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FORMULATION AND EVALUATION OF POLIHEXANIDE LOADED LIPOSOSMAL GEL FOR THE TREATMENT OF DIABETIC FOOT ULCER

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ABSTRACT

Diabetic foot ulcers (DFU) are severe complications of diabetes that often result in delayed wound healing, persistent infection, and an increased risk of lower-limb amputation. Conventional topical therapies frequently fail because they lack adequate drug retention and sustained antimicrobial activity at the wound site. To address these limitations, this study formulated and evaluated a polihexanide-loaded liposomal gel using soya lecithin, cholesterol, and a Carbopol 934 hydrogel base. Five liposomal formulations were prepared via the thin-film hydration method, with the optimized formulation (F5) demonstrating a particle size of 93.26 nm, a polydispersity index of 0.342, and an entrapment efficiency of 92.24%. Scanning electron microscopy confirmed that the vesicles were spherical with a smooth surface morphology. The resulting liposomal gel exhibited favorable physicochemical properties, including a pH of 6.12, a viscosity of 6513 cps, and good spreadability, while showing no signs of skin irritation. Furthermore, the formulation achieved sustained drug release with 97.89% cumulative release over 12 hours, following the Higuchi kinetic model ($R^2 = 0.9778$) to indicate a diffusion-controlled mechanism. Overall, the polihexanide-loaded liposomal gel represents a promising topical drug delivery system capable of providing prolonged antimicrobial action for improved diabetic wound management.

KEYWORDS: Polihexanide, Liposomes, Liposomal Gel, Diabetic Foot Ulcer, Nanotechnology, Controlled Drug Release, Wound Healing.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The prevalence of diabetes has increased rapidly worldwide and has become a major public health concern. Long-standing diabetes is associated with several complications, among which diabetic foot ulcer (DFU) is considered one of the most serious because of its high morbidity, prolonged hospitalization, risk of infection, and lower-limb amputation.

Diabetic foot ulcer is a chronic, non-healing wound occurring below the ankle in patients with diabetes. It develops mainly due to peripheral neuropathy, peripheral arterial disease, repeated trauma, infection, and impaired wound healing. Approximately 15–25% of diabetic patients develop a foot ulcer during their lifetime, and DFU is a leading cause of non-traumatic lower-extremity amputation. Persistent hyperglycemia causes oxidative stress, endothelial dysfunction, chronic inflammation, impaired angiogenesis, and reduced collagen synthesis, all of which delay wound healing. In addition, bacterial colonization and biofilm formation further complicate treatment by reducing the effectiveness of conventional antimicrobial therapy. Current treatment strategies include glycemic control, wound debridement, antibiotic therapy, antiseptics, wound dressings, pressure off-loading, and negative pressure wound therapy. However, conventional topical formulations such as creams, ointments, and solutions often exhibit poor penetration into the wound bed, limited residence time, and inadequate sustained drug release.

Polihexanide (polyhexamethylene biguanide, PHMB) is a broad-spectrum antimicrobial agent widely used in chronic wound management. It is effective against Gram-positive bacteria, Gram-negative bacteria, fungi, and biofilm-forming microorganisms. PHMB exhibits excellent tissue compatibility, low cytotoxicity, and a low tendency to induce microbial resistance, making it suitable for long-term wound care. Nanotechnology-based drug delivery systems have emerged as promising alternatives for chronic wound management. Liposomes are phospholipid vesicles capable of encapsulating both hydrophilic and lipophilic drugs. They improve drug stability, enhance skin penetration, provide localized delivery, and offer controlled and sustained drug release. Incorporation of drug-loaded liposomes into hydrogel systems further improves wound hydration, viscosity, spreadability,

and residence time at the application site.

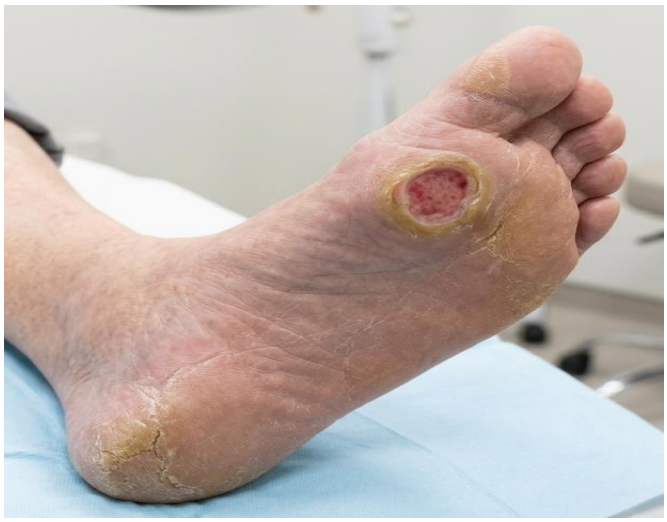

Figure 1: Diabetic foot ulcer.

