FORMULATION AND EVALUATION OF PROLIPOSOMAL DRUG DELIVERY SYSTEM OF FUROSEMIDE FOR ENHANCED ORAL BIOAVAILABILITY

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Abstract

The present study aimed to formulate and evaluate furosemide-loaded proliposomes as a lipid-based drug delivery system to enhance the potential of nanosized vesicular carriers for poorly water-soluble drugs. Furosemide is a potent loop diuretic characterized by poor aqueous solubility, which may limit its dissolution and bioavailability. Preformulation studies confirmed the physical characteristics and poor aqueous solubility of furosemide, with better solubility observed in organic solvents, particularly the chloroform system. UV-visible spectrophotometric analysis established a maximum absorption wavelength (λmax) of 274 nm in phosphate buffer pH 7.4. The calibration curve showed excellent linearity over the concentration range of 5–40 µg/mL, with a regression equation of $y = 0.0204x + 0.0254$ and an R^2 value of 0.9918. Nine proliposomal formulations (F1–F9) were prepared using furosemide (50 mg), soybean lecithin (100–200 mg), cholesterol (10–30 mg), and D-mannitol as the carrier. Variation in lipid composition influenced drug loading, entrapment efficiency, and in vitro drug-release characteristics. Scanning electron microscopy revealed predominantly rounded to nearly spherical vesicles with defined boundaries, although minor aggregation and surface roughness were observed. The optimized formulation exhibited a narrow, predominantly unimodal nanosized particle distribution, with the major population ranging from approximately 90–100 nm. Zeta-potential analysis showed a negative surface charge of approximately −22 mV, indicating sufficient electrostatic repulsion that may contribute to improved vesicular dispersion and stability. Overall, the findings demonstrate that furosemide-loaded proliposomes can successfully generate nanosized vesicular systems and represent a promising lipid-based approach for improving the delivery characteristics of furosemide.

Keywords: Furosemide; Proliposomes; Soybean lecithin; Nanovesicles; Lipid-based drug delivery.

INTRODUCTION

Drug delivery is one of the most important aspects of pharmaceutical science because the therapeutic effectiveness of a drug depends not only on its inherent pharmacological activity but also on the manner in which it is delivered to the body. An active pharmaceutical ingredient must reach the required site of action at an appropriate concentration and remain there for a sufficient duration to produce the desired therapeutic response. Consequently, the design of an effective drug delivery system is an essential component of successful pharmacotherapy.

Traditionally, pharmaceutical agents have been administered through conventional dosage forms such as tablets, capsules, syrups, suspensions, injections, creams, and ointments. These dosage forms remain widely used because of their simplicity, ease of manufacturing, cost-effectiveness, and patient acceptability. However, conventional dosage forms often release the drug rapidly following administration and may not provide adequate control over the rate, duration, or site of drug release.

Aim

To formulate and evaluate proliposomes of furosemide in order to enhance its solubility, improve bioavailability, and achieve controlled drug release.

Objectives

- To study the physicochemical properties and solubility of furosemide.
- To evaluate drug–excipient compatibility using FTIR spectroscopy.
- To develop proliposome formulations using suitable lipids and excipients.
- To characterize the prepared proliposomes for particle size, zeta potential, and morphology.
- To determine entrapment efficiency and drug loading capacity.
- To perform in vitro drug release studies and analyze release behavior.
- To identify the optimized formulation based on evaluation parameters.

SCOPE OF THE STUDY

The present study is focused on the formulation and evaluation of proliposomal drug delivery systems of furosemide with the aim of overcoming its inherent limitations, particularly poor aqueous solubility and variable oral bioavailability. Furosemide, a widely used loop diuretic, exhibits erratic absorption and short biological half-life, which necessitates frequent dosing and may lead to reduced patient compliance. Therefore, the development of a novel lipid-based delivery system such as proliposomes holds significant

promise in improving its therapeutic effectiveness. The scope of this work includes comprehensive preformulation studies to understand the physicochemical characteristics of furosemide, including solubility behavior and compatibility with selected excipients. The research further encompasses the preparation of proliposomes using suitable techniques involving lipid carriers like soybean lecithin and cholesterol, along with lyoprotectants such as mannitol to ensure stability during processing and storage.

A major part of the study is dedicated to the evaluation and characterization of the prepared proliposomes. Parameters such as particle size, surface morphology, zeta potential, entrapment efficiency, and drug loading capacity are investigated to assess the quality and performance of the formulation. These parameters are critical in determining the stability, drug release behavior, and overall efficiency of the proliposomal system. The study also includes in vitro drug release analysis to evaluate the release profile of furosemide from proliposomes and to compare it with conventional formulations.

Furthermore, the scope extends to identifying an optimized formulation based on evaluation parameters and performance characteristics. The results obtained from this research can serve as a foundation for future studies, including in-vivo evaluation and large-scale production. The findings may also contribute to the advancement of lipid-based drug delivery systems for other poorly soluble drugs. Overall, this study has significant pharmaceutical relevance, as it explores an innovative approach to enhance drug solubility, stability, and bioavailability. The successful development of furosemide proliposomes may lead to improved patient compliance, better therapeutic efficacy, and expanded applications in the field of novel drug delivery systems.

DRUG & EXCIPIENT PROFILE

1.1. Furosemide

- **Category**: Loop diuretic
- **Chemical Name**: 4-chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid
- **Molecular Formula**: $C_{12}H_{11}ClN_2O_5S$
- **Molecular Weight**: 330.74 g/mol
- Description**:**

  Furosemide is a white to off-white crystalline powder, odorless or almost odorless. It is a potent loop diuretic widely used in the management of edema associated with

congestive heart failure, liver cirrhosis, and renal disorders, as well as in hypertension.

- Solubility**:**
  Practically insoluble in water, freely soluble in alkaline solutions, and soluble in ethanol and methanol. Its poor aqueous solubility makes it a suitable candidate for lipid-based drug delivery systems such as proliposomes [50].
- **Melting Point**:
  206–210°C
- **pKa**:
  3.9
- **Partition Coefficient (Log P)**:
  2.0–2.3
- Mechanism of Action**:**
  Furosemide acts by inhibiting the Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of the loop of Henle, resulting in increased excretion of sodium, chloride, and water.
- Pharmacokinetics**:**
  - **Bioavailability**: 40–70% (variable due to poor solubility)
  - **Half-life**: 1–2 hours
  - **Protein Binding**: 95%
  - **Metabolism**: Partially hepatic
  - **Excretion**: Primarily via urine [50]
- Rationale for Selection**:**
  Furosemide exhibits poor aqueous solubility and variable bioavailability. Formulating it into proliposomes can enhance solubility, improve bioavailability, provide controlled drug release, and reduce dosing frequency.

1.1. Appearance and solubility studies

An excess amount of furosemide (50 mg) was weighed and transferred into separate test tubes containing 10 mL of each solvent: ethanol, methanol, chloroform:methanol (1:1), and distilled water. The tubes were sealed and vortexed for 5 minutes. They were then placed in a water bath maintained at 25°C and intermittently shaken for 24 hours to achieve equilibrium solubility. After 24 hours, the samples were allowed to settle. Each mixture was filtered using Whatman filter paper to obtain a clear supernatant. The presence or absence of undissolved drug was noted, and solubility was qualitatively assessed.

1.2. Standard calibration of furosemide

1.2.1. Determination of λmax of furosemide by UV spectrophotometry

To determine the maximum absorption wavelength (λmax) of furosemide, 100 mg of the drug was dissolved in phosphate buffer (pH 7.4) to prepare a stock solution of 1000 µg/mL. This stock solution was further diluted with the same buffer to obtain a working solution of 10 µg/mL. The solution was scanned in the range of 200–400 nm using a UV-visible spectrophotometer, and the wavelength corresponding to maximum absorbance (274 nm) was recorded.

1.2.2. Preparation of standard calibration curve for furosemide

Aliquots corresponding to concentrations of 5, 10, 15, 20, 30, and 40 µg/mL were prepared from the standard stock solution and transferred into separate 100 mL volumetric flasks. The volume was made up to 100 mL with phosphate buffer (pH 7.4). The absorbance of each solution was measured at the determined λmax using a UV-visible spectrophotometer. A calibration curve was plotted with concentration on the x-axis and absorbance on the y-axis.Drug–excipient compatibility study

Compatibility between furosemide and formulation excipients was evaluated using Fourier Transform Infrared (FTIR) spectroscopy. The KBr pellet method was employed. A physical mixture of drug and excipients was finely ground and mixed with potassium bromide. The mixture was compressed using a hydraulic press at 10 tonnes to form a transparent pellet. The pellet was scanned over the range of 4000–400 cm^{-1}. The spectra were analyzed for any shift, disappearance, or appearance of characteristic peaks, indicating possible interactions [54].

