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The Effect of Lyophilization on Solubility and Dissolution Enhancement of Furosemide: In Vitro Assessment and Kinetic Modeling

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ABSTRACT

Background: Furosemide, a BCS class IV diuretic, demonstrates inadequate water solubility and restricted intestinal permeability, leading to suboptimal oral bioavailability. Enhancing its dissolution is essential for improving therapeutic efficacy. This study assessed the effect of lyophilization on the in-vitro solubility profile of furosemide relative to the pure drug, Lasix (reference product), and a commercially available generic formulation. **Methods:** A lyophilized formulation of furosemide was developed using sucrose as a cryoprotectant and subjected to freeze-drying under regulated circumstances. Dissolution testing was conducted with a USP type 2 paddle apparatus in 0.1 N HCl, and drug concentrations were measured via UV-Vis spectrophotometry (λmax = 229 nm). Dissolution kinetics were analyzed using the Korsmeyer–Peppas model, and the dissolution profiles were evaluated with the difference factor (f_1). **Results:** The lyophilized formulation exhibited markedly superior solubility relative to the other formulations. Kinetic release adhered to a Fickian diffusion mechanism (n = 0.051), having enhanced porosity and partial amorphization. Conversely, the pure drug demonstrated a more gradual release profile (n = 0.667). The f_1 value between Lasix and the lyophilized formulation was above 160, signifying considerable dissimilarity. **Conclusion:** Lyophilization significantly enhanced the solubility of furosemide by altering its physicochemical properties. Nonetheless, owing to the significant disparities in dissolution patterns, further bioequivalence and pharmacokinetic investigations are necessary to validate clinical interchangeability.

KEYWORDS: Furosemide, lyophilization, dissolution enhancement, solubility enhancement, dissolution

INTRODUCTION

Furosemide is a potent loop diuretic frequently prescribed for the management of edema associated with congestive heart failure, liver cirrhosis, and renal dysfunction, and for controlling hypertension. Despite its clinical utility, furosemide exhibits low aqueous solubility and poor intestinal permeability, categorizing it under Biopharmaceutics Classification System (BCS) class IV. These physicochemical constraints lead to inconsistent and significantly low oral bioavailability, with a tendency to display considerable inter- and intrasubject variability (*1*). Numerous formulation strategies have been explored to overcome solubility barriers, including solid dispersions, salt formation, cyclodextrin inclusion complexes, self-emulsifying drug delivery systems, and nanosuspensions (*2*–*7*). However, each method carries specific limitations in terms of scalability, stability, and reproducibility.

Lyophilization (freeze-drying) is gaining traction as a versatile technique for enhancing the dissolution of poorly soluble drugs (*8*). It transforms the active pharmaceutical ingredient (API) into an amorphous, porous structure with significantly higher surface area, thereby facilitating faster wetting and dissolution upon contact with gastrointestinal fluids (*9*). Previous studies have demonstrated the ability of lyophilization to improve the dissolution rate and bioavailability of BCS Class IV drugs (*10*). Despite these advances, limited literature

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exists on the application of lyophilization to furosemide, especially in comparison with commercial reference and generic formulations.

This study aimed to examine the impact of lyophilization on the dissolution characteristics of furosemide, a BCS class IV drug, by comparing the dissolution profiles of the lyophilized formulation with those of the pure drug, the reference product (Lasix), and a marketed generic formulation. This study aims to determine if lyophilization could serve as an effective method to enhance the solubility and release properties of furosemide.

METHODS

Materials

Pure furosemide was procured from Pioneer (Iraq). Lasix 40-mg tablets (Sanofi, France; batch 4LU9A; expiration 02/2027) were used as the reference listed drug and purchased from the local market, as well as a generic furosemide tablet formulation (Accord Healthcare, UK; batch 09A246; expiration 09/2026). Sucrose (used as a cryoprotectant) and analytical-grade reagents were sourced from Alpha Chemica (India). All chemicals used were of pharmaceutical or analytical grade.

Tablet Characterization

Commercial tablets were measured for basic physical attributes using a digital caliper. Lasix 40 mg (Sanofi) was a white, round, scored tablet with an average diameter of 8 mm and a thickness of 2 mm. The generic furosemide tablets were also white and round, with an average diameter of 7 mm and a thickness of 2 mm.

