

FORMULATION, DEVELOPMENT, OPTIMIZATION AND EVALUATION OF A MARINE PEPTIDE-LOADED LIPOSOMAL HYDROGEL FOR ENHANCED BURN WOUND THERAPY

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ABSTRACT

Background: Marine peptides are described in the supplied work as bioactive molecules with antibacterial, antioxidant, anti-inflammatory and wound-healing properties. Their formulation use can be limited by instability and degradation. The present study developed a liposomal hydrogel intended to provide protected and sustained topical delivery for burn-wound therapy. **Methods:** Marine peptide was characterized by preformulation studies and then formulated into liposomes using the Thin Film Hydration Method. Response Surface Methodology with Box–Behnken Design was used to optimize soy phosphatidylcholine and cholesterol levels. The optimized liposomes were incorporated into Carbopol® 940 hydrogel, which was optimized using a second design. Physicochemical evaluation, in vitro release, kinetic modelling, RP-HPLC validation and three-month stability testing were performed. **Results:** TF6 liposomes showed 148.6 ± 3.2 nm particle size, PDI 0.214 ± 0.01 , zeta potential -31.8 ± 1.4 mV, entrapment efficiency $91.84 \pm 1.08\%$, drug loading $8.62 \pm 0.24\%$ and drug content $99.12 \pm 0.84\%$. Optimized hydrogel F6 showed pH 6.48 ± 0.03 , viscosity $20,560 \pm 245$ cP, spreadability 26.82 ± 0.54 g·cm/s, extrudability 485 ± 12 g, gel strength 52.4 ± 1.6 s and drug content $99.24 \pm 0.78\%$. Drug release reached $94.85 \pm 1.24\%$ at 24 h. Higuchi kinetics gave the highest reported fit ($R^2 = 0.992$), while the Korsmeyer–Peppas exponent was $n = 0.68$. **Conclusion:** The supplied experimental data support successful development of a nanosized marine peptide liposomal hydrogel with high encapsulation, suitable topical physicochemical properties, sustained release and satisfactory three-month stability.

KEYWORDS: Marine peptide; liposome; Carbopol 940; hydrogel; Box–Behnken design; burn wound; sustained release; RP-HPLC.

1. INTRODUCTION

Burn injury compromises the protective function of the skin and may be associated with infection, dehydration, persistent inflammation, oxidative stress and delayed tissue repair. The supplied thesis describes skin as a multifunctional barrier involved in protection, thermoregulation, immune response, sensation, metabolism and wound healing. Burn repair progresses through overlapping hemostasis, inflammation, proliferation and remodeling phases, and severe injury can disturb these processes.

Conventional topical treatments can be limited by short residence time, inadequate penetration, frequent application and formulation-related constraints. The supplied rationale therefore focuses on a system capable of maintaining the active peptide at the wound surface while providing controlled release. Liposomes can act as protective vesicular carriers, whereas hydrogels can provide a hydrated matrix with prolonged residence.

Marine peptides in the supplied material are natural bioactive peptides obtained from fish, algae, sponges, mollusks and marine microorganisms. The source attributes antibacterial, antioxidant, anti-inflammatory, regenerative and wound-healing activities to this class. The present formulation combines the peptide with a liposomal carrier and a Carbopol® 940 hydrogel base.

1.1 Study rationale

The formulation rationale is based on the instability of peptide molecules in conventional formulations and the need for improved local delivery. Liposomal encapsulation was intended to protect the peptide and improve delivery characteristics, while hydrogel incorporation was intended to improve topical retention and handling.

1.2 AIM

To formulate, develop, validate and evaluate a marine peptide-loaded liposomal hydrogel for enhanced burn wound therapy.

1.3 OBJECTIVES

- Characterize the marine peptide.
- Formulate and optimize marine peptide-loaded liposomes.
- Characterize the optimized liposomal formulation.
- Develop and optimize the marine peptide-loaded liposomal hydrogel.
- Develop and validate an RP-HPLC method according to ICH Q2(R2).
- Evaluate physicochemical quality, in vitro release, release kinetics and stability.

2. MATERIALS AND METHODS

The supplied study used pharmaceutical- or analytical-grade materials. Marine peptide was the active pharmaceutical ingredient; soy phosphatidylcholine was the liposome-forming lipid; cholesterol was the membrane stabilizer; Carbopol® 940 was the gelling agent; propylene glycol and glycerin were used as humectant/moisturizing components; triethanolamine adjusted pH; methyl paraben and propyl paraben served as preservatives; purified water was the vehicle.

Material	Grade	Purpose
Marine peptide	Pharmaceutical	API
Soy phosphatidylcholine	Pharmaceutical	Liposome-forming lipid
Cholesterol	Pharmaceutical	Membrane stabilizer
Carbopol® 940	Pharmaceutical	Gelling agent
Triethanolamine	Analytical	pH adjustment
Propylene glycol	Pharmaceutical	Humectant/penetration enhancer
Glycerin	Pharmaceutical	Moisturizer/humectant
Methyl paraben	Pharmaceutical	Preservative
Propyl paraben	Pharmaceutical	Preservative
Purified water	IP grade	Vehicle

2.1 Liposome formulation and optimization

Marine peptide-loaded liposomes were prepared using the Thin Film Hydration Method. Soy phosphatidylcholine (X1) was tested at 100, 150 and 200 mg and cholesterol (X2) at 20, 40 and 60 mg. Particle size, PDI, entrapment efficiency, zeta potential and cumulative release were treated as key responses. Response Surface Methodology with Box–Behnken Design was implemented using Design-Expert® software.

2.2 Hydrogel optimization

The optimized TF6 liposomal dispersion was incorporated into Carbopol® 940 hydrogel. Carbopol concentration (0.50–1.50% w/w) and propylene glycol concentration (5–15% w/w) were varied. Viscosity, spreadability, drug content, release and pH were evaluated.

2.3 Analytical and release studies

UV–Visible spectroscopy was used during preformulation, while RP-HPLC was developed for quantitative analysis. In vitro release was assessed using a Franz Diffusion Cell with phosphate buffer pH 7.4 as receptor medium for 24 h. Release data were fitted to Zero-order, First-order, Higuchi and Korsmeyer–Peppas models. Experiments were reported as mean $\pm$ SD (n = 3).