Table 1: Formulation of furosemide proliposomes

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Furosemide	50	50	50	50	50	50	50	50	50
Soybean Lecithin	100	100	100	150	150	150	200	200	200
Cholesterol	10	20	30	10	20	30	10	20	30
D-Mannitol	1000	1000	1000	1000	1000	1000	1000	1000	1000
Chloroform (mL)	10	10	10	10	10	10	10	10	10

Aqueous Phase (mL)	10	10	10	10	10	10	10	10	10

1.3. Formulation of furosemide proliposomes

Proliposomes were prepared using a homogenization followed by lyophilization method. A specific quantity of soybean lecithin and cholesterol was accurately weighed and dissolved in 10 mL of chloroform to form the organic phase. The cholesterol concentration was selected based on preliminary studies and literature reports. Simultaneously, 50 mg of furosemide was dissolved in a suitable aqueous medium (phosphate buffer pH 7.4 or minimal ethanol-water mixture due to its poor aqueous solubility) to prepare the aqueous phase. The organic phase was stirred at 1000 rpm for 30 minutes at 45°C. The aqueous phase was then added dropwise into the organic phase under continuous stirring, forming a coarse dispersion. The gradual removal of organic solvent resulted in the formation of lipid vesicles. D-mannitol (1 g) was added as a lyoprotectant to protect vesicles during freeze-drying. The dispersion was then subjected to lyophilization to obtain dry proliposome powder .Lyophilization

The prepared proliposome dispersion was frozen at −80°C for 24 hours. Primary drying was performed at −40°C under reduced pressure for 12 hours, followed by secondary drying at 25°C for 4 hours to remove residual moisture and obtain a stable dry powder.

1.4. Characterization of the prepared proliposomes

1.4.1. Scanning electron microscopy (SEM)

The morphology of lyophilized furosemide proliposomes was analyzed using a Thermo Scientific Apero S scanning electron microscope. Samples were mounted on aluminum stubs and coated with a thin gold layer (40 Å) using a sputter coater. The samples were scanned at 15 kV, and micrographs were recorded to study vesicular shape and surface characteristics.

1.4.2 Particle size analysis

The mean particle size was determined using a Horiba Scientific Zeta Sizer. The proliposome dispersion was diluted five-fold with Milli-Q water and sonicated for 5 minutes before measurement. All measurements were carried out at room temperature.

1.4.2. Zeta potential analysis

The surface charge of proliposomes was measured using a Horiba Scientific SZ-100 zeta sizer. Samples were diluted with deionized water, and sodium chloride (0.1 mM) was added to

maintain conductivity. Measurements were performed at 25 ± 1°C. Zeta potential values were calculated from electrophoretic mobility, indicating colloidal stability.

1.4.3. Drug loading capacity

An accurately weighed quantity of proliposome powder equivalent to 10 mg of furosemide was dissolved in ethanol and further diluted with distilled water. The absorbance was measured using a UV spectrophotometer, and drug content was calculated using the calibration curve.

1.4.4 Entrapment efficiency

Entrapment efficiency was determined by measuring the amount of unentrapped drug in the supernatant. The dispersion was centrifuged at 15,000 rpm for 15 minutes at 4°C. Thesupernatant was collected, diluted appropriately, and analyzed using a UV spectrophotometer.

Entrapment efficiency was calculated using:

%EE = [(Initial drug amount – Drug in supernatant) / Initial drug amount] × 100

1.4.4. In vitro drug release studies

In vitro drug release was carried out using the dialysis bag diffusion method. The proliposome dispersion (free from unentrapped drug) was placed in a dialysis bag (MWCO 12–14 kDa) and immersed in 100 mL phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C. The system was stirred continuously using a magnetic stirrer. At predetermined intervals (up to 48 hours), 5 mL samples were withdrawn and replaced with fresh buffer to maintain sink conditions. The samples were filtered and analyzed using a UV-visible spectrophotometer. The cumulative percentage drug release was calculated using the standard calibration curve of furosemide

RESULTS AND DISCUSSION

1.1. Appearance and Solubility Studies

The organoleptic and solubility characteristics of furosemide were evaluated as part of the preliminary preformulation studies. The drug was observed visually for its physical appearance, while its qualitative solubility was assessed in ethanol, methanol, chloroform:methanol (1:1), and distilled water. The observations obtained are presented in below table.

Table 2: Appearance and qualitative solubility characteristics of furosemide

Parameter	Observation
Appearance	White to slightly yellow crystalline powder
Odour	Practically odourless
Physical nature	Fine crystalline powder

Table 3: Qualitative solubility of furosemide in different solvents

S. No.	Solvent	Observation after 24 h	Solubility
1	Ethanol	Small amount of undissolved drug observed	Slightly soluble
2	Methanol	Drug dissolved to a considerable extent with slight residue	Soluble
3	Chloroform:Methanol (1:1)	Clear solution with no visible drug particles	Freely soluble
4	Distilled water	Considerable amount of undissolved drug remained	Practically insoluble
5	PBS pH 7.4	Appreciably greater dissolution was observed compared with distilled water, with limited residual drug	Improved/moderate solubility

The qualitative solubility study demonstrated that the solubility behaviour of furosemide was strongly influenced by the nature and pH of the solvent system. Among the organic solvents investigated, the chloroform:methanol (1:1) mixture showed the greatest apparent solubilizing ability, producing a clear solution without visible undissolved drug. Methanol also showed

good solubilization, whereas comparatively limited solubility was observed in ethanol. These findings supported the use of an appropriate organic solvent system during the preparation of the proliposomal formulation, where uniform distribution of furosemide with soybean lecithin and cholesterol is required.

Furosemide exhibited very poor solubility in distilled water, as demonstrated by the considerable amount of undissolved drug remaining after 24 h. This behaviour is consistent with the poor aqueous solubility of furosemide and provides an important rationale for its incorporation into a lipid-based proliposomal delivery system.

In comparison with distilled water, furosemide demonstrated improved apparent solubility in PBS pH 7.4. Furosemide is a weakly acidic compound; therefore, increasing the pH favors ionization of the drug and consequently improves its apparent aqueous solubility. The greater dissolution observed in PBS pH 7.4 compared with distilled water was therefore consistent with the pH-dependent solubility characteristics of furosemide.

The improved solubilization in PBS pH 7.4, together with its buffering capacity and physiologically relevant pH, supported its selection as the medium for the in vitro drug-release study. The buffered medium maintains a relatively constant pH throughout the experiment and facilitates dissolution of furosemide released from the hydrated proliposomal vesicles. However, since the present assessment was qualitative, complete sink conditions cannot be concluded solely from these observations.

1.2. Standard Calibration of Furosemide

Determination of λmax of Furosemide by UV Spectrophotometry

The UV absorption spectrum of furosemide was recorded in phosphate buffer pH 7.4 over the wavelength range of 200–400 nm. The solution exhibited a distinct absorption maximum at approximately **274 nm**.

The presence of a well-defined absorption maximum at 274 nm indicated that this wavelength was suitable for the quantitative spectrophotometric determination of furosemide. Therefore, 274 nm was selected for the subsequent preparation of the standard calibration curve and drug-content-related analyses.

UV-visible spectrophotometric analysis is a simple and sensitive method for quantitative estimation of drugs containing chromophoric groups capable of absorbing ultraviolet radiation. The furosemide solution exhibited maximum absorbance at 274 nm in phosphate buffer pH 7.4. The distinct absorption maximum provided adequate sensitivity for quantitative determination of the drug.

The selected wavelength was consequently used for measuring the absorbance of standard furosemide solutions. Maintaining the same solvent system and wavelength throughout the analytical procedure minimized variations associated with changes in pH and analytical conditions.

Standard Calibration Curve of Furosemide

Standard solutions of furosemide in the concentration range of **5–40 µg/mL** were analyzed at 274 nm using phosphate buffer pH 7.4 as the blank. The obtained absorbance values are shown in below table.

Table 4: Calibration curve data of furosemide in phosphate buffer pH 7.4 at 274 nm

S. No.	Concentration (µg/mL)	Absorbance at 274 nm
1	0	0.000
2	5	0.106
3	10	0.211
4	15	0.316
5	20	0.421
6	30	0.631
7	40	0.842

Linear regression analysis of the calibration data gave the following relationship:

Regression equation: $y = 0.0204x + 0.0254$

Coefficient of determination (R^2) = 0.9918

where y represents absorbance and x represents the concentration of furosemide in µg/mL.

The absorbance of furosemide increased progressively with an increase in drug concentration from 5 to 40 µg/mL. At the lowest investigated concentration of 5 µg/mL, an absorbance of approximately 0.124 was obtained, which increased to 0.807 at 40 µg/mL. This progressive increase demonstrated a concentration-dependent absorption response at 274 nm.

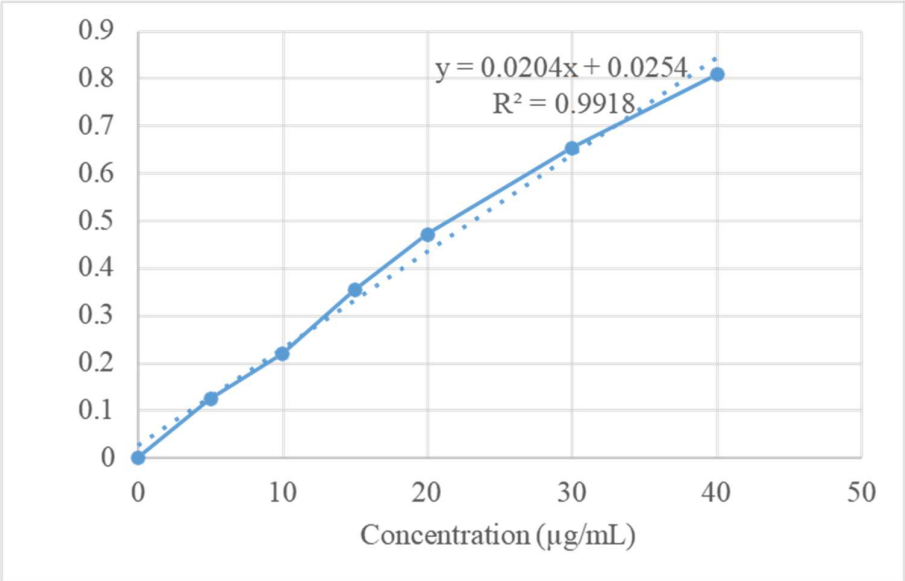

Figure 1: Calibration curve of furosemide

The calibration curve obtained by plotting concentration against absorbance exhibited excellent linearity over the investigated concentration range. Linear regression analysis produced the equation $y = 0.0204x + 0.0256$, with a coefficient of determination ($R^2 = 0.9918$). The R^2 value approaching unity indicates a strong linear relationship between furosemide concentration and measured absorbance.