1.1 Etiology of Diabetic Foot Ulcer (DFU)

Diabetic foot ulcer (DFU) develops due to multiple factors associated with long-standing diabetes mellitus. The primary causes include peripheral neuropathy, peripheral arterial disease, repetitive foot trauma, foot deformities, infection, and uncontrolled hyperglycemia. Peripheral neuropathy reduces pain sensation, allowing minor injuries to remain unnoticed and progress into chronic ulcers. Peripheral arterial disease decreases blood flow to the lower extremities, resulting in inadequate oxygen and nutrient supply required for tissue repair. Persistent hyperglycemia impairs immune function, collagen synthesis, and angiogenesis, thereby delaying wound healing. Microbial colonization and biofilm formation further complicate the healing process by increasing the risk of chronic infection and tissue destruction.

1.2 Management of Diabetic Foot Ulcer (DFU)

Management of diabetic foot ulcer requires a multidisciplinary approach aimed at promoting wound healing, controlling infection, and preventing amputation. The major components include strict glycemic control, regular wound assessment, surgical debridement of necrotic tissue, infection management using appropriate antimicrobial agents, pressure off-loading with specialized footwear or casts, vascular assessment, and patient education. Advanced wound dressings and topical drug delivery systems are increasingly used to maintain a moist wound environment and accelerate tissue regeneration. Early diagnosis and timely intervention significantly improve healing outcomes and reduce complications.

1.3 Prevention and Treatment of Diabetic Foot Ulcers

Prevention is the most effective strategy for reducing the incidence of diabetic foot ulcers. Regular foot examination, proper foot hygiene, appropriate footwear, daily self-inspection, smoking cessation, and optimal blood glucose control play important roles in preventing ulcer formation. Once an ulcer develops, treatment includes wound cleansing, debridement, infection control, moisture-balanced dressings, pressure off-loading, and advanced therapies such as growth factors, negative pressure wound therapy, skin substitutes, and nanotechnology-based drug delivery systems. These approaches help accelerate wound closure and minimize the risk of lower-limb amputation.

1.4 Polyhexanide (PHMB)

Polyhexanide, also known as polyhexamethylene biguanide (PHMB), is a broad-spectrum antimicrobial polymer widely used in modern wound care. It exhibits potent activity against Gram-positive and Gram-negative bacteria, fungi, and biofilm-forming microorganisms while demonstrating excellent tissue compatibility and low cytotoxicity. PHMB acts by disrupting the microbial cell membrane, leading to leakage of intracellular components and subsequent cell death. Due to its low potential for antimicrobial resistance and favorable safety profile, PHMB is extensively incorporated into wound irrigation solutions, dressings, hydrogels, and liposomal formulations for the treatment of chronic wounds, including diabetic foot ulcers. Its sustained antimicrobial activity and ability to support a moist wound environment make it a promising therapeutic agent for advanced wound-healing applications.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

All chemicals and reagents used in this study were of analytical grade and were used without further purification. Polihexanide was obtained as a gift sample from Natural Remedies, India. Soya lecithin and cholesterol were procured from Sigma-Aldrich. Carbopol 934, triethanolamine, propylene glycol, methyl paraben, methanol, acetone, disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from standard commercial suppliers.

Table 1: Chemicals Used in the Study.

S. No.	Chemicals/Reagents	Company
1	Polihexanide	Natural Remedies (Gift Sample)
2	Soya Lecithin	Sigma-Aldrich
3	Cholesterol	Sigma-Aldrich
4	Acetone	Rankem
5	Carbopol 934	Sulab
6	Triethanolamine	Loba Chemie
7	Propylene Glycol	Merck
8	Methyl Paraben	Merck
9	Disodium Hydrogen Phosphate	Merck
10	Potassium Dihydrogen Phosphate	Merck
11	Sodium Chloride	Merck
12	Methanol	Rankem

2.1.2 Glassware

The laboratory glassware used throughout the study was thoroughly cleaned, dried, and calibrated before use.

Table 2: Glassware Used.

S. No.	Glassware	Company
1	Test Tubes	Borosil
2	Petri Dishes	Borosil
3	Glass Rod	Borosil
4	Beaker	Borosil
5	Conical Flask	Borosil
6	Measuring Cylinder	Borosil
7	Glass Slide	Borosil
8	Volumetric Flask	Borosil

2.1.3 Instruments

The analytical instruments employed during the study are listed below.

Table 3: Instruments Used.

S.No.	Instrument	Model
1	Boiling Water Bath	Universal
2	Magnetic Stirrer	Remi
3	Analytical Balance	Sirtech
4	UV–Visible Spectrophotometer	Systronic 2202
5	Vortex Shaker	Scientech SE-146
6	Melting Point Apparatus	Tempo
7	FTIR Spectrophotometer	Perkin Elmer Spectrum BX
8	Hot Air Oven	Scientech
9	Lyophilizer	Laborax
10	Zetasizer	Malvern Instruments

11	Scanning Electron Microscope	Hitachi High-Tech
12	Digital pH Meter	EI
13	Brookfield Viscometer	DV-II+ Pro (LV)
14	Dialysis Bag	Sigma-Aldrich
15	Desiccator	Polycab

2.2 Preformulation Studies

Preformulation studies were performed to evaluate the physicochemical properties of polihexanide before formulation development. The studies included organoleptic evaluation, solubility determination, pH measurement, melting point determination, UV spectrophotometric analysis, and Fourier Transform Infrared (FTIR) spectroscopy.

2.2.1 Organoleptic Evaluation

The organoleptic properties of polihexanide, including colour, odour, appearance, and physical state, were evaluated by visual inspection.