3. Preformulation Results

The preformulation stage established the physical and analytical characteristics of the marine peptide. The sample was reported as an off-white to pale yellow amorphous powder with a fine, uniform and free-flowing texture.

Sr. No.	Parameter	Observation
1	Colour	Off-white to pale yellow
2	Odour	Characteristic
3	Physical State	Amorphous powder
4	Appearance	Fine and uniform
5	Texture	Free-flowing, non-gritty

3.1 Solubility

Solubility varied substantially among the tested solvents. DMSO showed the highest measured value (52.38 ± 0.61 mg/mL), followed by phosphate buffer pH 7.4 (28.15 ± 0.36 mg/mL) and distilled water (24.83 ± 0.42 mg/mL). Chloroform showed only 0.42 ± 0.03 mg/mL. The source interprets the profile as supporting aqueous hydration for liposome preparation.

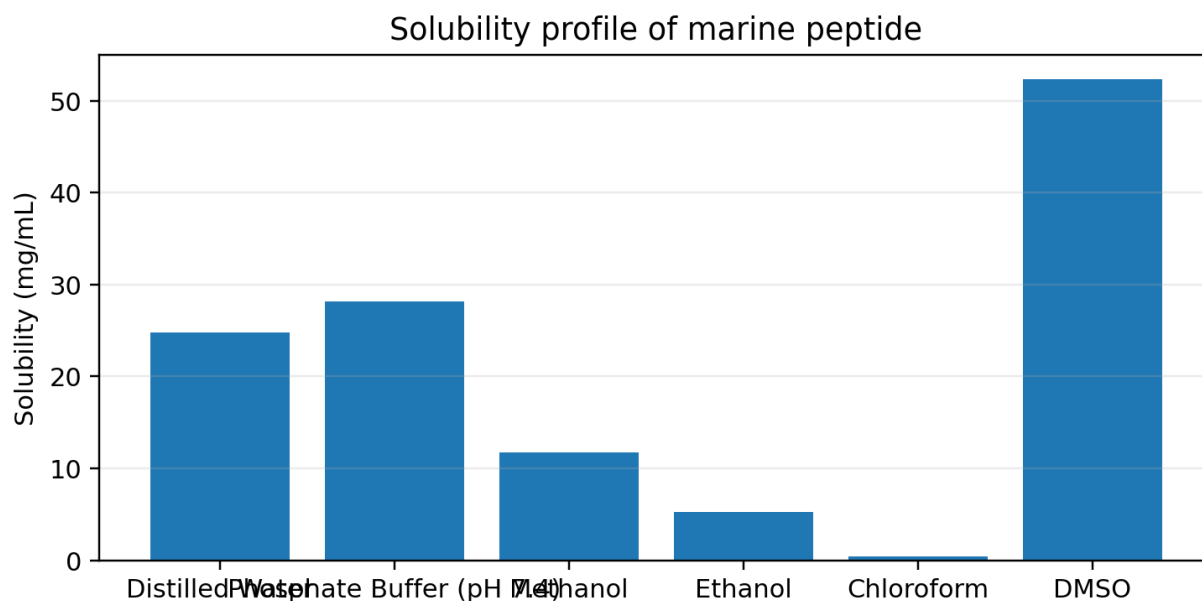


Figure 1: Solubility profile derived from the supplied.

3.2 pH and UV spectrum

The pH of a 1% w/v aqueous peptide solution was 6.92 ± 0.04 . UV–Visible scanning from 200–400 nm in phosphate buffer pH 7.4 produced a sharp maximum at 220 nm, which was selected for analytical estimation.

Sr. No.	Parameter	Observation
1	Solvent	Phosphate Buffer (pH 7.4)
2	Scanning Range	200–400 nm
3	λ_{max}	220 nm
4	Peak Nature	Sharp and well-defined

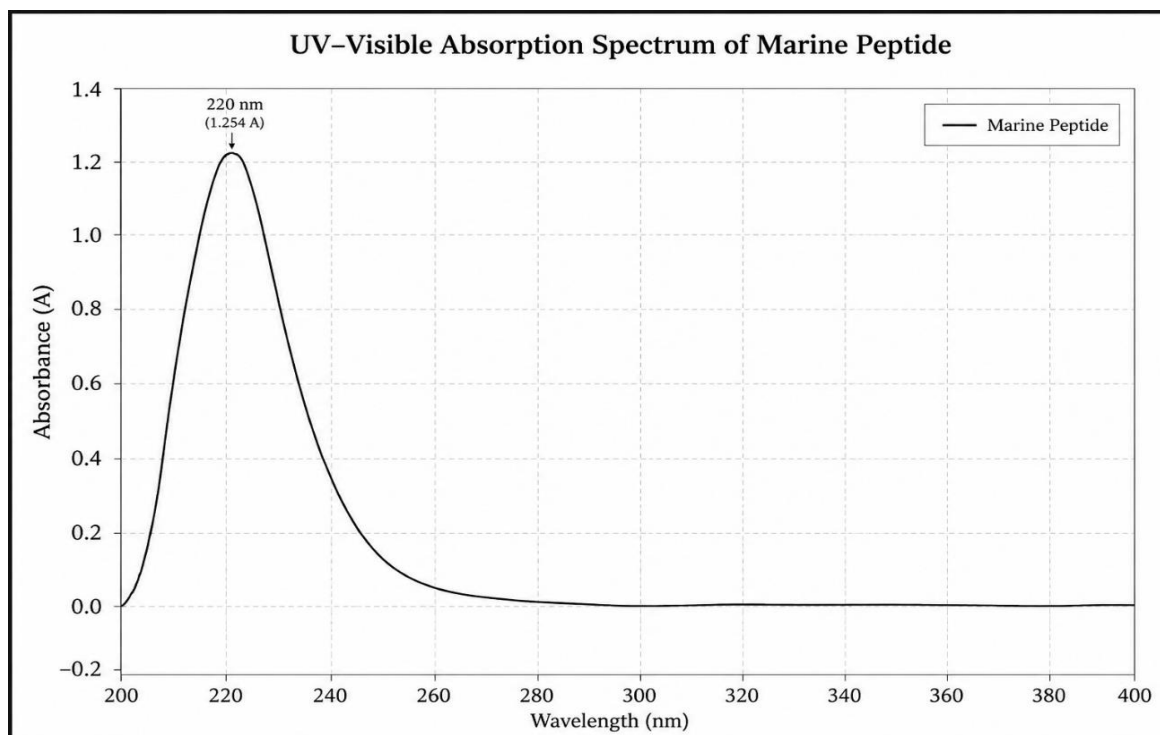


Figure 2: Supplied UV–Visible absorption spectrum of marine peptide.