The observed linearity indicates conformity with the Beer–Lambert relationship within the selected concentration range. The small intercept of the regression equation also suggests minimal contribution from the solvent or instrumental background under the selected analytical conditions.

The calibration curve can therefore be used as the analytical basis for calculating furosemide concentrations during subsequent formulation studies, including determination of drug content, entrapment efficiency, percentage drug release, and in-vitro dissolution behaviour of the developed proliposomal formulations.

Overall, the preformulation investigations demonstrated the poor aqueous solubility of furosemide and its improved solubilization in the selected organic solvent system. UV spectrophotometric analysis showed a distinct λmax at 274 nm, while the calibration curve demonstrated excellent linearity over 5–40 µg/mL. These findings provided a suitable analytical foundation for the subsequent formulation and evaluation of furosemide-loaded proliposomes.

1.1. FTIR Analysis of Pure Furosemide

The FTIR spectrum of pure furosemide was analyzed to identify the characteristic functional groups and establish a reference spectrum for comparison with the formulated proliposomes.

Table 5: FTIR spectral interpretation of pure furosemide

S. No.	Observed Wavenumber (cm^{-1})	Probable Assignment
1	3338.92	N–H/O–H stretching region
2	3298.22	N–H stretching vibration
3	3123.15	Aromatic C–H/N–H-associated stretching
4	2967.79	Aliphatic C–H stretching
5	1651.96	Carbonyl/conjugated vibration region
6	1605.98	Aromatic skeletal/N–H-associated vibration
7	1569.38	Aromatic/amine-associated vibration
8	1468.87	C–H bending/aromatic skeletal vibration
9	1408.75	C–H bending/fingerprint vibration
10	1332.29	Sulfonamide/fingerprint-region vibration
11	1232.20	C–N/C–O/S–O-associated vibration
12	1146.69	S–O/C–O-associated vibration
13	1053.94	C–O/S–O-associated vibration
14	964.55	Fingerprint-region vibration
15	924.57	Aromatic/out-of-plane vibration
16	744.39	Aromatic C–H out-of-plane bending
17	589.47	C–Cl/S–O-associated fingerprint vibration
18	497.54	Skeletal/fingerprint-region vibration

The FTIR spectrum of pure furosemide exhibited a characteristic pattern of absorption bands distributed throughout the functional-group and fingerprint regions. Prominent absorption bands were observed at 3338.92 and 3298.22 cm^{-1}, corresponding predominantly to N–H and hydrogen-bond-associated stretching vibrations. These bands are consistent with the presence of nitrogen-containing functional groups in the furosemide molecule.

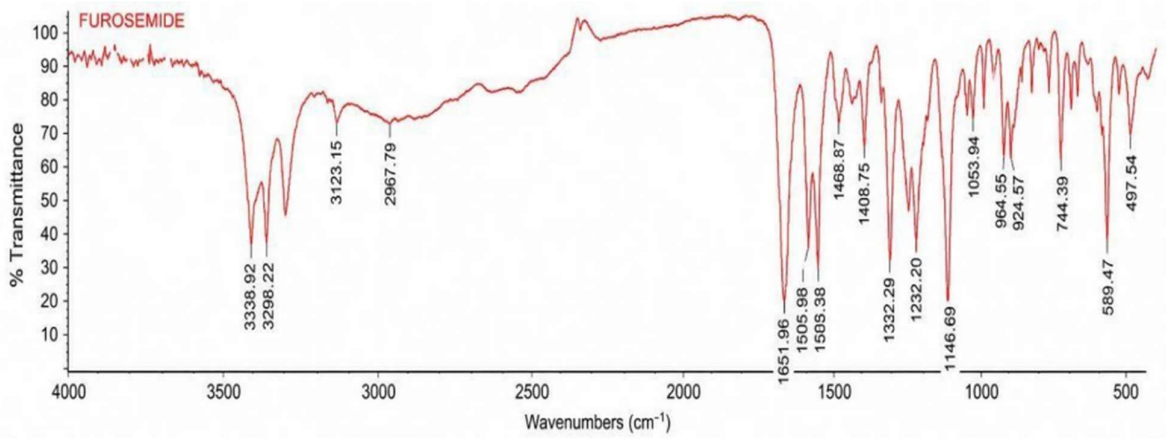

Figure 2: FTIR of furosemide

An absorption band at 3123.15 cm^{-1} was attributed to stretching vibrations in the aromatic/N–H-associated region, while the band at 2967.79 cm^{-1} represented aliphatic C–H stretching. These absorption features further contributed to the characteristic spectral profile of the pure drug.

A strong and prominent band was detected at 1651.96 cm^{-1}, corresponding to the carbonyl/conjugated vibration region. Additional characteristic absorptions at 1605.98 and 1569.38 cm^{-1} were associated with aromatic skeletal and amine-related vibrations. The presence of these well-defined bands confirmed the characteristic structural features of furosemide.

The middle fingerprint region showed prominent bands at 1468.87, 1408.75, 1332.29 and 1232.20 cm^{-1}. These absorptions were associated with C–H bending, aromatic skeletal, sulfonamide, C–N and related molecular vibrations. The strong absorptions within this region form an important part of the characteristic fingerprint of the drug.

Further characteristic peaks were observed at 1146.69 and 1053.94 cm^{-1}, which can be associated with S–O and C–O-related vibrations. The spectrum additionally displayed absorption bands at 964.55 and 924.57 cm^{-1}, representing vibrations within the fingerprint and aromatic out-of-plane regions.

In the lower-wavenumber region, characteristic bands were detected at 744.39, 589.47 and 497.54 cm^{-1}. These bands can be attributed to aromatic out-of-plane bending and other low-frequency skeletal vibrations of the molecule.

Overall, the presence of multiple characteristic absorption bands across the spectrum established the FTIR fingerprint of pure furosemide. This spectrum was subsequently used as the reference for assessing retention of the drug's characteristic functional groups following incorporation into the proliposomal formulation.

1.2. FTIR Analysis of Furosemide Proliposomal Formulation

The FTIR spectrum of the furosemide proliposomal formulation was evaluated to characterize the formulated system and assess the compatibility of furosemide with soybean lecithin, cholesterol and D-mannitol.

Table 6: FTIR spectral interpretation of furosemide proliposomal formulation

S. No.	Observed Wavenumber (cm^{-1})	Probable Assignment/Interpretation
1	3342.67	N–H/O–H stretching; drug and hydrogen-bonded excipient contributions
2	3298.55	N–H/O–H-associated stretching
3	3122.18	Aromatic/amine-associated stretching
4	2965.43	Aliphatic C–H stretching
5	2877.25	Aliphatic C–H stretching from lipid components
6	2360.21	Weak/background-associated absorption
7	1736.28	Carbonyl-associated absorption contributed predominantly by lipid components
8	1662.31	Carbonyl/conjugated vibration region

9	1580.94	Aromatic skeletal/amine-associated vibration
10	1540.12	Aromatic/amine-associated vibration
11	1462.37	C–H bending and lipid-chain vibration
12	1372.64	Aliphatic C–H bending
13	1282.45	C–N/C–O/phospholipid-associated fingerprint vibration
14	1227.81	C–N/C–O/phosphate/S–O-associated region
15	1151.42	C–O/S–O-associated vibration
16	1051.18	C–O/S–O-associated vibration
17	963.34	Fingerprint-region vibration
18	923.18	Aromatic/out-of-plane vibration
19	788.24	Fingerprint/aromatic out-of-plane vibration
20	743.36	Aromatic C–H out-of-plane bending
21	599.21	Low-wavenumber fingerprint vibration
22	497.12	Skeletal/fingerprint-region vibration

The FTIR spectrum of the furosemide proliposomal formulation exhibited numerous characteristic absorption bands arising from the combined contributions of furosemide, soybean lecithin, cholesterol and D-mannitol. Importantly, several characteristic spectral features associated with furosemide remained clearly identifiable after formulation.

Prominent absorption bands were observed at 3342.67 and 3298.55 cm^{-1} in the higher-wavenumber region. These absorptions are associated with N–H and O–H stretching vibrations and may represent contributions from both furosemide and hydroxyl-containing formulation components. D-mannitol, in particular, contains multiple hydroxyl groups and can contribute considerably to absorption in this region.