Figure 2: Organoleptic characteristics of pure polihexanide.

2.2.2 Solubility Study

The qualitative solubility of polihexanide was determined in methanol, ethanol, distilled water, dimethyl sulfoxide (DMSO), and chloroform according to USP guidelines. Approximately 1 mg of drug was added separately to each solvent, mixed thoroughly, and observed for solubility.

2.2.3 pH Determination

The pH of polihexanide solution was measured using a calibrated digital pH meter. Approximately 2 mg of drug was dissolved in 10 mL of distilled water, and the pH was recorded at room temperature.

2.2.4 Melting Point Determination

The melting point of polihexanide was determined by the open capillary method using a Thiele's tube apparatus. The temperature at which the drug completely melted was recorded.

2.2.5 UV Spectrophotometric Method Development

2.2.5.1 Preparation of Standard Stock Solution

A standard stock solution (1000 µg/mL) of polihexanide was prepared in methanol and further diluted to obtain a working standard solution (100 µg/mL) for analytical studies.

2.2.5.2 Determination of λmax

CA 10 µg/mL working solution was scanned over the wavelength range of **200–800 nm** using a UV–Visible spectrophotometer with methanol as blank. The wavelength corresponding to maximum absorbance (λmax) was selected for further analysis.

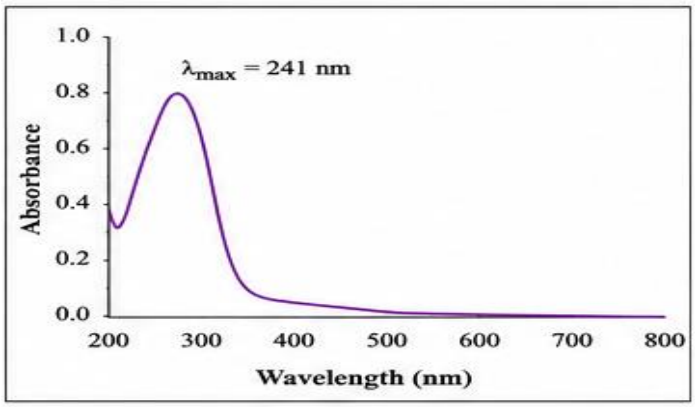

Figure 3: UV absorption spectrum of polihexanide.

2.2.5.3 Calibration Curve

Standard solutions containing **5–30 µg/mL** of polihexanide were prepared from the working stock solution. The absorbance of each solution was recorded at **241 nm**, and a calibration curve was constructed by plotting concentration against absorbance.

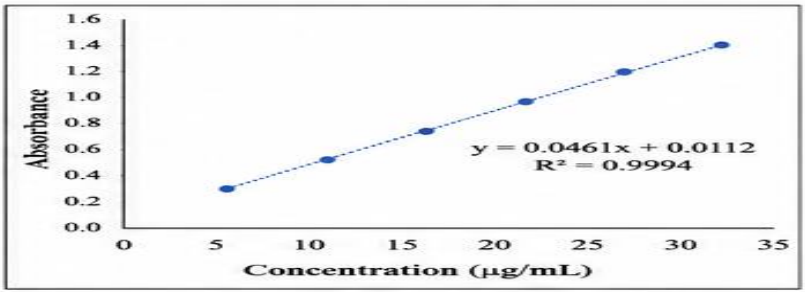

Figure 4: Calibration curve of polihexanide.

2.2.5.4 Precision Study

The developed analytical method was evaluated for precision by performing both intra-day and inter-day studies. The absorbance values were measured at fixed concentrations, and the percentage relative standard deviation (%RSD) was calculated.

2.2.5.5 Ruggedness Study

Ruggedness was evaluated by performing the analysis using two different analysts under identical experimental conditions, and the results were compared.

2.2.5.6 Robustness Study

Robustness of the analytical method was assessed by introducing small deliberate variations in analytical conditions and observing their effect on absorbance.

2.2.6 Fourier Transform Infrared (FTIR) Spectroscopy

Drug–excipient compatibility was evaluated using FTIR spectroscopy by the potassium bromide (KBr) pellet method. Spectra were recorded over the range of **4000–400 cm^{-1}**, and characteristic functional groups of the drug and optimized formulation were compared.

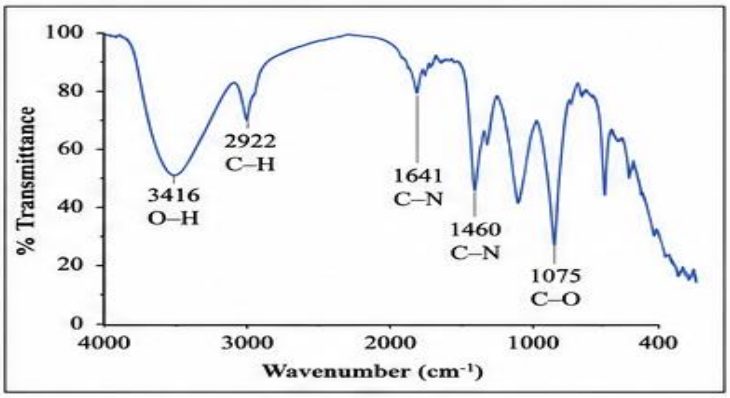

Figure 4: FTIR spectrum of pure polihexanide.