4. Spectroscopic and Thermal Characterization

The UV calibration dataset covered 2–20 µg/mL. Absorbance increased progressively across the concentration range.

Sr. No.	Concentration (µg/mL)	Absorbance*
1	2	0.128 ± 0.002
2	4	0.249 ± 0.003
3	6	0.372 ± 0.004
4	8	0.496 ± 0.003
5	10	0.618 ± 0.004
6	12	0.742 ± 0.005
7	14	0.865 ± 0.004
8	16	0.989 ± 0.005
9	18	1.111 ± 0.006
10	20	1.235 ± 0.005

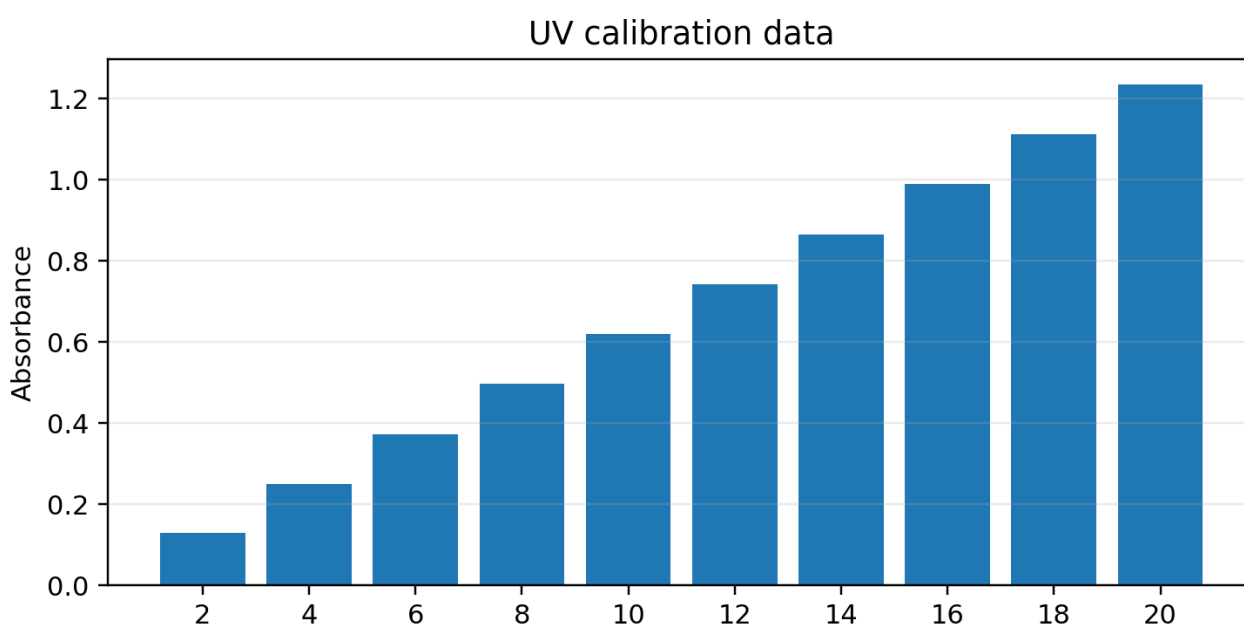


Figure 3: UV calibration data of marine peptide.

4.1 FTIR characterization

FTIR showed characteristic bands at 3295 cm⁻¹ (N–H/Amide A), 2932 cm⁻¹ (aliphatic C–H), 1652 cm⁻¹ (Amide I), 1541 cm⁻¹ (Amide II), 1452 cm⁻¹ (CH₂ bending), 1245 cm⁻¹ (Amide III) and 1086 cm⁻¹ (C–O/C–N stretching).

Sr. No.	Observed Peak (cm ⁻¹)	Functional Group	Assignment
1	3295	N–H Stretching	Amide A
2	2932	C–H Stretching	Aliphatic C–H
3	1652	C=O Stretching	Amide I
4	1541	N–H Bending / C–N Stretching	Amide II
5	1452	C–H Bending	CH ₂ Bending
6	1245	C–N Stretching	Amide III
7	1086	C–O / C–N Stretching	Peptide Backbone