Experimental Methods

Preparation of Lyophilized Formulation

A furosemide-sucrose solution was prepared in distilled water containing 10% w/w furosemide and 90% w/w sucrose after freeze-drying. The solution was first homogenized, then pre-frozen at −55 °C. Primary drying was performed using a laboratory freeze dryer (Alpha 1-2 LSCbasic, Germany) under a vacuum of 0.01 mbar at −55 °C for 10 hours, followed by secondary drying at 0 °C to remove residual moisture for 12 hours. The formulations were prepared and stored in 10 mL type I glass vials, sealed with laboratory-grade rubber stoppers and secured with Parafilm. Samples were kept in desiccators at room temperature (approximately 25 °C), protected from light, and tested within 3 days of lyophilization (*11*). The outcome of the process was a lyophilized powder obtained as uniform, white, and porous cakes with no evidence of collapse or shrinkage during processing. No final dosage form was developed at this stage.

Solubility Studies

The inherent aqueous solubility of pure furosemide was determined under standardized conditions to establish a baseline for comparison with formulated products. Excess pure furosemide was introduced to 10 mL of distilled water and incubated at 37 °C with continuous agitation for 72 hours to attain equilibrium. The solubility experiment was performed in triplicate (n = 3). After filtration through 0.45-µm syringe filters, the saturated concentration was measured using UV-Vis spectrophotometry (UV-1800, Shimadzu, Japan) (*12*).

Identification and Calibration

Furosemide was identified by UV-Vis spectroscopy, with a maximum absorbance (λmax) observed at 229 nm in 0.1 N HCl (*13*). A calibration curve was established using standard solutions ranging from 0.1–0.7 µg/mL. The calibration equation was derived by plotting absorbance against concentration, demonstrating linearity with R^2 = 0.998.

In Vitro Dissolution Testing

Dissolution profiles were assessed using the USP apparatus 2 (paddle method; PTWS 120D, Pharma Test, Germany), with 900 mL of (0.1 N HCl) as the dissolution medium. The pH of the medium was verified using a digital pH meter (3510, Jenway, UK). The temperature was maintained at 37 ± 0.5 °C, and the paddle speed was set at 50 rpm. Powdered samples were placed inside a small semi-permeable membrane pouch. The pouch was gently opened at the surface of the dissolution medium to allow uniform and immediate dispersion while preventing floating or adhesion to the vessel walls. For all formulations, the sample amount used in the dissolution test provided an equivalent furosemide dose of 40 mg. This corresponded to 40 mg of raw furosemide, 40 mg furosemide-equivalent from the physical mixture, and 400 mg of lyophilized powder based on a 10% drug loading. Samples were withdrawn at predetermined intervals (0, 5, 10, 15, 30, 45, and 60 minutes), filtered using 0.45-µm polytetrafluoroethylene (PTFE) membrane syringe filters, and analyzed at 229 nm using UV spectrophotometry (*14*).

Data Analysis

Dissolution data were analyzed using DD Solver version 1.0. The goodness of fit was assessed using the maximum correlation coefficient (R^2) and the minimum Akaike Information Criterion (AIC) values. Model-independent comparison between formulations was carried out using the difference factor (f_1). All experiments were performed in triplicate, and the data are expressed as mean ± SD.

Statistical analysis was conducted using one-way ANOVA, with differences considered statistically significant at a p-value less than 0.05 (*15*).

RESULTS

Solubility

The intrinsic saturation solubility of pure furosemide in distilled water at 37 °C was established as 1.9 ± 0.5 µg/mL. This value is in close agreement with previously published data, which reported a solubility of 1.8 ug/mL under similar conditions (*16*). The lyophilized formulation exhibited more than a 10-fold increase in solubility relative to the pure drug.

Dissolution Testing

The cumulative percentage of drug released over time for each formulation is summarized in Table 1 and graphically represented in Figure 1. The lyophilized formulation exhibited a markedly enhanced dissolution profile compared to all other formulations. A rapid initial release was observed, with approximately 86% drug release within the first 5 minutes, followed by sustained release reaching nearly 90% at 60 minutes.

Table 1. Cumulative Drug Release (%) of Different Furosemide Formulations at Various Time Intervals