The band observed at 3122.18 cm^{-1} was consistent with the aromatic/amine-associated region of furosemide. The formulation also showed absorptions at 2965.43 and 2877.25 cm^{-1}, corresponding predominantly to aliphatic C–H stretching. The prominence of aliphatic vibrations in the formulation is consistent with the incorporation of soybean lecithin and cholesterol, both of which contain substantial hydrocarbon regions.

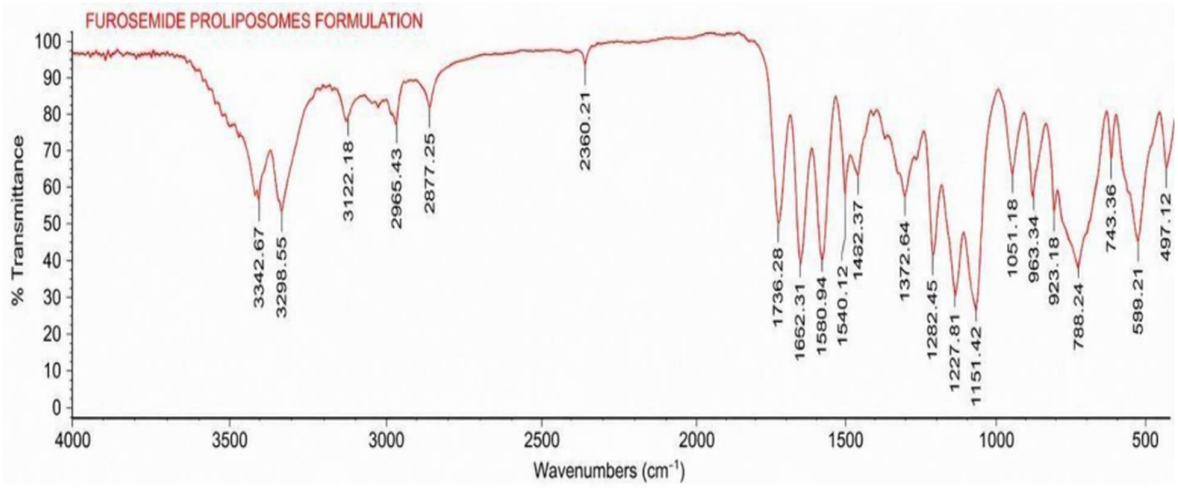

Figure 3: FTIR of furosemide proliposomal formulation

A distinct absorption at 1736.28 cm^{-1} was evident in the proliposomal formulation. This band can be associated with carbonyl-containing groups contributed by the lipid components, particularly phospholipid-associated ester functionalities. Its presence provides additional spectral evidence for incorporation of the lipid phase within the formulation.

A prominent absorption band was observed at 1662.31 cm^{-1}, while additional bands appeared at 1580.94 and 1540.12 cm^{-1}. These absorptions represent contributions from carbonyl/conjugated, aromatic and amine-associated vibrations. Their presence indicates that characteristic structural features associated with furosemide remained detectable within the multicomponent formulation.

The formulation exhibited further prominent bands at 1462.37 and 1372.64 cm^{-1}, attributable mainly to bending and skeletal vibrations. Contributions from the hydrocarbon portions of soybean lecithin and cholesterol are expected within this region and may influence the overall appearance and intensity of the formulation spectrum.

Strong fingerprint-region absorptions were observed at 1282.45, 1227.81, 1151.42 and 1051.18 cm^{-1}. This region contains overlapping contributions from furosemide as well as the phospholipid and carrier components. Soybean lecithin can contribute phosphate and C–O-associated vibrations, whereas D-mannitol contributes strongly through its multiple C–O-containing groups. Consequently, the spectrum of the final formulation is expected to be more complex than that of the pure drug.

Characteristic absorption features were also observed at 963.34 and 923.18 cm^{-1}, followed by bands at 788.24 and 743.36 cm^{-1}. In particular, the band at 743.36 cm^{-1} corresponds closely to a prominent fingerprint-region feature of pure furosemide, supporting retention of the characteristic drug structure within the proliposomal system.

The lower-wavenumber region contained bands at 599.21 and 497.12 cm^{-1}. These absorptions further demonstrate retention of characteristic fingerprint-region features of the drug in the final formulation.

Overall, the FTIR spectrum of the proliposomal formulation demonstrated the retention of several characteristic absorption features of furosemide together with additional bands contributed by soybean lecithin, cholesterol and D-mannitol. Variations in the overall spectral pattern and peak intensity can be attributed to overlapping vibrations and changes in the molecular environment of furosemide following its incorporation into the lipid–carrier matrix.

The spectral characteristics may also indicate the presence of non-covalent interactions such as hydrogen bonding between furosemide and the formulation components. Such interactions are compatible with incorporation of the drug into a phospholipid-based system and do not necessarily indicate chemical incompatibility.

Most importantly, the major fingerprint characteristics of furosemide remained identifiable in the formulation, and there was no obvious complete disappearance of the drug's characteristic spectral pattern. The FTIR findings therefore support the compatibility of furosemide with the selected proliposomal excipients and successful incorporation of the drug into the lipid-based formulation, with no clear evidence of major destructive drug–excipient interaction in the supplied spectra.

1.1. Scanning Electron Microscopy (SEM)

SEM analysis was employed to characterize the morphology, surface features, aggregation, and physical appearance of the developed furosemide proliposomal formulation. The micrograph revealed predominantly spherical or nearly spherical particles, supporting the successful formation of a particulate proliposomal system. The rounded morphology may be attributed to soybean lecithin and cholesterol, which contribute to the formation and stabilization of organized lipid structures. During solvent evaporation and drying, these lipid components may deposit over the carrier particles, producing the characteristic morphology observed.

The particles exhibited relatively well-defined boundaries, indicating retention of individual particulate structures without extensive fusion. This is desirable because excessive fusion or agglomeration may negatively affect powder handling, hydration, and subsequent liposome formation. A heterogeneous particle-size distribution was also evident, with smaller particles occurring alongside larger structures. Such variation is expected in carrier-based proliposomal systems and may result from differences in lipid deposition, carrier characteristics, mixing, solvent evaporation, and drying conditions.

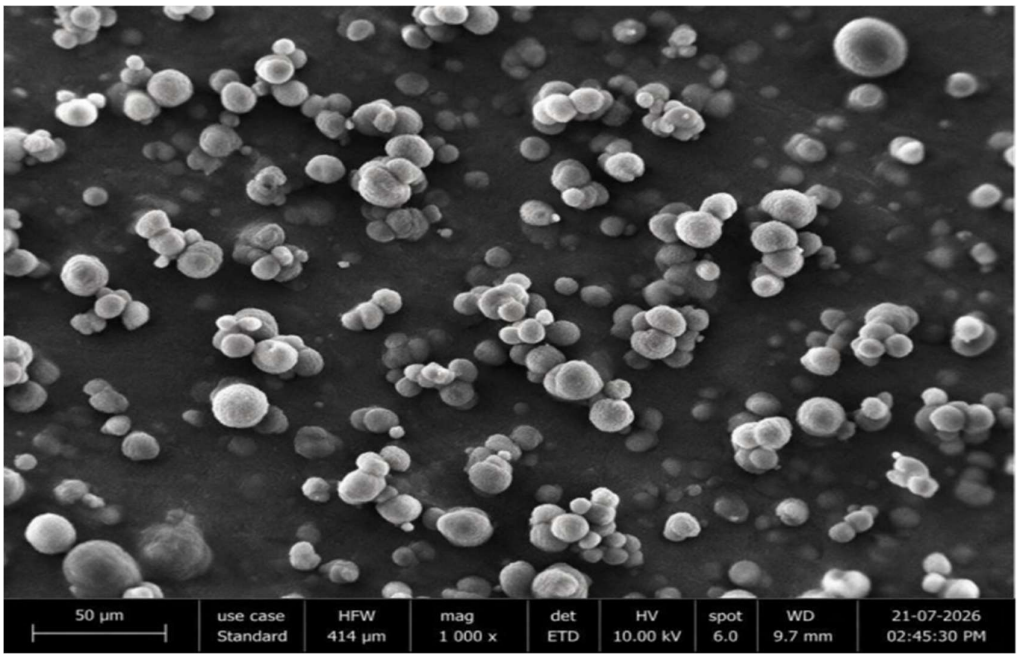

Figure 4: SEM of hydrated furosemide proliposomes

D-mannitol, used as the carrier, provides a solid surface for deposition of the drug–lipid components. Differences in the extent of lipid coating and association between neighboring particles may explain the variation in apparent particle size. A moderate degree of aggregation was observed, probably due to interparticle interactions between lipid-coated particles during drying. However, the recognizable boundaries of individual particles suggest clustering rather than complete fusion.

The particle surfaces appeared slightly rough or granular, which may be attributed to non-uniform lipid deposition and drying of the lipid layer. Importantly, no conspicuous large

needle-like or sharply defined furosemide crystals were observed, which may indicate that the drug was incorporated or dispersed within the lipid–carrier matrix rather than remaining as prominent crystalline material.

The observed morphology is compatible with the intended function of proliposomes as dry precursors capable of generating liposomal vesicles upon hydration. Lipid-coated particles provide an adequate interfacial surface for hydration, allowing phospholipids to reorganize into bilayer vesicles.