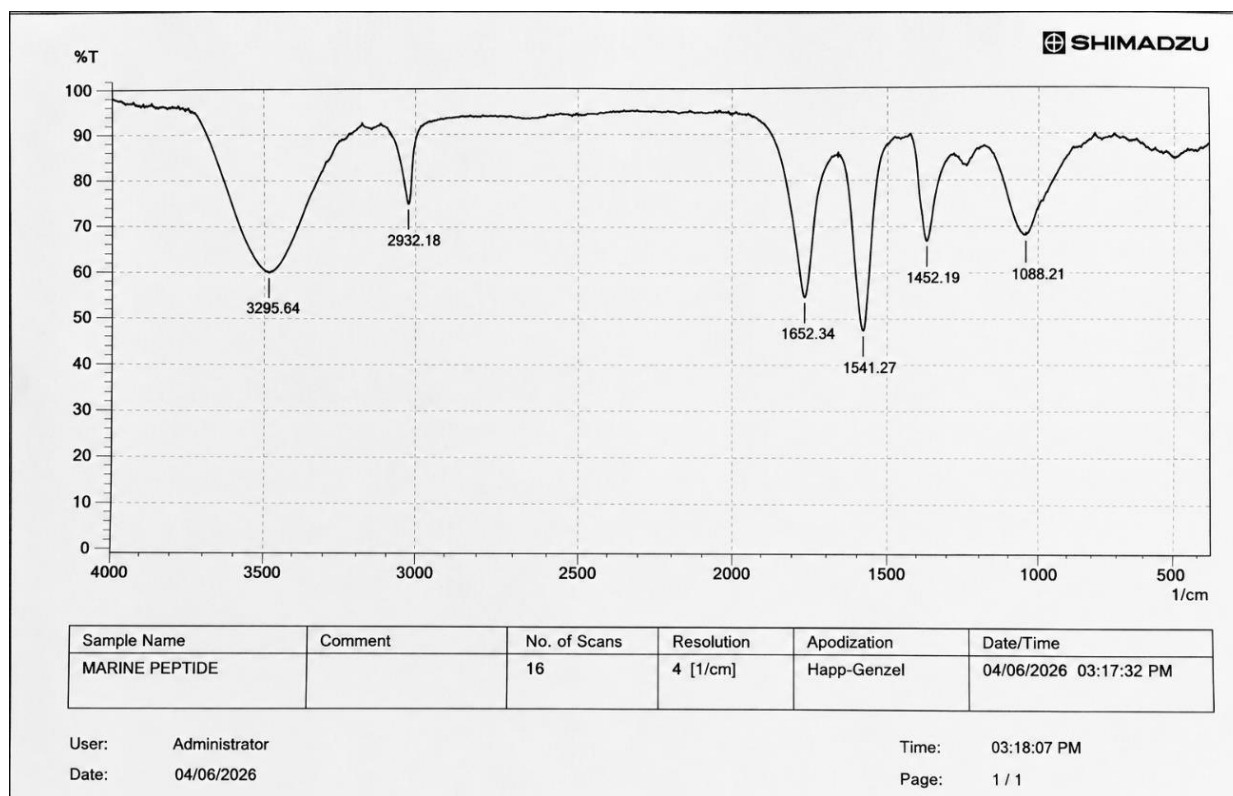


Figure 4: Supplied FTIR spectrum of marine peptide.

4.2 DSC

DSC showed onset 164.8°C, peak 169.6°C, endset 173.4°C and enthalpy 84.5 J/g. The supplied discussion interprets this as supporting adequate thermal stability for the formulation process.

Sr. No.	Parameter	Observation
1	Onset Temperature (°C)	164.8
2	Peak Temperature (°C)	169.6
3	Endset Temperature (°C)	173.4
4	Enthalpy (ΔH) (J/g)	84.5
5	Thermal Event	Sharp Endothermic Peak

5. Drug–Excipient Compatibility

The physical mixture study compared FTIR and DSC characteristics of marine peptide with the selected excipients. The supplied interpretation reports retention of characteristic FTIR peaks without significant shifts, absence of new/disappearing major peaks, and retention of the DSC event with slight reduction in intensity.

Sr. No.	Evaluation Parameter	Observation
1	FTIR Analysis	Characteristic peaks of marine peptide retained without significant shift
2	New Absorption Peaks	Not observed
3	Disappearance of Characteristic Peaks	Not observed
4	DSC Endothermic Peak	Retained with slight reduction in intensity
5	Thermal Transition	No significant change observed
6	Compatibility Assessment	Compatible

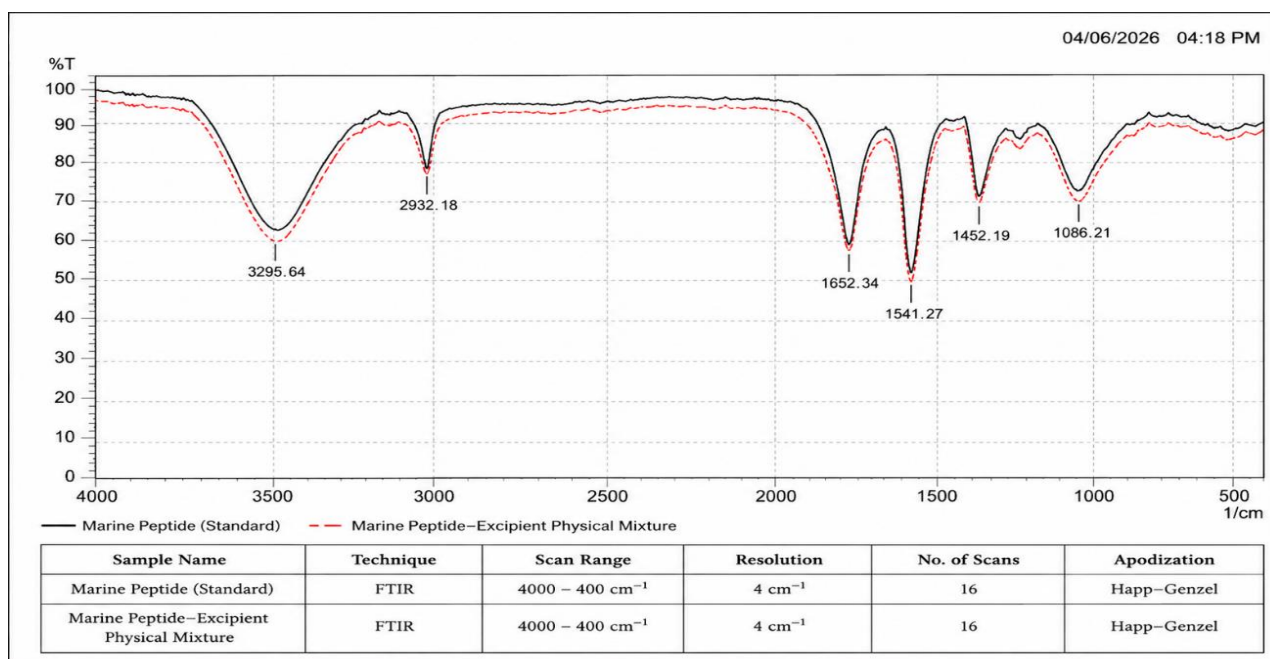


Figure 5: Supplied FTIR comparison of marine peptide and physical mixture.