Time (min)	Pure furosemide	Lasix	Generic	Lyophilized
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	1.6 ± 0.5	24.6 ± 2.1	22.3 ± 1.8	86.7 ± 3.5
10	7.3 ± 0.9	31.5 ± 2.4	29.7 ± 2.2	71.6 ± 3.1
15	9.6 ± 1.1	34.9 ± 2.0	32.2 ± 2.3	79.3 ± 2.8
20	11.3 ± 1.4	39.5 ± 2.6	34.9 ± 2.7	83.5 ± 3.6
25	12.1 ± 1.2	44.2 ± 2.8	34.8 ± 2.4	80.6 ± 3.0
30	15.4 ± 1.7	40.7 ± 2.2	36.1 ± 2.6	86.9 ± 4.1
35	16.7 ± 1.5	42.0 ± 2.5	39.3 ± 2.1	87.7 ± 3.7
40	18.8 ± 1.9	46.3 ± 3.0	47.8 ± 3.4	88.5 ± 4.2
45	20.0 ± 2.0	39.6 ± 2.7	48.7 ± 3.1	88.8 ± 3.9
50	20.5 ± 1.8	44.1 ± 2.9	51.6 ± 3.6	87.5 ± 4.0
55	21.7 ± 2.2	46.4 ± 3.1	54.0 ± 3.8	89.1 ± 4.3
60	21.1 ± 1.9	53.3 ± 3.5	52.2 ± 3.2	88.5 ± 3.8

Values are expressed as mean ± SD (n = 3).

In contrast, the pure drug showed a significantly slower release profile, not exceeding 22% at 60 minutes. The reference product (Lasix) and the generic formulation demonstrated moderate release rates, with cumulative releases of approximately 53% and 52%, respectively, at 60 minutes.

Kinetic Modeling

The dissolution data of the four tested formulations were analyzed using DD Solver, applying the Korsmeyer–Peppas model to evaluate the release kinetics (*15*, *17*). The selection of the model was guided by its superior goodness-of-fit parameters, as indicated by the highest R^2 and lowest AIC values when compared to alternative models, including zero-order, first-order, and Higuchi models.

The values of the release exponent (n), kinetic constant (k), R^2, and AIC for each formulation are summarized in Table 2. The Korsmeyer–Peppas model consistently exhibited the lowest AIC values, confirming its superiority over zero-order, first-order, and Higuchi models in describing the release kinetics of all formulations. The lyophilized formulation exhibited the lowest n-value (0.051), consistent with a predominantly Fickian diffusion release pattern. In contrast, pure furosemide (n = 0.667) showed a non-Fickian release mechanism involving both diffusion and matrix relaxation. The reference product (Lasix) and the generic formulation also demonstrated Fickian diffusion patterns but with slower release rates.

Table 2. Korsmeyer–Peppas Kinetic Model Parameters for Furosemide Formulations

Formulation	n-value	k (min^{-n})	R^2	AIC
Pure furosemide	0.667	1.5	0.9748	40.2
Lasix	0.243	18.05	0.9533	63.94
Generic furosemide	0.378	11.27	0.9704	60.77
Lyophilized formulation	0.051	71.725	0.9715	72.75

n = release exponent; k = kinetic constant (min^{-n}); R^2 = correlation coefficient; AIC = Akaike Information Criterion.

Figure 2 further illustrates the observed versus predicted dissolution profiles, demonstrating the close agreement of the model with the experimental data for Lasix, the generic formulation, pure furosemide, and the lyophilized product.

Difference Factor (f_1) Analysis

To further evaluate the similarity between the lyophilized formulation and marketed products, f_1 was calculated based on model-independent comparison criteria recommended by the U.S. Food and Drug Administration (FDA) (*18*). An f_1 value between 0 and 15 indicates that the two dissolution profiles are similar. In the present study, only the generic formulation (f_1 = 8.45) met this criterion, demonstrating statistical similarity to the reference product (Lasix). By contrast, the pure drug (f_1 = 26.13) and the lyophilized formulation (f_1 = 162.23) exceeded the threshold, indicating substantial dissimilar-