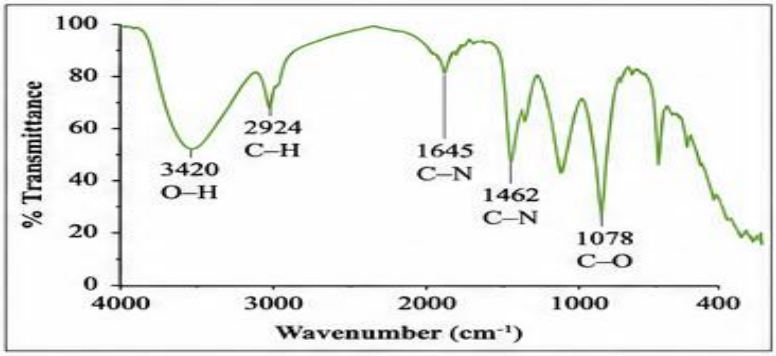

Figure 5: FTIR spectrum of optimized liposomal formulation.

2.3 Preparation of Polihexanide-Loaded Liposomes

Polihexanide-loaded liposomes were prepared by the thin-film hydration method. Soya lecithin and cholesterol were dissolved in a chloroform:methanol (1:1) mixture, followed by the addition of polihexanide. The solvent was removed using a rotary evaporator to form a thin lipid film. The film was hydrated with phosphate buffer (pH 7.4), vortexed for 15 min, and sonicated for different time intervals to obtain nanosized liposomes.

Table 4: Composition of Polihexanide-Loaded Liposomes.

Ingredients	F1	F2	F3	F4	F5
Polihexanide (mg)	100	100	100	100	100
Soya Lecithin (mg)	100	150	200	250	300
Cholesterol (mg)	300	250	200	150	100
Vortex Time (min)	15	15	15	15	15
Sonication Time (min)	20	30	40	50	60

2.4 Evaluation of Polihexanide-Loaded Liposomes

2.4.1 Particle Size Analysis

The particle size and polydispersity index (PDI) of the prepared liposomes were determined using a Malvern Zetasizer at **25°C** after suitable dilution with distilled water.

2.4.2 Entrapment Efficiency

Entrapment efficiency was determined by ultracentrifugation at **15,000 rpm for 30 min**. The amount of free drug present in the supernatant was estimated using a UV–Visible spectrophotometer, and the percentage entrapment efficiency was calculated.

$$\text{Entrapment efficiency \%} = \frac{\text{Total Drug} - \text{Free Drug}}{\text{Total Drug}} \text{ X } 100$$

2.4.3 Scanning Electron Microscopy (SEM)

The optimized liposomal formulation was coated with a thin layer of gold and examined under a scanning electron microscope to evaluate the surface morphology and vesicle shape.

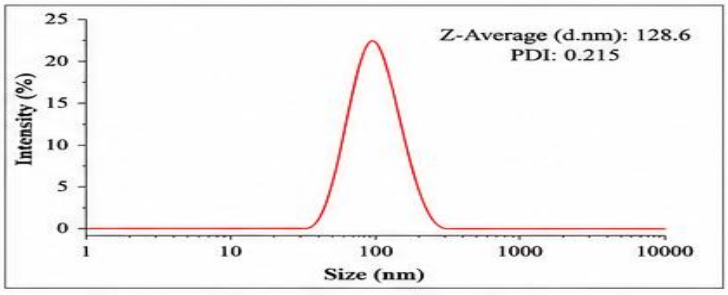

Figure 6: Particle size distribution of optimized liposomes.

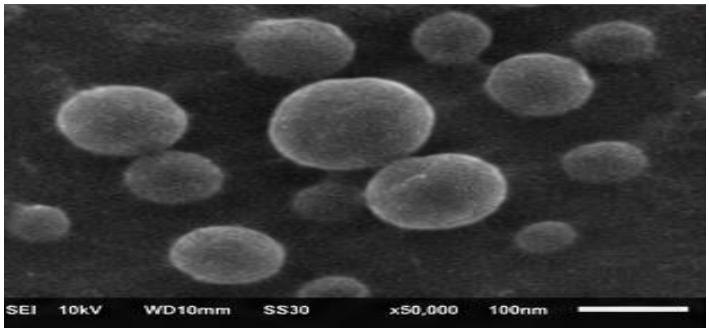

Figure 7: SEM image of optimized liposomes.

2.5 Preparation of Polihexanide-Loaded Liposomal Gel

The optimized liposomal dispersion was incorporated into a Carbopol 934 gel base. Carbopol 934 was hydrated in warm distilled water, followed by the addition of carboxymethyl cellulose and methyl paraben. Triethanolamine was added to adjust the pH, and propylene glycol was incorporated as a permeation enhancer. The optimized liposomes were mixed into the gel under continuous stirring until a smooth and homogeneous gel was obtained.

Table 5: Composition of Liposomal Gel.

Ingredient	Quantity
Carbopol 934	1.0 g
Carboxymethyl Cellulose	1.0 g
Propylene Glycol	0.5 mL
Methyl Paraben	0.2 mL
Optimized Liposomes	100 µL
Triethanolamine	q.s.
Distilled Water	100 mL

2.6 Evaluation of Liposomal Gel

2.6.1 Physical Appearance

The prepared gel was evaluated visually for colour, appearance, homogeneity, and consistency.