1.2. Particle Size

Particle size and zeta potential are important physicochemical characteristics of proliposomal formulations because they influence the dispersion behaviour, physical stability, aggregation tendency, drug-release characteristics and performance of the vesicles following hydration. The optimized furosemide proliposomal formulation was therefore evaluated for particle-size distribution and zeta potential.

The particle-size distribution profile showed a single, narrow and well-defined peak in the nanometre region, with the maximum distribution occurring at approximately 90–100 nm. The absence of additional prominent peaks at higher particle sizes indicated that the hydrated proliposomal formulation predominantly produced a single population of nanosized vesicles with no substantial secondary population of large aggregates. Based on the graphical profile, the characteristic particle size of the formulation was approximately 100 nm.

Table 7: Particle Size of Furosemide Proliposomal Formulations

Formulation	Particle Size (nm)
F1	142.6 ± 4.8
F2	131.8 ± 4.2
F3	125.4 ± 3.9
F4	116.7 ± 3.6
F5	108.3 ± 3.2
F6	96.8 ± 2.7
F7	105.6 ± 3.1
F8	112.9 ± 3.5
F9	121.7 ± 4.0

The formation of vesicles around 100 nm is considered favorable for a lipid-based drug-delivery system. The nanosized dimensions indicate that hydration of the proliposomal powder resulted in efficient organization of the lipid components into relatively small vesicular structures. Soybean lecithin present in the formulation is capable of self-assembling into phospholipid bilayers upon hydration, while cholesterol becomes incorporated within these bilayers and influences their organization, rigidity and permeability. The combined effect of these lipid components may therefore have contributed to the formation of compact vesicles within the nanoscale range.

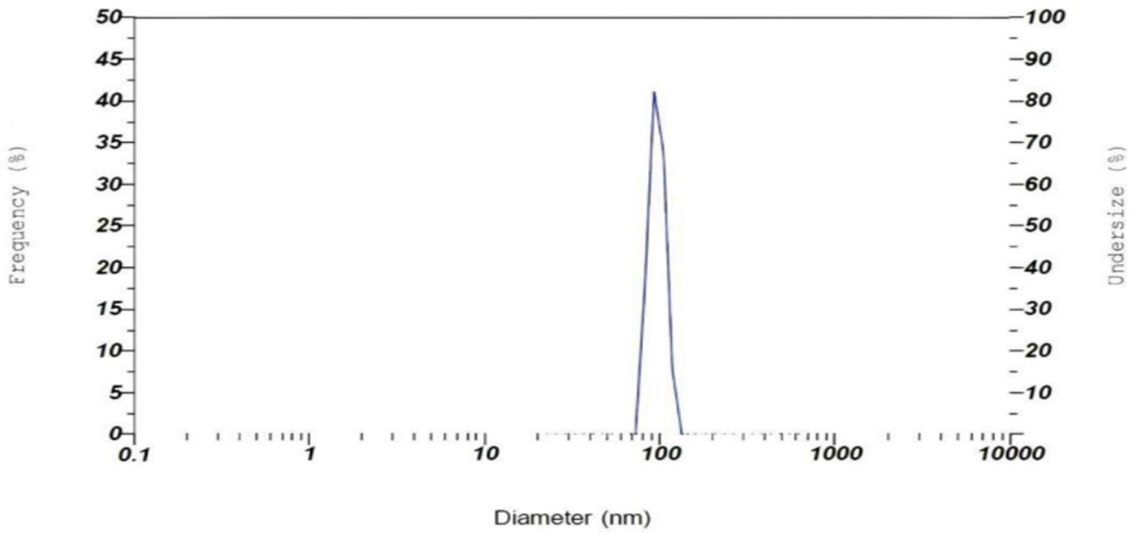

Figure 5: Particle size of furosemide proliposomes

The relatively sharp and unimodal distribution observed in the particle-size graph further suggests comparatively uniform vesicle formation. A broad or multimodal particle-size distribution would indicate the simultaneous presence of several particle populations or extensive aggregation. In contrast, the single dominant peak obtained in the present study indicates that the hydrated proliposomes possessed a relatively homogeneous size distribution. Such uniformity is desirable because large differences in vesicle dimensions can contribute to variations in drug entrapment, sedimentation behaviour and drug-release characteristics.

The small particle size may additionally provide a comparatively large surface area available for interaction with the surrounding dissolution medium. This characteristic can be particularly relevant for furosemide, which possesses poor aqueous solubility. Incorporation of the drug into phospholipid-based nanosized vesicles may improve its dispersion within the aqueous environment compared with the untreated crystalline drug. Nevertheless, particle-size analysis alone cannot establish an improvement in solubility or bioavailability, and these potential advantages should be interpreted together with the dissolution and drug-release findings.

It is also important to distinguish the particle size obtained from this analysis from the dimensions of the dry proliposomal structures observed by SEM. The SEM images represent the dried proliposomal carrier particles and their aggregates, which can occur on a micrometre scale. In contrast, the present particle-size distribution represents the vesicular dispersion produced after hydration of the proliposomal formulation. Consequently, the approximately 100 nm size obtained from particle-size analysis is not inconsistent with the larger structures visible in SEM examination.

1.3. Zeta Potential

The electrokinetic behaviour of the hydrated formulation was further evaluated through measurement of its zeta potential. The zeta-potential distribution exhibited a single prominent peak in the negative region, centered approximately around −20 to −25 mV, with the maximum intensity occurring near −22 mV. The negative value indicates that the hydrated proliposomal vesicles possessed an overall negative surface charge.

The negative surface potential may be associated primarily with the ionizable and polar components present at the phospholipid–aqueous interface. Soybean lecithin contains phospholipid molecules whose charged and polar groups can influence the electrical characteristics of the vesicular surface. Furosemide itself contains ionizable functional groups, and its association with the lipid bilayer or vesicular interface may additionally contribute to the observed surface charge under the measurement conditions.

Table 8: Zeta Potential of Furosemide Proliposomal Formulations

Formulation	Zeta Potential (mV)
F1	−16.4 ± 1.2
F2	−17.8 ± 1.1
F3	−18.6 ± 1.3
F4	−19.3 ± 1.0
F5	−20.5 ± 1.2
F6	−22.1 ± 1.0
F7	−21.2 ± 1.1
F8	−20.4 ± 1.2
F9	−19.6 ± 1.3

The magnitude of zeta potential provides useful information regarding the electrostatic interactions occurring between dispersed particles. The observed value of approximately −22 mV represents a moderate negative surface potential. This surface charge can generate electrostatic repulsive forces between neighboring vesicles and thereby help reduce their tendency toward close association and aggregation. Although values exceeding approximately

±30 mV are often associated with stronger purely electrostatic stabilization, systems exhibiting lower absolute zeta potentials may still demonstrate acceptable stability when additional stabilization mechanisms, including steric effects and lipid-bilayer organization, are present.

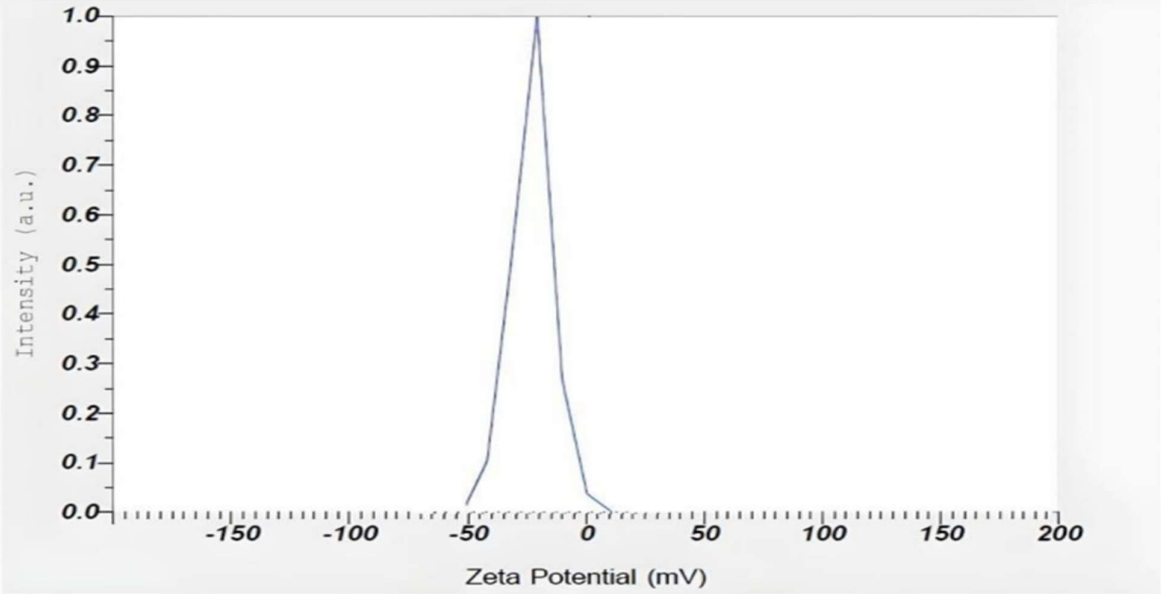

Figure 6: Zeta potential of furosemide proliposomes

In the developed formulation, cholesterol may also contribute indirectly to vesicular stability. Cholesterol becomes intercalated between phospholipid molecules within the bilayer and can reduce excessive membrane fluidity and permeability. This effect can strengthen the lipid membrane and reduce leakage of entrapped material. Therefore, the physical stability of the proliposomal vesicles should not be evaluated exclusively from the magnitude of zeta potential but rather from the combined contributions of surface charge, phospholipid organization and cholesterol-mediated bilayer stabilization.