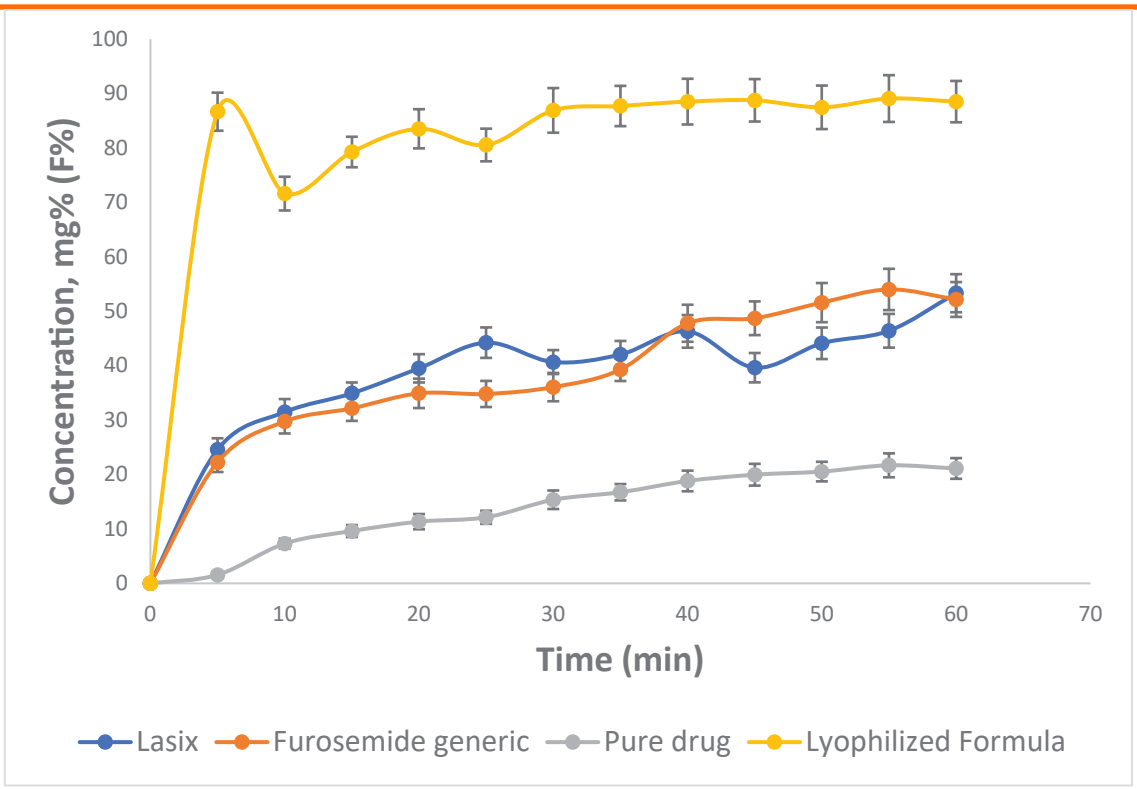

Figure 1. Dissolution profiles of furosemide formulations over 60 minutes in 0.1 N HCl.

