: higher speeds provide more mechanical energy—as intense shear forces, turbulence, and cavitation—leading to more efficient particle breakdown and smaller particle sizes. This trend was seen across all stabilizer concentrations.

The role of the stabilizer, Poloxamer 407, was more complex, showing a non-linear relationship with particle size. At low concentrations (2%), the stabilizer might not cover the vast new surface area created during homogenization, leading to re-aggregation or Ostwald ripening, where smaller particles dissolve and redeposit on larger ones, increasing the mean particle size. At optimal concentrations, like the 5% in Fs6, Poloxamer 407 can effectively cover the drug surface and provide a dense steric barrier that stabilizes the small particles. However, at very high concentrations, the increased viscosity of the medium can reduce the homogenization process's effectiveness by lessening the force and frequency of particle collisions, which could explain some of the complex trends seen.

Choosing Fs6 as the optimized batch, even though Fs8 had a smaller particle size, highlights a key aspect of pharmaceutical development. The much lower drug content in the batches made at the highest speed of 15,000 rpm (Fs7, Fs8, Fs9) suggests that the extreme energy might have caused some drug degradation or loss. Therefore, Fs6 is a robust "sweet spot"—the conditions (10,000 rpm) were strong enough for significant size reduction and fast dissolution but gentle enough to preserve the drug's chemical integrity, as shown by its high drug content of 95%. This shows that formulation optimization is a multi-parameter process where the goal is not just to maximize one parameter (like minimizing particle size) but to achieve the most effective and stable overall formulation that meets all critical quality standards.

Interpreting the Physicochemical Hallmarks of a Robust Nanosystem

The final characteristics of the optimized formulation (Fs6) strongly suggest a well-made and physically stable nanosystem. A mean particle size of 384 nm is well within the desired nanometer range and represents a huge increase in surface area compared to raw or even micronized furosemide. The polydispersity index (PDI) of 0.132 is especially good,

Table 4: Kinetic Modeling of In Vitro Dissolution Data for Optimized Formulation (Fs6)

Model	*Rate Constant (K)*	*Coefficient of Determination (R2)*	*Release Exponent (n)*
Zero-order	9.897	0.989	-
First-order	0.231	0.895	-
Higuchi	31.78	0.954	-
Korsmeyer-Peppas	-	0.976	0.496

as a value below 0.2 usually means a very narrow and uniform particle size distribution. A monodisperse system is highly desirable because it minimizes the risk of Ostwald ripening during storage and leads to more predictable and reproducible dissolution and bioavailability.

The zeta potential of -30.7 mV gives more insight into the system's stability. While Poloxamer 407 is a non-ionic stabilizer that works mainly through steric hindrance, a zeta potential of this size (where absolute values over 30 mV are generally considered to provide good electrostatic stability) is significant. This suggests a powerful dual-stabilization mechanism. The surface charge likely comes from the preferential adsorption of anions (like hydroxyl ions) from the water onto the furosemide crystal surface. This creates a negative charge layer that causes electrostatic repulsion between particles. This repulsion works with the physical steric barrier from the extended PEO chains of the adsorbed Poloxamer 407. This synergistic combination of steric and electrostatic stabilization creates a very robust system that is highly resistant to aggregation, a more sophisticated and effective approach than relying on just one mechanism. Finally, FTIR and DSC characterization confirmed that the drug remained chemically stable and mostly crystalline, ensuring that the performance improvement was due to physical modification (nanosizing and partial surface amorphization) rather than an unintended chemical change.

Mechanistic Insights into Drug Release Kinetics

The dissolution results clearly achieved the study's main goal. The nanosuspension's ability to release almost the entire drug load in 10 minutes shows the power of the nanosizing approach. The analysis of the release kinetics for the optimized batch (Fs6) showed an interesting but explainable combination of results: the data best fit a Zero-order model, while the Korsmeyer-Peppas analysis indicated a Fickian diffusion mechanism.

This apparent contradiction can be explained by considering the unique properties of a nanosuspension. The underlying physical process of dissolution is indeed Fickian diffusion: individual drug molecules must diffuse from the particle's solid surface, across a stagnant aqueous boundary layer, and into the bulk dissolution medium. The Korsmeyer-Peppas exponent of n≈0.5 correctly identifies this as the fundamental, microscopic rate-limiting mechanism. The reason the process can be accurately described by a Zero-order model (with an R2 of 0.989) is the quasi-constant surface area of the system during the initial, rapid phase of dissolution. According to the Noyes-Whitney equation, the overall dissolution rate of a powder is directly proportional to the total surface area of the particles. In a nanosuspension, the total number of particles is huge. Over a short time like 10 minutes, only a very small fraction of the total drug mass dissolves. As a result, the change in the radius of the individual particles is negligible, and therefore the change in the total surface area of the entire system is also negligible. Since the surface area remains effectively constant, the dissolution rate also remains constant. A constant rate of release is, by definition, zero-order kinetics. Therefore, Fickian diffusion is the *microscopic mechanism* at the particle level, while the *macroscopic observation* of the system's release over this initial phase is a zero-order profile. This interpretation shows a deep understanding of dissolution theory and accurately describes the system's behavior.

Stability Profile and Practical Implications

The 30-day stability study confirmed that the optimized furosemide nanosuspension is physically and chemically stable when refrigerated. The slight increase in particle size at room temperature is a common issue for high-energy colloidal systems like nanosuspensions and highlights the importance of controlling storage conditions to maintain product performance. Practically, this suggests that if this formulation were developed into a commercial liquid product, it would likely need refrigerated storage and a "shake well before use" label.

Alternatively, and more commonly for marketed nanosuspension products, the liquid nanosuspension could be turned into a solid dosage form to improve its long-term stability at room temperature.Techniques like lyophilization (freeze-drying), as done for the DSC sample in this study, or spray drying can be used to remove the water, leaving a solid powder of stabilized nanoparticles that can be easily reconstituted or put into tablets or capsules. This approach would significantly improve the product's shelf-life and ease of transport and storage, making it a more viable strategy for commercial development.

Conclusion

This study successfully created and characterized stable furosemide nanosuspensions using a scalable and economical high-speed homogenization technique. Through a systematic 3^2 factorial design, an optimized formulation stabilized with 5% w/v Poloxamer 407 and made at 10,000 rpm was identified. This optimized formulation showed a desirable set of physicochemical properties, including a mean particle size of 384 nm, a narrow particle size distribution (PDI = 0.132), and excellent physical and chemical stability, especially when refrigerated.

Most importantly, the nanosizing process led to a dramatic improvement in the in vitro dissolution rate of furosemide, a very challenging BCS Class IV drug. The optimized formulation released over 95% of its drug content within 10 minutes in a simulated gastric environment. This rapid and nearly complete dissolution, caused by the huge increase in drug surface area, is a highly viable and targeted strategy to overcome the dissolution-rate-limited absorption of furosemide in its critical gastric absorption window. The findings of this research show that the nanosuspension approach has great promise for improving the therapeutic consistency and oral bioavailability of furosemide and could be a valuable platform technology for other poorly soluble drugs with similar biopharmaceutical challenges.

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