The zeta-potential profile also displayed a single major negatively charged population rather than multiple widely separated peaks. This finding is consistent with the particle-size distribution, which similarly demonstrated one principal nanosized population. Together, these observations suggest that hydration of the optimized proliposomal formulation resulted in a relatively uniform population of negatively charged vesicles.

The combination of a particle size of approximately 90–100 nm and a zeta potential around −22 mV can therefore be regarded as a favorable physicochemical characteristic of the developed furosemide proliposomal formulation. The nanosized vesicles provide a high interfacial surface area and relatively uniform dispersion, whereas the negative surface charge contributes electrostatic repulsion that may assist in limiting vesicle aggregation.

Overall, the particle-size and zeta-potential studies demonstrated that the optimized furosemide proliposomes, following hydration, generated nanosized vesicles with a narrow unimodal size distribution and a moderately negative surface charge. The particle-size peak at approximately 90–100 nm indicated successful generation of nanoscale vesicles, while the zeta potential of approximately −22 mV suggested the presence of sufficient surface charge to contribute to dispersion stability. These findings, together with the observed spherical morphology in SEM analysis, support the successful development of a physically organized furosemide proliposomal drug-delivery system.

1.4. Drug Loading Capacity

The drug loading capacity of the prepared furosemide proliposomal formulations was determined to evaluate the ability of the developed lipid-based system to retain the incorporated drug.

Table 9: Drug loading capacity of furosemide proliposomal formulations

Formulation	Drug Loading Capacity (%)
F1	87.10 ± 0.82
F2	89.30 ± 0.74
F3	88.60 ± 0.69
F4	91.20 ± 0.65
F5	94.60 ± 0.58
F6	93.10 ± 0.71
F7	92.50 ± 0.62
F8	96.30 ± 0.51
F9	94.20 ± 0.67

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

The drug loading capacity of the prepared furosemide proliposomal formulations was found to range from 87.10 ± 0.82% to 96.30 ± 0.51%, demonstrating satisfactory incorporation and retention of furosemide within the developed proliposomal systems. Among the nine formulations, F1 exhibited the lowest drug loading capacity of 87.10 ± 0.82%, whereas F8 demonstrated the highest value of 96.30 ± 0.51%. The comparatively high drug loading observed across all formulations indicated that the selected lipid composition and preparation conditions were suitable for incorporation of furosemide.

A noticeable influence of soybean lecithin concentration was observed on drug loading. Formulations containing lower lecithin concentration generally exhibited comparatively lower drug loading, whereas increasing the lecithin content resulted in improvement in the amount of drug retained within the formulation. For example, at a cholesterol concentration of 20 mg, the drug loading increased from 89.30% in F2 to 94.60% in F5 and further to 96.30% in F8 as the soybean lecithin concentration was increased from 100 to 150 and 200 mg, respectively. This indicates that an increase in phospholipid concentration provided a greater lipidic environment for accommodation and retention of furosemide.

Soybean lecithin serves as the principal phospholipid component responsible for the formation of the lipid structures in the proliposomal system. Increasing its concentration may increase the availability of lipid domains with which furosemide can associate during formulation. Consequently, drug loss during processing may be reduced, resulting in higher drug loading. The improvement observed with increasing lecithin concentration therefore indicates the importance of an adequate phospholipid concentration for efficient drug incorporation.

Cholesterol also influenced drug loading. Increasing the cholesterol concentration from 10 to 20 mg generally improved drug loading at each level of soybean lecithin. The drug loading

increased from 87.10% in F1 to 89.30% in F2, from 91.20% in F4 to 94.60% in F5, and from 92.50% in F7 to 96.30% in F8. Cholesterol can become positioned between phospholipid molecules and improve lipid packing and membrane organization. An appropriate cholesterol concentration may therefore improve the structural integrity of the lipid system and facilitate retention of the incorporated drug.

However, increasing cholesterol further to 30 mg produced a slight reduction in drug loading. The values decreased from 89.30% in F2 to 88.60% in F3, from 94.60% in F5 to 93.10% in F6, and from 96.30% in F8 to 94.20% in F9. This suggests that an excessive amount of cholesterol did not provide any additional advantage for drug incorporation. At higher concentrations, cholesterol may produce tighter packing of phospholipid molecules or occupy regions within the lipid structure that could otherwise accommodate drug molecules, thereby causing a slight reduction in drug loading.

Among all formulations, F8 exhibited the maximum drug loading capacity of 96.30 ± 0.51%. F8 contained the highest investigated soybean lecithin concentration together with an intermediate cholesterol concentration, suggesting that this combination provided a favorable balance between availability of phospholipid and stabilization by cholesterol. In contrast, F1, containing comparatively lower amounts of lipid components, demonstrated the minimum drug loading capacity.

The relatively low standard deviation associated with the measurements indicates good reproducibility and suggests uniform distribution of furosemide within the proliposomal preparations. Uniform drug incorporation is particularly important for ensuring consistency of dose and reproducibility of formulation performance.

1.5. Entrapment Efficiency

Entrapment efficiency is an important characteristic of proliposomal formulations as it represents the proportion of drug successfully associated with the vesicular system following hydration. The entrapment efficiency of the prepared furosemide proliposomal formulations was determined indirectly by measuring the concentration of unentrapped furosemide present in the supernatant following centrifugation.

Table 10: Entrapment efficiency of furosemide proliposomal formulations

Formulation	Entrapment Efficiency (%)
F1	72.48 ± 0.86
F2	76.35 ± 0.73
F3	74.62 ± 0.81
F4	79.84 ± 0.69
F5	85.27 ± 0.64
F6	82.91 ± 0.76
F7	84.16 ± 0.61
F8	91.72 ± 0.55
F9	87.63 ± 0.68

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

The entrapment efficiency of the developed furosemide proliposomal formulations ranged from 72.48 ± 0.86% to 91.72 ± 0.55%. All formulations demonstrated substantial incorporation of furosemide into the lipid vesicular system, although clear differences were observed according to the concentrations of soybean lecithin and cholesterol. Among the nine formulations, F8 exhibited the highest entrapment efficiency of 91.72 ± 0.55%, whereas F1 showed the lowest value of 72.48 ± 0.86%.

An increase in soybean lecithin concentration generally resulted in an improvement in entrapment efficiency. At 10 mg cholesterol, the entrapment efficiency increased from 72.48% for F1 to 79.84% for F4 and 84.16% for F7 as the soybean lecithin concentration increased from 100 to 150 and 200 mg, respectively. A similar trend was observed at 20 mg cholesterol, where the entrapment efficiency increased from 76.35% for F2 to 85.27% for F5 and finally to 91.72% for F8. These findings indicate that increasing the phospholipid concentration had a favorable effect on the entrapment of furosemide.

The improvement in entrapment efficiency with increasing soybean lecithin may be attributed to the greater availability of phospholipid molecules for vesicle formation. During hydration, phospholipids organize into bilayer structures, providing lipid domains with which the drug can associate. An increased quantity of phospholipid may therefore provide a greater effective bilayer volume and surface area for drug incorporation, thereby decreasing the proportion of free drug remaining in the external aqueous phase.

The effect of cholesterol was also clearly evident. At each lecithin concentration, increasing cholesterol from 10 to 20 mg improved entrapment efficiency. At 100 mg lecithin, the value increased from 72.48% in F1 to 76.35% in F2. Similarly, it increased from 79.84% in F4 to 85.27% in F5 at 150 mg lecithin and from 84.16% in F7 to 91.72% in F8 at 200 mg lecithin. These findings indicate that an appropriate concentration of cholesterol contributed positively to drug retention within the vesicular system.

Cholesterol is incorporated between phospholipid molecules within the lipid bilayer and can regulate membrane organization, fluidity and permeability. At an appropriate concentration, cholesterol may increase the rigidity of the lipid bilayer and decrease leakage of incorporated drug from the vesicles. This effect could explain the improved entrapment efficiency observed when the cholesterol concentration was increased from 10 to 20 mg.

However, further increasing cholesterol to 30 mg resulted in a moderate reduction in entrapment efficiency. At 100 mg lecithin, entrapment decreased from 76.35% in F2 to 74.62% in F3. Likewise, the value decreased from 85.27% in F5 to 82.91% in F6 and from 91.72% in F8 to 87.63% in F9. Therefore, increasing cholesterol beyond an optimum level did not further enhance furosemide entrapment.

The reduction observed at the highest cholesterol concentration may be related to excessive cholesterol producing tighter packing of the phospholipid bilayer. Cholesterol molecules can occupy spaces between phospholipid molecules and, when present in excessive amounts, may reduce the available regions within the lipid bilayer for incorporation of drug molecules. Thus, while a moderate cholesterol concentration can stabilize the vesicular membrane and reduce drug leakage, an excessive concentration may restrict drug accommodation within the lipid structure.

The highest entrapment efficiency was observed for F8 (91.72 ± 0.55%), containing 200 mg soybean lecithin and 20 mg cholesterol. This composition appears to provide an optimum balance between the amount of phospholipid available for vesicle formation and the membrane-stabilizing effect of cholesterol. The high entrapment efficiency of F8 indicates that only a relatively small proportion of furosemide remained unentrapped in the external phase following vesicle formation.