2.6.2 pH Determination

The pH of the gel was measured using a calibrated digital pH meter at room temperature.

2.6.3 Viscosity

The viscosity of the gel was measured using a Brookfield viscometer with spindle No. 61 at **100 rpm**.

2.6.4 Spreadability

Spreadability was determined using the glass slide method and calculated using the following equation:

$$S = \frac{M \times L}{T}$$

Where **M** is the applied weight, **L** is the length of the glass slide, and **T** is the time required for spreading.

2.6.5 Skin Irritation Study

The optimized formulation was applied to the forearm of healthy human volunteers after obtaining informed consent. The application site was observed for 24 h for any signs of erythema, itching, or irritation.

2.6.6 In Vitro Drug Release Study

The in vitro drug release of the optimized polihexanide-loaded liposomal gel was evaluated using the dialysis bag diffusion method**.** The gel was placed in a pre-soaked dialysis membrane and immersed in 100 mL phosphate buffer (pH 7.4) maintained at **37 ± 2°C** with continuous stirring at 100 rpm**.** Samples **(**2 mL**)** were withdrawn at predetermined time intervals and replaced with fresh buffer to maintain sink conditions. The drug content was analyzed using a UV–Visible spectrophotometer at 241 nm. The cumulative drug release was calculated, and the release data were fitted to Zero-order, First-order, Higuchi, and Korsmeyer–Peppas kinetic models to determine the drug release mechanism.

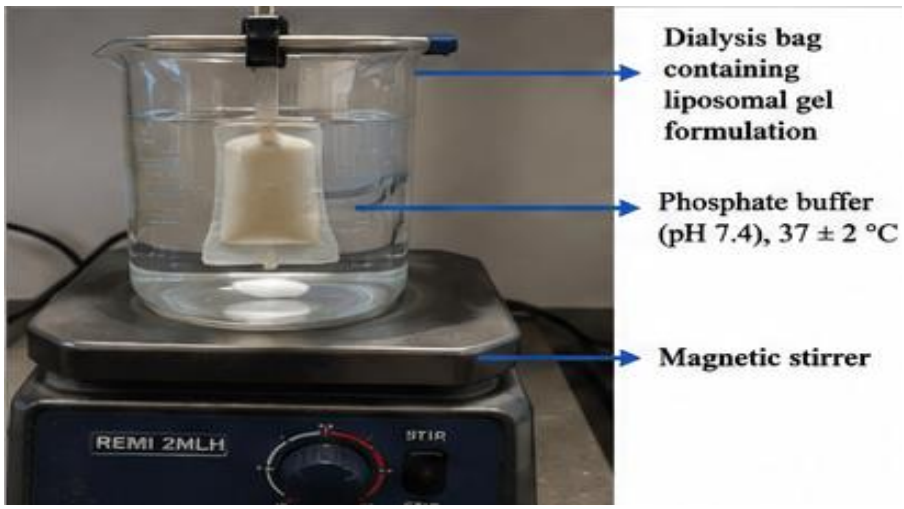

Figure 8: Experimental setup for the in vitro drug release study.

2.7 Drug Release Kinetic Analysis

The in vitro drug release data were fitted to different kinetic models to determine the drug release mechanism.

1. Zero-Order Model
2. First-Order Model
3. Higuchi Model
4. Korsmeyer–Peppas Model

Table 6: Interpretation of Drug Release Mechanism.

Diffusion Exponent (n)	Drug Transport Mechanism	Release Behaviour
0.45	Fickian diffusion	Diffusion-controlled release
0.45–0.89	Non-Fickian (Anomalous) diffusion	Combined diffusion and polymer relaxation
0.89	Case II transport	Zero-order release
>0.89	Super Case II transport	Polymer relaxation-controlled release

3. RESULTS AND DISCUSSION

3.1 Preformulation Studies

3.1.1 Organoleptic Evaluation

The organoleptic properties of pure polihexanide were evaluated and found to comply with the reported pharmacopoeial characteristics. The drug appeared as an off-white, odourless, solid powder, indicating its suitability for formulation development.

Table 7: Organoleptic Properties of Polihexanide.

Parameter	Observation
Colour	Off-white
Odour	Odourless
Physical state	Solid
Appearance	Fine powder

3.1.2 Solubility Study

Polihexanide exhibited excellent solubility in water, methanol, and DMSO, moderate solubility in ethanol, and slight solubility in chloroform. These findings facilitated the selection of suitable solvents during liposome preparation.

Table 8: Solubility Profile of Polihexanide.

Solvent	Observation
Water	Freely soluble
Methanol	Freely soluble
DMSO	Freely soluble
Ethanol	Soluble
Chloroform	Slightly soluble

3.1.3 pH Determination

The pH of polihexanide solution was found to be **6.13**, indicating near-neutral characteristics suitable for topical formulations.

Table 9: pH of Polihexanide.

Drug	pH
Polihexanide	6.13

3.1.4 Melting Point

The observed melting point of polihexanide was **123°C**, which agreed with the reported reference range (120–125°C), confirming drug purity.