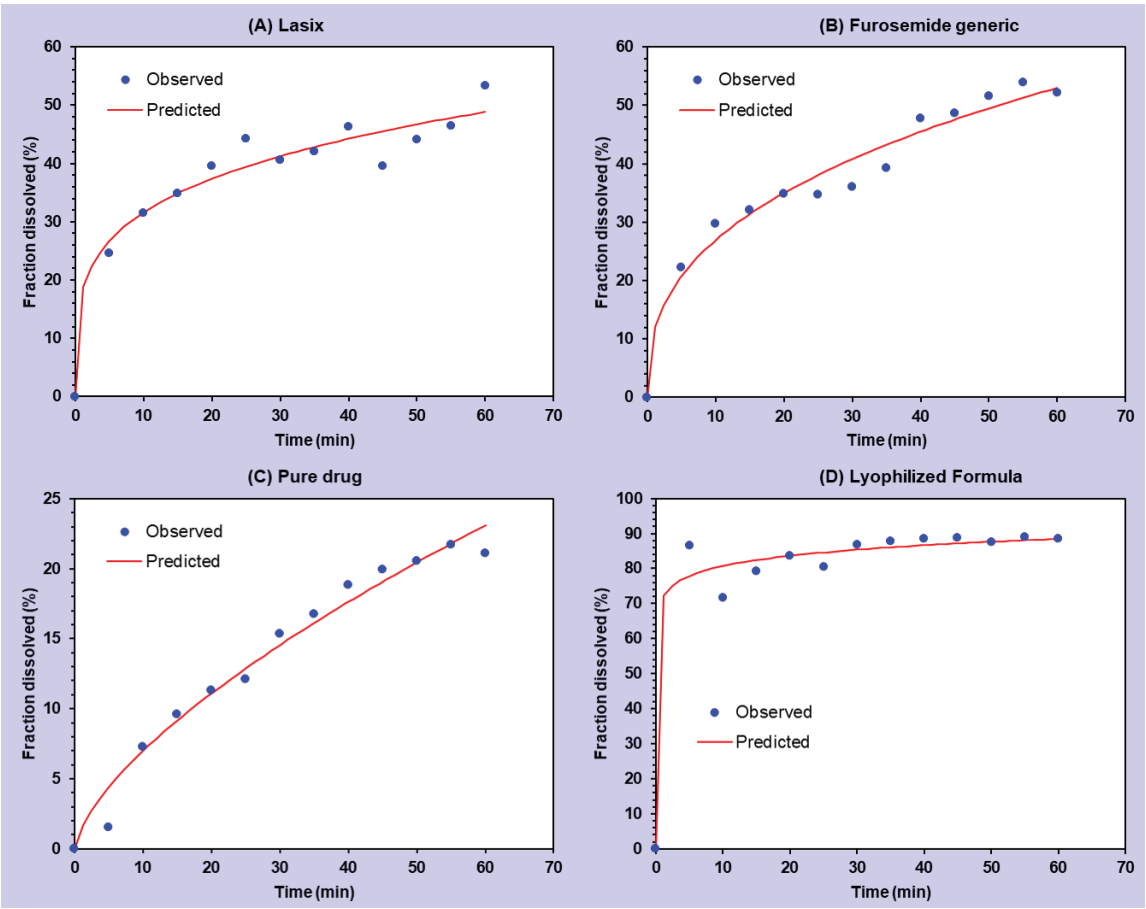

*Figure 2. Observed (dots) and predicted (lines) dissolution profiles of (**A**) Lasix, (**B**) generic furosemide, (**C**) pure drug, and (**D**) lyophilized formula, fitted using the Korsmeyer–Peppas model.*

ity, with the lyophilized product showing the greatest deviation.

DISCUSSION

The present study evaluated the impact of lyophilization on the solubility and dissolution performance of furosemide, a BCS class IV drug characterized by poor aqueous solubility and limited intestinal permeability. The findings demonstrated that lyophilization significantly improved both the solubility and dissolution rate of furosemide when compared to the pure drug, the reference product (Lasix), and a marketed generic formulation.

The marked increase in solubility observed for the lyophilized formulation may be attributed to several physicochemical modifications introduced by lyophilization, including increased porosity, reduced crystallinity, enhanced wettability, and improved particle dispersion. Comparable results have been reported in prior investigations, indicating that freeze-drying markedly enhances the solubility of weakly water-soluble pharmaceuticals (*19–21*).

The rapid release of the lyophilized formulation can be attributed to its highly porous matrix, increased surface area, reduced particle aggregation, and possible partial amorphization of the drug substance. These factors facilitate improved water penetration and accelerated drug dissolution (*22*, *23*).

The superiority of the Korsmeyer–Peppas model was confirmed as the best fit model for describing the release profiles. These findings are consistent with previous reports indicating that lyophilized systems favor diffusion-based release due to their porous structure (*24*).

Despite its superior dissolution performance, the lyophilized formulation exhibited an f_1 value of 162.23 when compared to Lasix, exceeding the FDA's similarity threshold ($f_1 \leq 15$). This confirms a substantial dissimilarity between the release profiles of the lyophilized product and the reference formulation. Therefore, although lyophilization markedly improves solubility and dissolution, it does not guarantee pharmaceutical equivalence without further in vivo bioequivalence assessments. Only the generic formulation demonstrated acceptable similarity to Lasix (f_1 = 8.45), as expected for an approved interchangeable product.

A key strength of this study is the successful application of lyophilization to substantially enhance the dissolution rate of furosemide, providing a promising strategy for improving oral delivery of BCS class IV drugs. Unlike most previous studies that relied primarily on solid dispersions or cyclodextrin complexes, this work demonstrates a process-driven enhancement approach. The comprehensive comparative analysis with both the reference (Lasix) and a marketed generic product allowed for a robust and clinically relevant evaluation of the formulation's performance. Collectively, these findings underscore the novelty of applying lyophilization as a formulation strategy for furosemide and highlight its potential significance as a platform technology for improving the biopharmaceutical properties of other poorly soluble drugs.

Despite these improvements, the lyophilization process yielded a powder that was stored in type I glass vials, serving as a proof-of-concept rather than a finished dosage form. The lyophilized product remained visually stable during short-term storage under desiccated conditions, showing no evidence of collapse, shrinkage, or discoloration. In contrast, Lasix and the generic tablets were commercially packaged with established stability data. Thus, accelerated and long-term stability studies are required to comprehensively evaluate the stability of the lyophilized formulation. Moreover, the study is limited by its exclusive reliance on in vitro data. Further in vivo pharmacokinetic and pharmacodynamic studies are necessary to assess the clinical relevance and ensure the biopharmaceutical safety and efficacy of the lyophilized formulation.

CONCLUSION

In this study, lyophilization proved to be an effective strategy to enhance the solubility and dissolution rate of furosemide, a poorly water-soluble BCS class IV drug. The lyophilized formulation demonstrated significantly faster drug release compared to both the pure drug and commercially available products, and the release profiles for the lyophilized product and the reference formulation lacked similarity.

DISCLOSURES

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