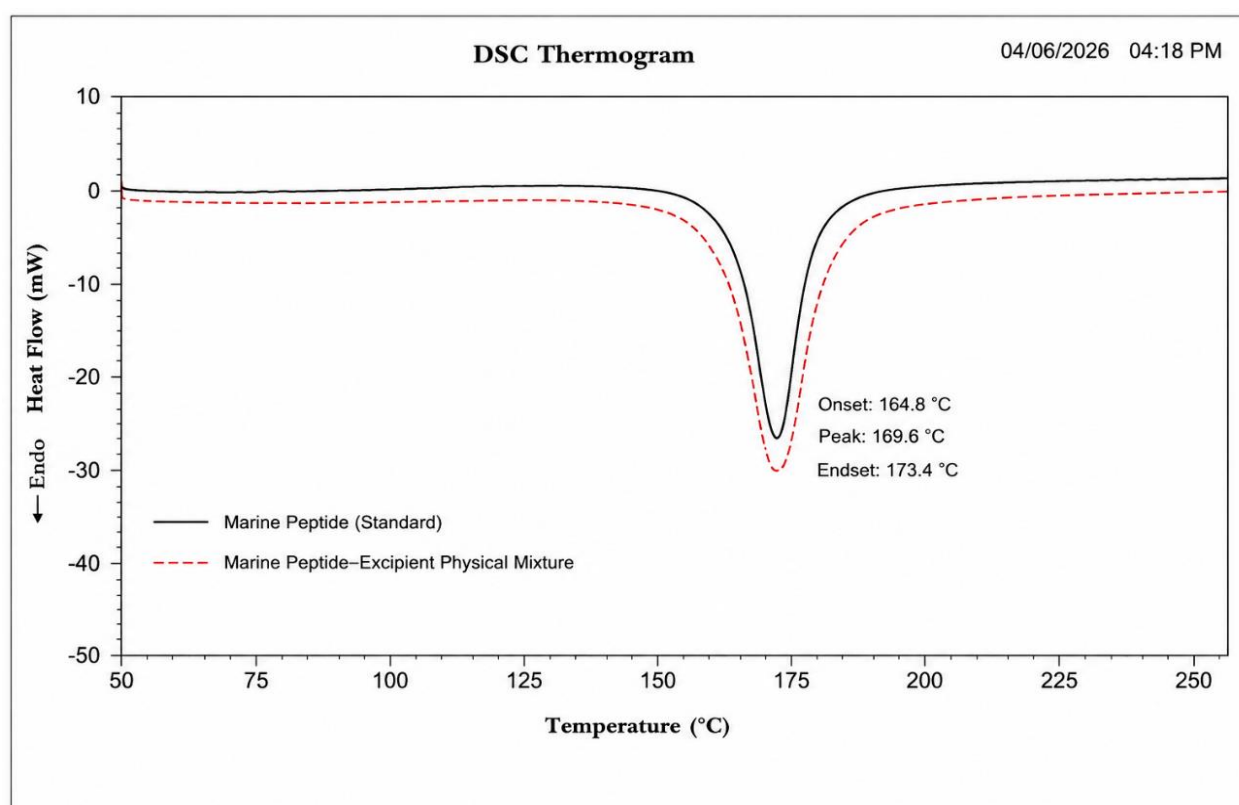


Figure 6: Supplied DSC comparison of marine peptide and physical mixture.

These compatibility observations supported continued formulation development. They should be interpreted as formulation compatibility findings and not as proof of clinical safety or efficacy.

6. Formulation and Optimization of Marine Peptide-Loaded Liposomes

Preliminary batches TF1–TF7 varied soy phosphatidylcholine and cholesterol. The qualitative observations showed improved vesicle formation as the lipid composition approached TF5–TF6.

Batch	Marine Peptide (mg)	Soy Phosphatidylcholine (mg)	Cholesterol (mg)	Observation
TF1	20	100	20	Large vesicles, moderate stability
TF2	20	150	20	Improved homogeneity
TF3	20	200	20	Stable vesicles
TF4	20	100	40	Slight aggregation observed
TF5	20	150	40	Good vesicle formation
TF6	20	200	40	Uniform, stable liposomes
TF7	20	100	60	Increased particle size
TF8	20	150	60	Slight aggregation
TF9	20	200	60	Stable but larger vesicles

Box–Behnken levels were 100, 150 and 200 mg phosphatidylcholine and 20, 40 and 60 mg cholesterol.

Factor	Independent Variable	Low (-1)	Medium (0)	High (+1)
X ₁	Soy Phosphatidylcholine (mg)	100	150	200
X ₂	Cholesterol (mg)	20	40	60

6.1 Optimization criteria

Optimization sought minimum particle size and PDI while maximizing entrapment efficiency, zeta potential and cumulative release. The supplied source states that polynomial models and ANOVA were used and that the desirability function identified TF6.

Parameter	Optimized Value
Particle Size (nm)	148.6 ± 3.2
Polydispersity Index	0.214 ± 0.01
Zeta Potential (mV)	−31.8 ± 1.4
Entrapment Efficiency (%)	91.84 ± 1.08
Drug Loading (%)	8.62 ± 0.24
Drug Release (24 h) (%)	94.72 ± 1.32

TF6 contained 20 mg marine peptide, 200 mg soy phosphatidylcholine and 40 mg cholesterol, with chloroform: methanol (2:1) for lipid dissolution and phosphate buffer pH 7.4 for hydration.

Ingredient	Quantity
Marine Peptide	20 mg
Soy Phosphatidylcholine	200 mg
Cholesterol	40 mg
Chloroform : Methanol (2:1)	10 mL
Phosphate Buffer (pH 7.4)	20 mL
Sonication Time	10 min

7. Liposomal Characterization: Particle Size and PDI

Particle size ranged from 148.6 ± 3.2 to 236.5 ± 4.8 nm. TF6 had the smallest reported value. Relative to TF1, the TF6 mean was approximately 37.2% lower.