Table 10: Melting Point of Polihexanide.

Drug	Observed	Reference
Polihexanide	123°C	120–125°C

3.1.5 UV Spectrophotometric Analysis

The maximum absorbance (λmax) of polihexanide was observed at **241 nm** in methanol.

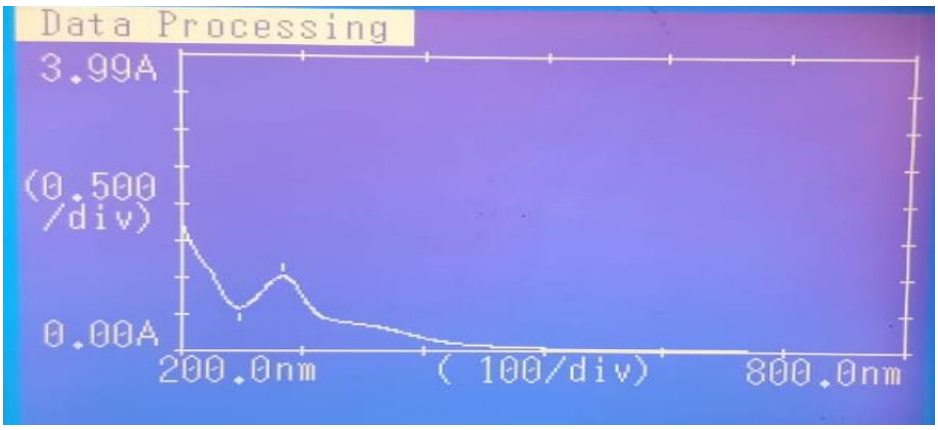

Figure 9: UV spectrum of polihexanide.

Table 11: λmax of Polihexanide.

Drug	λmax
Polihexanide	241 nm

3.1.6 Calibrtion Curve

The calibration curve demonstrated good linearity within the concentration range of **5–30 µg/mL** with a regression equation of **y = 0.0257x + 0.0031** and a correlation coefficient (**R^2 = 0.9938**), confirming the suitability of the analytical method.

Table 12: Calibration Data.

Concentration (µg/mL)	Absorbance
5	0.104
10	0.207
15	0.329
20	0.436
25	0.542
30	0.676

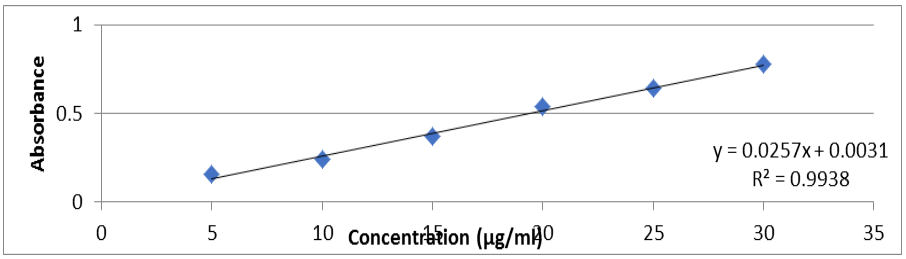

Figure 10: Calibration curve of polihexanide.

3.1.7 Method Validation

The developed UV method exhibited excellent precision, ruggedness, and robustness. The %RSD values for repeatability, intra-day, inter-day precision, ruggedness, and robustness were all **below 2%**, indicating acceptable analytical performance according to ICH guidelines.

Table 13: Summary of Method Validation.

Parameter	Result
Precision (%RSD)	0.48
Intra-day Precision (%RSD)	0.63
Inter-day Precision (%RSD)	0.60
Ruggedness (%RSD)	0.48–0.86
Robustness (%RSD)	0.47–0.48

3.1.8 FTIR Analysis

The FTIR spectrum of pure polihexanide exhibited all characteristic functional group peaks without significant deviation from the reported spectrum. The optimized formulation also

retained these characteristic peaks, indicating no chemical interaction between the drug and formulation excipients.