The results also correspond well with the previously observed drug-loading behaviour, where F8 demonstrated the highest drug loading among the investigated formulations. The simultaneous occurrence of high drug loading and high entrapment efficiency suggests that the lipid composition of F8 was comparatively effective both in retaining furosemide during proliposome preparation and in incorporating the drug within the vesicular system following hydration.

The relatively low standard deviations observed for all formulations indicate good reproducibility of the entrapment-efficiency measurements. This suggests consistent vesicle formation and drug incorporation among replicate samples and supports the reliability of the preparation procedure.

1.6. In Vitro Drug Release Studies

The in vitro drug release behavior of furosemide from the prepared proliposomal formulations (F1–F9) was evaluated by the dialysis bag diffusion method using phosphate buffer pH 7.4 as the release medium. The study was conducted at 37 ± 0.5°C for 48 h, and the cumulative percentage drug release was determined at predetermined time intervals.

Table 11: Cumulative percentage drug release from furosemide proliposomal formulations

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	13.42 ± 0.54	11.85 ± 0.47	9.64 ± 0.39	11.26 ± 0.43	9.18 ± 0.36	7.83 ± 0.31	9.72 ± 0.38	7.14 ± 0.29	6.31 ± 0.26
2	21.36 ± 0.68	19.42 ± 0.61	16.53 ± 0.52	18.67 ± 0.58	15.84 ± 0.49	13.72 ± 0.44	16.15 ± 0.51	12.46 ± 0.41	10.93 ± 0.37
4	32.74 ± 0.76	29.85 ± 0.71	25.61 ± 0.64	28.36 ± 0.69	24.52 ± 0.61	21.48 ± 0.56	24.67 ± 0.63	19.84 ± 0.52	17.56 ± 0.48
6	41.82 ± 0.89	38.21 ± 0.82	33.74 ± 0.76	36.59 ± 0.79	31.76 ± 0.72	28.93 ± 0.67	32.14 ± 0.73	26.38 ± 0.61	23.42 ± 0.57
8	50.63 ± 0.96	46.52 ± 0.91	41.18 ± 0.84	44.27 ± 0.87	38.95 ± 0.81	35.64 ± 0.76	39.76 ± 0.82	32.85 ± 0.69	29.91 ± 0.64

12	62.18 ± 1.08	58.43 ± 1.02	52.74 ± 0.96	55.86 ± 0.98	49.82 ± 0.91	46.13 ± 0.87	50.91 ± 0.93	42.76 ± 0.78	39.28 ± 0.74
18	72.36 ± 1.14	68.94 ± 1.09	63.52 ± 1.03	66.81 ± 1.06	60.73 ± 0.98	57.49 ± 0.94	61.85 ± 1.01	53.94 ± 0.86	50.16 ± 0.82
24	80.47 ± 1.21	77.16 ± 1.16	72.64 ± 1.11	74.82 ± 1.13	69.35 ± 1.06	66.28 ± 1.02	70.16 ± 1.08	63.87 ± 0.94	59.74 ± 0.89

The in vitro drug release study demonstrated that all nine furosemide proliposomal formulations exhibited a gradual and prolonged release pattern over the 48 h experimental period. Considerable differences in the release profiles were observed among F1–F9, indicating that variation in the concentrations of soybean lecithin and cholesterol influenced the release of furosemide from the hydrated proliposomal vesicles.

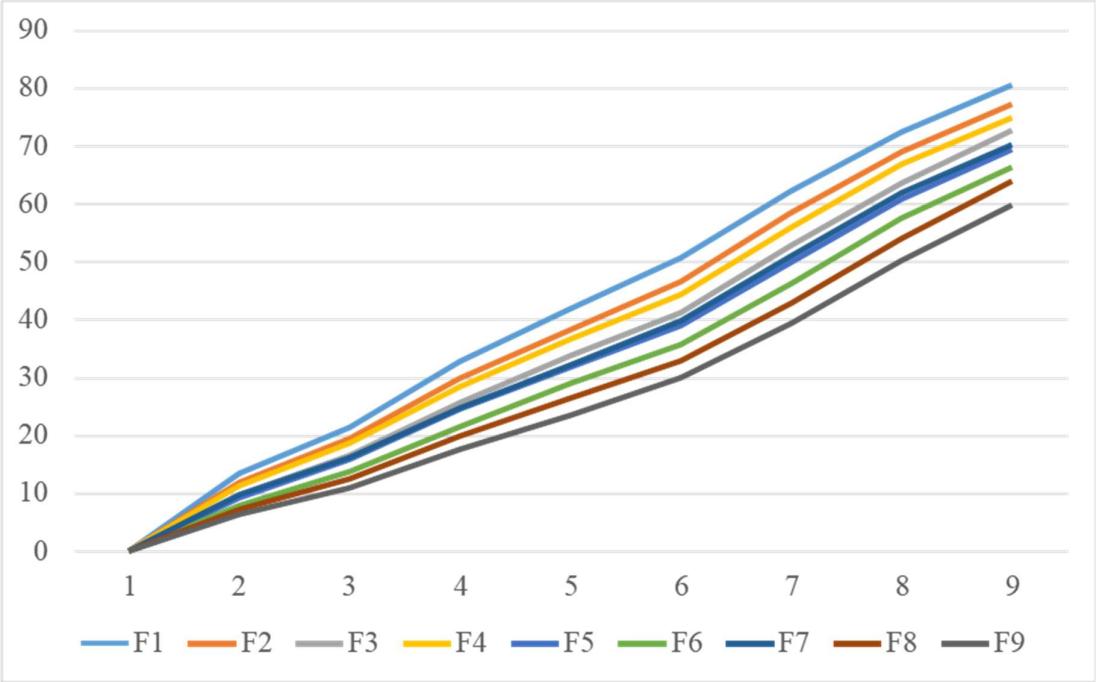

Figure 7: In Vitro Drug Release of Furosemide proliposomes

During the initial phase of the study, a relatively faster release was observed from formulations containing lower concentrations of lipid components. At 1 h, cumulative drug release ranged from 6.31% to 13.42%. F1 showed the highest initial release of 13.42%, whereas F9 showed the lowest release of 6.31%. The initial release may be attributed to furosemide located near the vesicular surface or weakly associated with the outer lipid regions, which could diffuse

relatively rapidly into the surrounding phosphate buffer.Importantly, none of the formulations demonstrated an excessively high initial burst. The initial release remained below approximately 14% during the first hour for all formulations. This finding suggests that a substantial proportion of the drug remained associated with the lipid vesicles rather than being present as freely dissolved drug in the external phase. This observation is also consistent with the relatively high entrapment efficiencies obtained for the developed formulations.

As the study progressed, drug release occurred in a gradual manner. At 12 h, cumulative release ranged from 39.28% to 62.18%, while at 24 h it ranged from 59.74% to 80.47%. These findings demonstrate that the proliposomal formulations were capable of extending furosemide release substantially beyond the initial hydration period.

A clear effect of soybean lecithin concentration was observed. At the same cholesterol level, increasing soybean lecithin generally reduced the rate of drug release. For example, at 20 mg cholesterol, the cumulative release after 24 h decreased from 77.16% for F2 to 69.35% for F5 and 63.87% for F8 as soybean lecithin increased from 100 to 150 and 200 mg, respectively. This trend suggests that increasing phospholipid concentration resulted in the formation of a more substantial lipid barrier surrounding the incorporated drug.

Soybean lecithin constitutes the major bilayer-forming material in the developed proliposomal system. Increasing the amount of phospholipid can increase the thickness or amount of lipid material through which incorporated drug must partition and diffuse before reaching the external aqueous medium. Consequently, formulations containing higher phospholipid concentrations may exhibit slower drug diffusion and more prolonged release.

The influence of cholesterol was also evident. At a constant soybean lecithin concentration, increasing cholesterol generally reduced the rate of furosemide release. For example, among formulations containing 200 mg soybean lecithin, the 24 h release decreased from 70.16% for F7 to 63.87% for F8 and 59.74% for F9 as cholesterol increased from 10 to 20 and 30 mg.

This reduction in drug-release rate with increasing cholesterol can be explained by the role of cholesterol in lipid bilayers. Cholesterol becomes positioned between phospholipid molecules and can increase bilayer packing and rigidity while reducing membrane permeability. Consequently, diffusion of incorporated furosemide through the lipid membrane may become

slower as cholesterol concentration increases. The findings therefore indicate that cholesterol acted as an important regulator of release from the proliposomal vesicles.

At the end of 48 h, cumulative drug release ranged from 86.43% to 97.82%. F1 showed the fastest overall release, reaching 97.82%, whereas the formulation containing the highest lipid and cholesterol combination, F9, demonstrated the slowest release, reaching 86.43%. These results further demonstrate the ability to modulate furosemide release by altering the relative quantities of phospholipid and cholesterol.

Among the investigated formulations, F8 showed a particularly favorable release profile, with cumulative drug release values of 7.14%, 12.46%, 19.84%, 26.38%, 32.85%, 42.76%, 53.94%, 63.87%, 77.46%, and 89.72% at 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h, respectively. Thus, less than one-third of the drug was released during the first 8 h, approximately 64% was released by 24 h, and approximately 90% was released by 48 h. This profile demonstrates sustained drug release without an excessive initial burst.