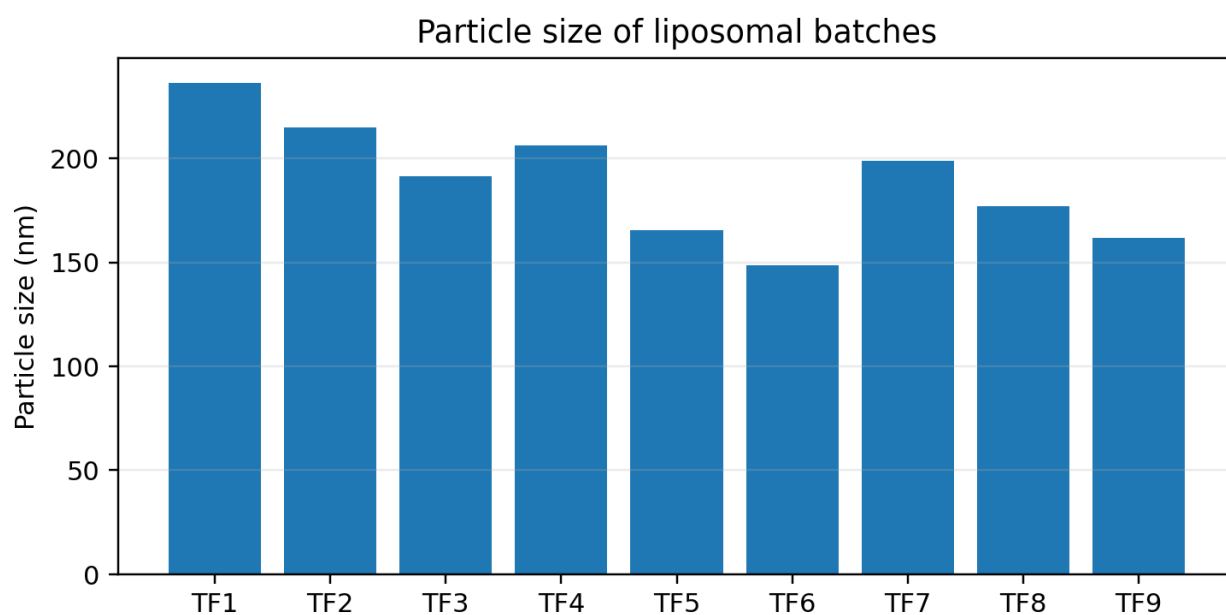


Figure 7: Particle size of liposomal batches TF1–TF7.

Sr. No.	Batch	Particle Size (nm)
1	TF1	236.5 ± 4.8
2	TF2	214.8 ± 3.9
3	TF3	191.6 ± 3.6
4	TF4	206.2 ± 4.1
5	TF5	165.4 ± 3.3
6	TF6	148.6 ± 3.2
7	TF7	198.8 ± 3.8
8	TF8	176.9 ± 3.5
9	TF9	161.8 ± 3.4

7.1 Polydispersity index

PDI ranged from 0.214 ± 0.01 to 0.391 ± 0.02 . TF6 had the lowest PDI, indicating the narrowest size distribution in the supplied dataset.

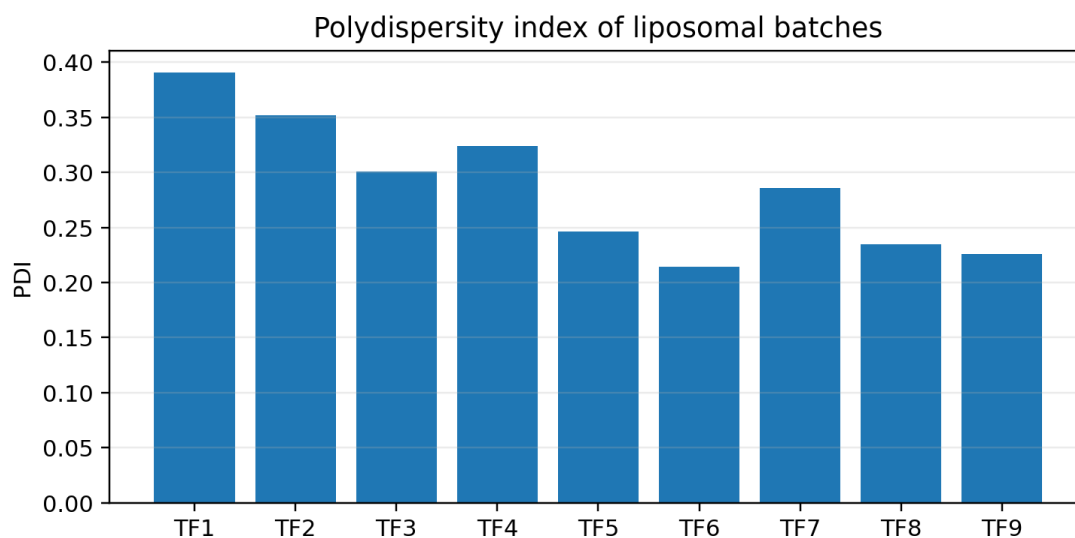


Figure 8: PDI of liposomal batches TF1–TF7.

Sr. No.	Batch	PDI
1	TF1	0.391 ± 0.02
2	TF2	0.352 ± 0.02
3	TF3	0.301 ± 0.01
4	TF4	0.324 ± 0.01
5	TF5	0.246 ± 0.01
6	TF6	0.214 ± 0.01
7	TF7	0.286 ± 0.01
8	TF8	0.235 ± 0.01
9	TF9	0.226 ± 0.01

8. Liposomal Surface Charge and Entrapment

Zeta potential ranged from -20.6 ± 1.1 to -31.8 ± 1.4 mV. TF6 showed the most negative value; the source interprets this as supportive of colloidal stability.