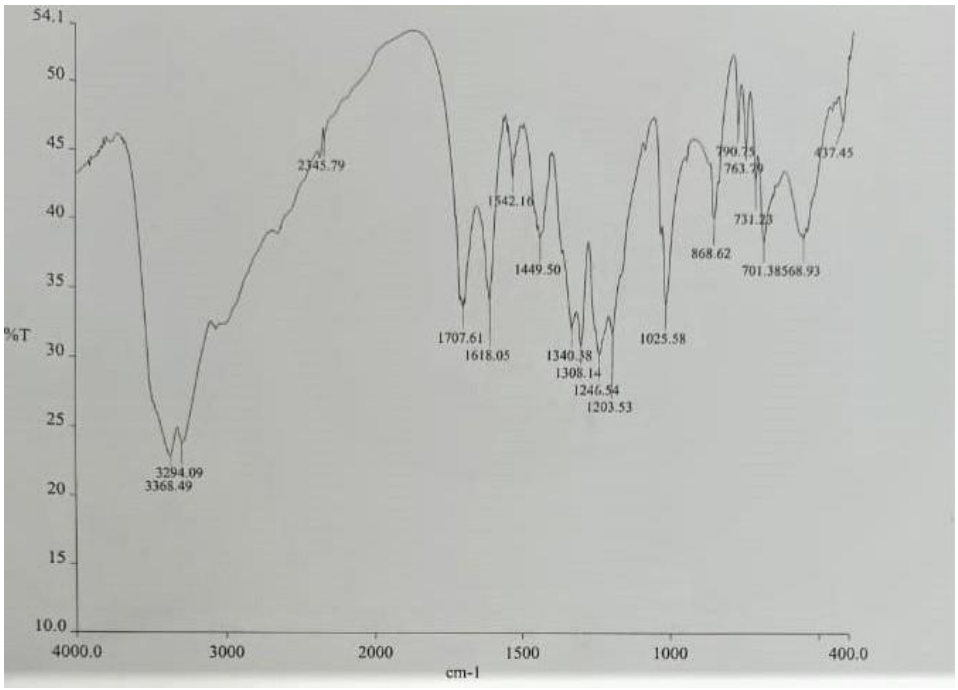

Figure 11. FTIR spectrum of pure polihexanide.

3.2 Evaluation of Polihexanide-Loaded Liposomes

3.2.1 Particle Size

Particle size analysis revealed that the prepared liposomes ranged from 93.26 to 541.43 nm**.** Formulation F5 exhibited the smallest particle size **(**93.26 nm**)** with an acceptable PDI, indicating a uniform nanosized dispersion.

Table 14: Particle Size Analysis.

Formulation	Particle Size (nm)	PDI
F1	541.43	0.265
F2	433.16	0.321
F3	197.34	0.301
F4	210.79	0.212
F5	93.26	0.342

3.2.2 Entrapment Efficiency

Entrapment efficiency ranged from **73.96% to 92.24%**. Formulation **F5** showed the highest drug entrapment (**92.24%**), indicating efficient encapsulation of polihexanide within the liposomal vesicles.

Table 15: Entrapment Efficiency.

Formulation	Entrapment Efficiency (%)
F1	73.96
F2	78.07
F3	90.38
F4	87.73
F5	92.24

3.2.3 Scanning Electron Microscopy

SEM analysis of the optimized formulation confirmed the formation of **spherical liposomal vesicles** with smooth surfaces and uniform morphology, supporting successful liposome preparation.

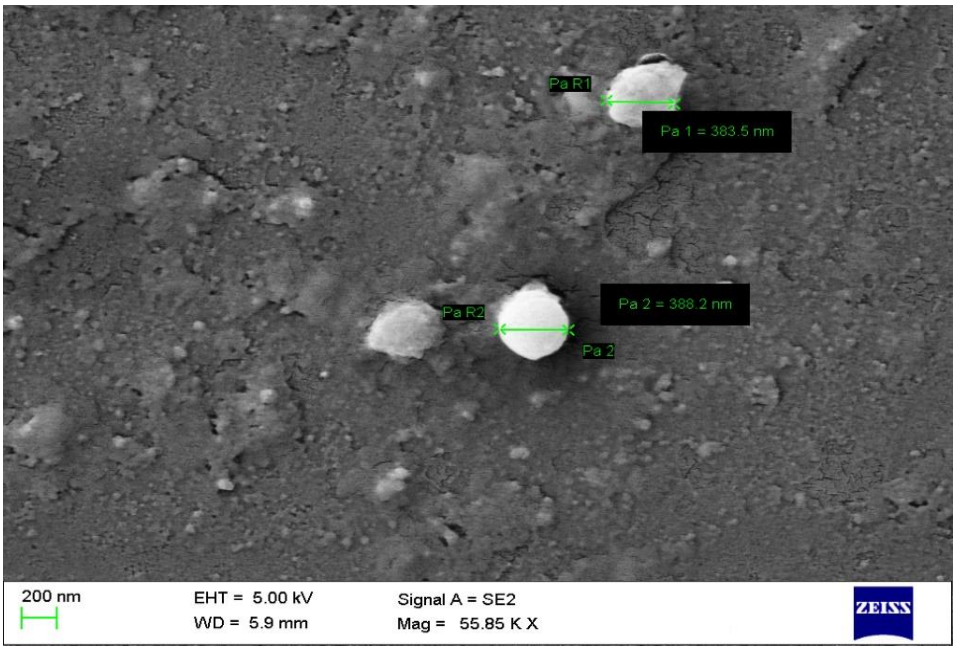

Figure 12: SEM image of optimized liposomes.

3.3 Evaluation of Liposomal Gel

3.3.1 Physical Characteristics

The optimized liposomal gel appeared **clear white, transparent, homogeneous, and odourless**, indicating good aesthetic properties suitable for topical application.

Table 16: Physical Characteristics.

Parameter	Result
Colour	Clear white
Odour	Odourless
Appearance	Transparent
Homogeneity	Homogeneous

3.3.2 pH, Viscosity and Spreadability

The optimized gel exhibited a pH of **6.12**, viscosity of **6513 cps**, and spreadability of **13.18 g·cm/s**, demonstrating compatibility with skin and ease of application.

Table 17: Physicochemical Properties.

Parameter	Result
pH	6.12
Viscosity	6513 cps
Spreadability	13.18 g·cm/s

3.3.3 Skin Irritation Study

No erythema, itching, or irritation was observed following topical application of the optimized gel, confirming its dermatological safety.