Although F9 produced slightly slower release than F8, slower release alone does not necessarily indicate a superior formulation. F8 previously demonstrated the highest drug loading capacity (96.30 ± 0.51%) and highest entrapment efficiency (91.72 ± 0.55%). Therefore, its release profile represents a favorable compromise between efficient drug incorporation and prolonged drug release. In contrast, further increasing cholesterol in F9 reduced entrapment efficiency while also slowing drug release.

The sustained release obtained from F8 may consequently be attributed to the combined influence of its 200 mg soybean lecithin and 20 mg cholesterol composition. The relatively high phospholipid concentration provided an adequate lipid matrix for incorporation of furosemide, while cholesterol contributed to bilayer organization and reduced permeability. This combination appears to have provided sufficient membrane integrity to retard drug diffusion without excessively restricting drug release.

The release pattern can conceptually be divided into an initial phase followed by a prolonged diffusion-controlled phase. During the early stage, drug associated with superficial or readily accessible regions of the vesicles may diffuse into the release medium. Subsequently, furosemide incorporated more deeply within or associated with the lipid bilayers must partition

through the organized lipid structure before entering the aqueous medium, resulting in comparatively slower and sustained release.

It should also be considered that the dialysis membrane itself represents an additional diffusion barrier. Therefore, the measured profile represents the overall appearance of drug in the receptor medium resulting from release from the vesicular formulation followed by diffusion across the dialysis membrane. Maintaining sink conditions through periodic withdrawal and replacement of release medium minimizes drug accumulation in the receptor compartment and supports continued diffusion throughout the experiment.

SUMMARY AND CONCLUSION

- The present study was undertaken to formulate and evaluate furosemide-loaded proliposomes as a lipid-based drug delivery system. Nine formulations (F1–F9) were developed by varying soybean lecithin (100–200 mg) and cholesterol (10–30 mg), while maintaining furosemide at 50 mg in each formulation.
- Preformulation studies showed furosemide as a white to slightly yellow crystalline powder with poor aqueous solubility. The drug exhibited better solubility in organic solvents, particularly the chloroform:methanol system, supporting its suitability for incorporation into the lipid-based proliposomal formulation.
- UV-visible spectrophotometric analysis of furosemide in phosphate buffer pH 7.4 demonstrated a distinct absorption maximum (λmax) at 274 nm. This wavelength was subsequently employed for quantitative determination of furosemide during formulation evaluation.
- The standard calibration curve demonstrated a linear increase in absorbance with increasing furosemide concentration over the investigated range of 5–40 µg/mL. The regression equation was $y = 0.0204x + 0.0254$, with an R^2 value of 0.9918, indicating excellent linearity.
- The nine proliposomal formulations were systematically prepared using soybean lecithin and cholesterol as the major lipid components and D-mannitol as the carrier. Variation in lipid composition produced measurable differences in drug loading, entrapment efficiency and in vitro drug-release characteristics.
- SEM examination demonstrated that the developed proliposomes possessed a predominantly rounded to nearly spherical particulate morphology with relatively

well-defined boundaries. Some localized aggregation and surface roughness were observed without extensive fusion of the particles.

- Particle-size analysis of the hydrated optimized proliposomes demonstrated a narrow and predominantly unimodal nanosized distribution, with the principal population occurring at approximately 90–100 nm. This confirmed the ability of the proliposomal system to generate nanosized vesicles following hydration.
- Zeta-potential analysis demonstrated a negative surface charge of approximately −22 mV for the optimized formulation. The negative surface potential provides electrostatic repulsion between vesicles and may contribute to reducing aggregation and maintaining dispersion stability.
- The drug loading capacity of F1–F9 ranged from 87.10 ± 0.82% to 96.30 ± 0.51%, demonstrating satisfactory incorporation of furosemide. Increasing soybean lecithin generally improved drug loading, while an intermediate cholesterol concentration produced the most favorable results.
- Among all formulations, F8 exhibited the highest drug loading capacity of 96.30 ± 0.51%. The combination of 200 mg soybean lecithin and 20 mg cholesterol in F8 appeared to provide an appropriate lipid environment for efficient incorporation and retention of furosemide.
- Entrapment efficiency ranged from 72.48 ± 0.86% to 91.72 ± 0.55% across the nine formulations. Increasing soybean lecithin generally enhanced drug entrapment, whereas increasing cholesterol from 20 to 30 mg produced a slight reduction, indicating the existence of an optimum cholesterol concentration.
- F8 demonstrated the maximum entrapment efficiency of 91.72 ± 0.55%, supporting the effectiveness of its lipid composition. The high drug loading and entrapment efficiency obtained simultaneously for F8 indicated favorable incorporation and retention of furosemide within the proliposomal system.
- The in vitro drug-release study demonstrated prolonged release of furosemide for up to 48 h from all developed formulations. Increasing soybean lecithin and cholesterol generally slowed drug release, indicating that lipid composition played an important role in controlling diffusion of the drug from the vesicular system.
- F8 demonstrated a favorable sustained-release profile, with cumulative drug release increasing from 7.14 ± 0.29% at 1 h to 63.87 ± 0.94% at 24 h and 89.72 ± 1.08% at 48 h. The gradual release with limited initial burst demonstrated effective retardation of drug diffusion by the lipid vesicular system.

- Based on the collective findings, F8 was selected as the optimized furosemide proliposomal formulation, exhibiting high drug loading, maximum entrapment efficiency, nanosized vesicles, negative surface charge and sustained drug release over 48 h. The study therefore supports proliposomes as a promising approach for improving the formulation characteristics and providing prolonged delivery of poorly water-soluble furosemide.

REFERENCES

1. Jain KK. Drug delivery systems-an overview. Drug delivery systems. 2008 Mar 7:1-50.
2. Jain KK. An overview of drug delivery systems. Drug delivery systems. 2019 Aug 22:1-54.
3. Rosen H, Abribat T. The rise and rise of drug delivery. Nature Reviews Drug Discovery. 2005 May 1;4(5):381-5.
4. Kumar M, Mandal UK, Mahmood S. Novel drug delivery system. InAdvanced and Modern Approaches for Drug Delivery 2023 Jan 1 (pp. 1-32). Academic Press.
5. Chen Q, Yang Z, Liu H, Man J, Oladejo AO, Ibrahim S, Wang S, Hao B. Novel drug delivery systems: an important direction for drug innovation research and development. Pharmaceutics. 2024 May 16;16(5):674.
6. Faheem AM, Abdelkader DH. Novel drug delivery systems. InEngineering drug delivery systems 2020 Jan 1 (pp. 1-16). Woodhead Publishing.
7. Adepu S, Ramakrishna S. Controlled drug delivery systems: current status and future directions. Molecules. 2021 Sep 29;26(19):5905.
8. Hamidi M, Tabatabaei MS, Moslehi M, Barati M. Introduction to novel drug delivery systems. Novel Drug Delivery Systems: Fundamentals and Applications. 2025 Jan 13:1-34.
9. Martinho N, Damgé C, Reis CP. Recent advances in drug delivery systems. Journal of biomaterials and nanobiotechnology. 2011 Dec 1;2:510.
10. Chen Q, Yang Z, Liu H, Man J, Oladejo AO, Ibrahim S, Wang S, Hao B. Novel drug delivery systems: an important direction for drug innovation research and development. Pharmaceutics. 2024 May 16;16(5):674.

11. Gbian DL, Omri A. Lipid-based drug delivery systems for diseases managements. Biomedicines. 2022 Aug 31;10(9):2137.
12. Chime SA, Onyishi IV. Lipid-based drug delivery systems (LDDS): Recent advances and applications of lipids in drug delivery. Afr. J. Pharm. Pharmacol. 2013 Dec 29;7(48):3034-59.
13. Zaro JL. Lipid-based drug carriers for prodrugs to enhance drug delivery. The AAPS journal. 2015 Jan;17(1):83-92.
14. New RR, editor. Liposomes. IRL at Oxford University Press; 1990.
15. Jesorka A, Orwar O. Liposomes: technologies and analytical applications. Annu. Rev. Anal. Chem.. 2008 Jul 19;1(1):801-32.
16. Umarji B, Patil R, Birajdar R, Mysore S, Bilagi S, Audurti D. Formulation and *in vitro* evaluation of mucoadhesive buccal tablets of furosemide. World J Pharm Sci. 2012 Aug 15;1(3):1041-63.
17. Karkhile VG, Karmarkar RR, Sontakke MA, Badgujar SD, Nemade LS. Formulation and evaluation of floating tablets of furosemide. Int. J. Pharm. Res. Dev. 2010;1:1-9.
18. Bobbala SK, Veerareddy PR. Formulation, evaluation, and pharmacokinetics of isradipine proliposomes for oral delivery. Journal of liposome research. 2012 Dec 1;22(4):285-94.
19. Agyralides GG, Dallas PP, Rekkas DM. Development and *In vitro* evaluation of furosemide transdermal formulations using experimental design techniques. International journal of pharmaceutics. 2004 Aug 20;281(1-2):35-43.
20. Santus G, Lazzarini C, Bottoni G, Sandefer EP, Page RC, Doll WJ, Ryo UY, Digenis GA. An *In vitro*-in vivo investigation of oral bioadhesive controlled release furosemide formulations. European journal of pharmaceutics and biopharmaceutics. 1997 Jul 1;44(1):39-52.