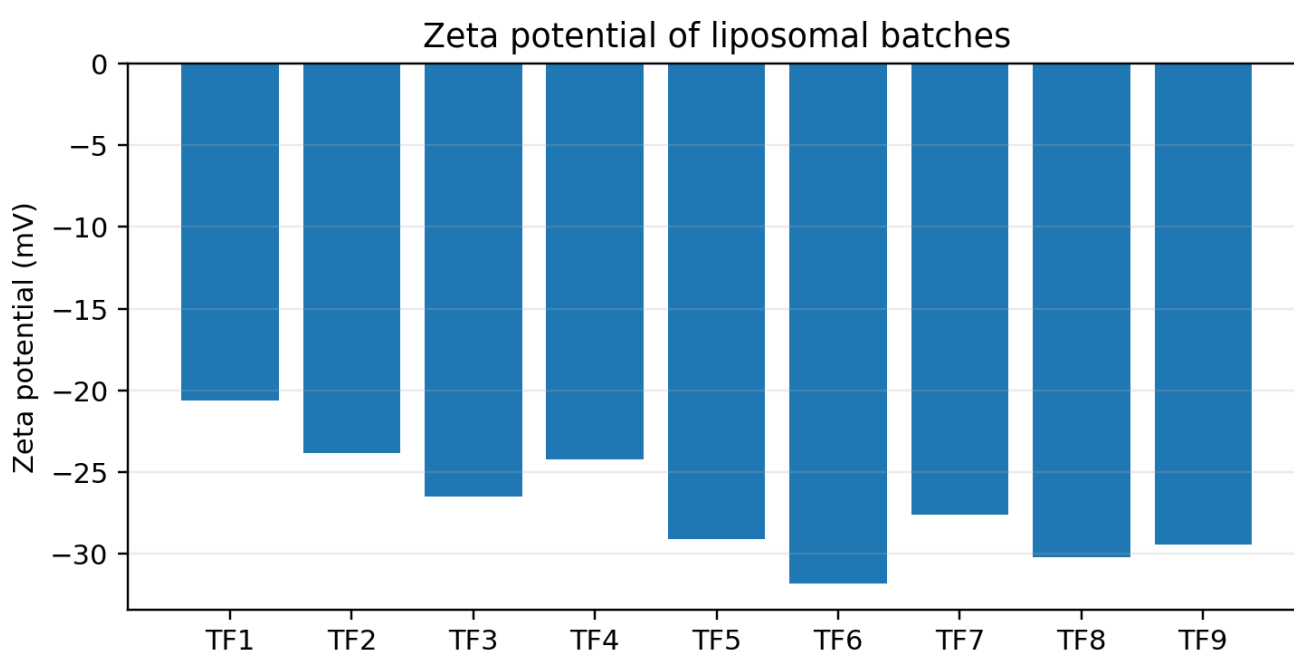


Figure 9: Zeta potential of liposomal batches.

Sr. No.	Batch	Zeta Potential (mV)
1	TF1	-20.6 ± 1.1
2	TF2	-23.8 ± 1.2
3	TF3	-26.5 ± 1.0
4	TF4	-24.2 ± 1.1
5	TF5	-29.1 ± 1.3
6	TF6	-31.8 ± 1.4
7	TF7	-27.6 ± 1.2
8	TF8	-30.2 ± 1.3
9	TF9	-29.4 ± 1.2

8.1 Entrapment efficiency

Entrapment efficiency ranged from $72.45 \pm 1.62\%$ to $91.84 \pm 1.08\%$. TF6 provided the highest value. The supplied discussion associates increasing phospholipid concentration with improved encapsulation, while excessive cholesterol could reduce entrapment.

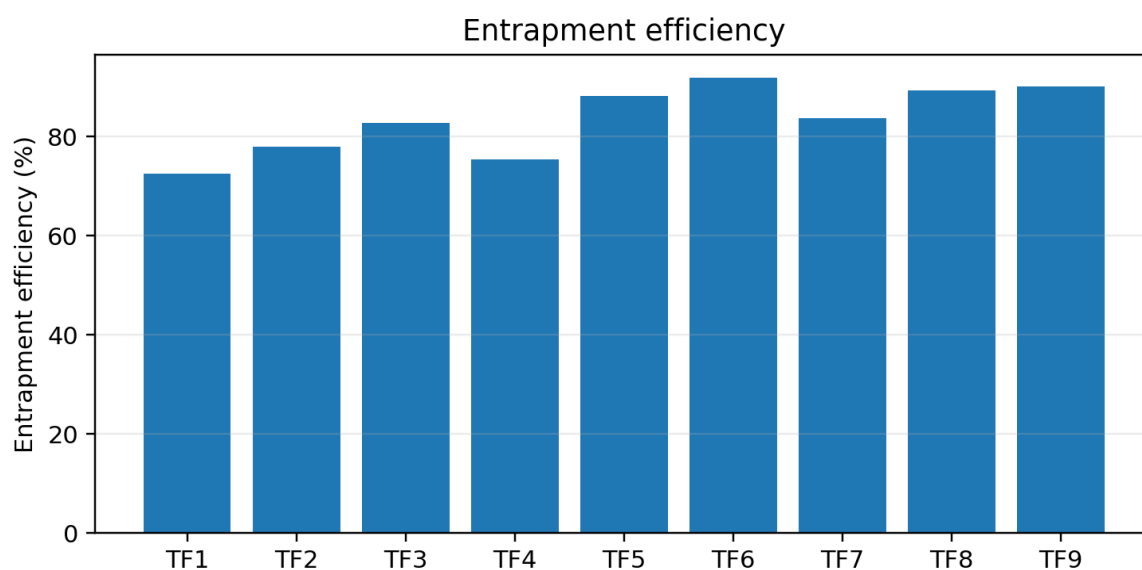


Figure 10: Entrapment efficiency of liposomal batches.

Sr. No.	Batch	Entrapment Efficiency (%)
1	TF1	72.45 ± 1.62
2	TF2	77.83 ± 1.34
3	TF3	82.64 ± 1.28
4	TF4	75.36 ± 1.42
5	TF5	88.12 ± 1.15
6	TF6	91.84 ± 1.08
7	TF7	83.56 ± 1.24
8	TF8	89.15 ± 1.12
9	TF9	90.08 ± 1.10

9. Liposomal Drug Loading and Drug Content

Drug loading ranged from $5.48 \pm 0.21\%$ to $8.62 \pm 0.24\%$, with TF6 highest. Drug content ranged from $90.24 \pm 1.18\%$ to $99.12 \pm 0.84\%$, also highest in TF6.