Table 18: Skin Irritation Study.

Formulation	Observation
Optimized Gel	Non-irritant

3.3.4 In Vitro Drug Release

The optimized liposomal gel exhibited sustained drug release, achieving **97.89% cumulative release within 12 h**. Drug release followed the **Higuchi model (R^2 = 0.9778)**, suggesting diffusion-controlled release from the gel matrix.

Table 19: In Vitro Drug Release.

Time (h)	Drug Released (%)
0	0
1	23.12
2	36.01
4	43.56
6	57.16
8	76.31
10	87.08
12	97.89

3.3.5 Release Kinetics

Table 20: Kinetic Model Fitting.

Model	R^2
Zero-order	0.9648
First-order	0.8128
Higuchi	0.9778
Korsmeyer–Peppas	0.6120

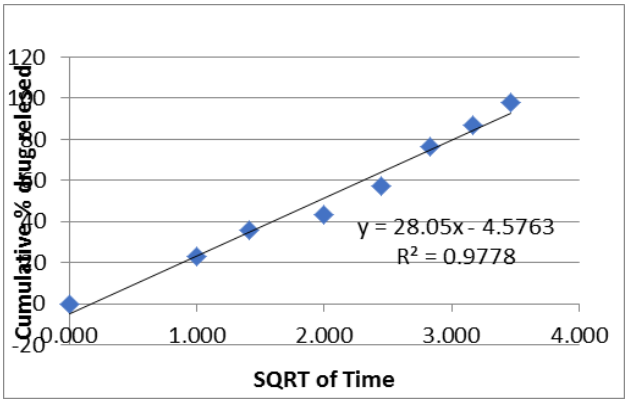

Figure 13: Zero-order release plot.

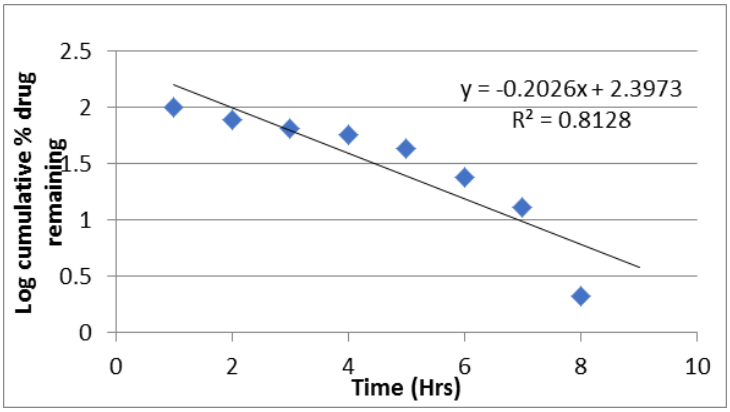

Figure 14: First-order release plot.

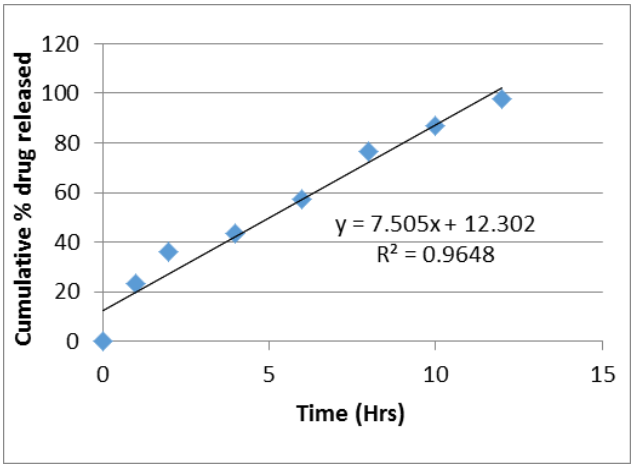

Figure 15: Higuchi release plot.

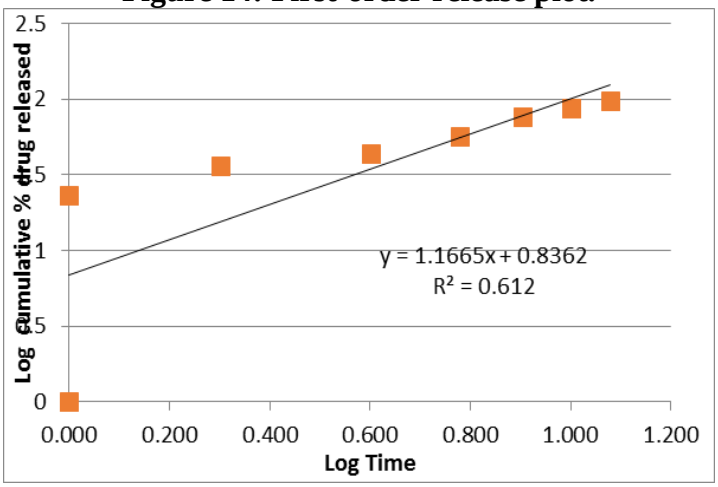

Figure 16: Korsmeyer–Peppas release plot.

The release kinetics demonstrated that the optimized formulation best fitted the Higuchi model**,** indicating that drug release occurred predominantly through diffusion from the liposomal gel matrix, providing a sustained release profile suitable for topical wound management.

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