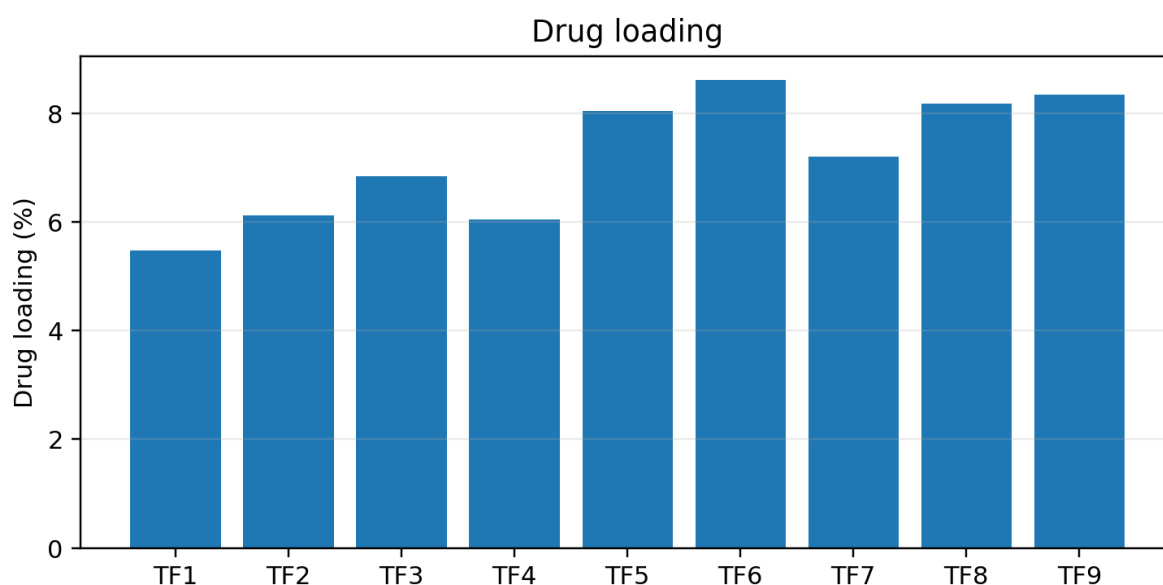


Figure 11: Drug loading of liposomal batches.

Sr. No.	Batch	Drug Loading (%)
1	TF1	5.48 ± 0.21
2	TF2	6.12 ± 0.18
3	TF3	6.84 ± 0.19
4	TF4	6.04 ± 0.20
5	TF5	8.04 ± 0.22
6	TF6	8.62 ± 0.24
7	TF7	7.21 ± 0.21
8	TF8	8.18 ± 0.23
9	TF9	8.35 ± 0.22

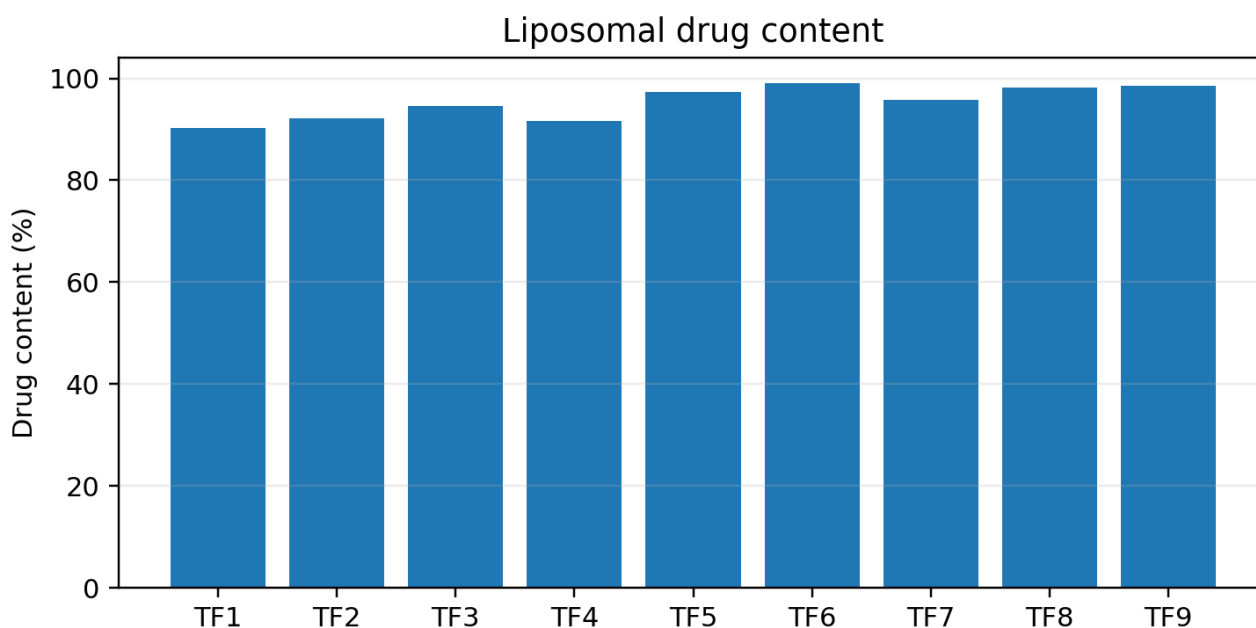


Figure 12: Drug content of liposomal batches.

Sr. No.	Batch	Drug Content (%)
1	TF1	90.24 ± 1.18
2	TF2	92.18 ± 1.12
3	TF3	94.52 ± 1.04
4	TF4	91.64 ± 1.15
5	TF5	97.26 ± 0.91
6	TF6	99.12 ± 0.84
7	TF7	95.84 ± 0.98
8	TF8	98.16 ± 0.88
9	TF9	98.54 ± 0.86

The combined profile of size, PDI, zeta potential, entrapment, loading and drug content provided the experimental basis for carrying TF6 forward.

10. Vesicle Morphology and Integrated Liposome Discussion

TEM examination of TF6 showed discrete, spherical vesicles with relatively smooth surfaces and limited aggregation. The supplied source notes agreement between the observed morphology and the DLS size data.

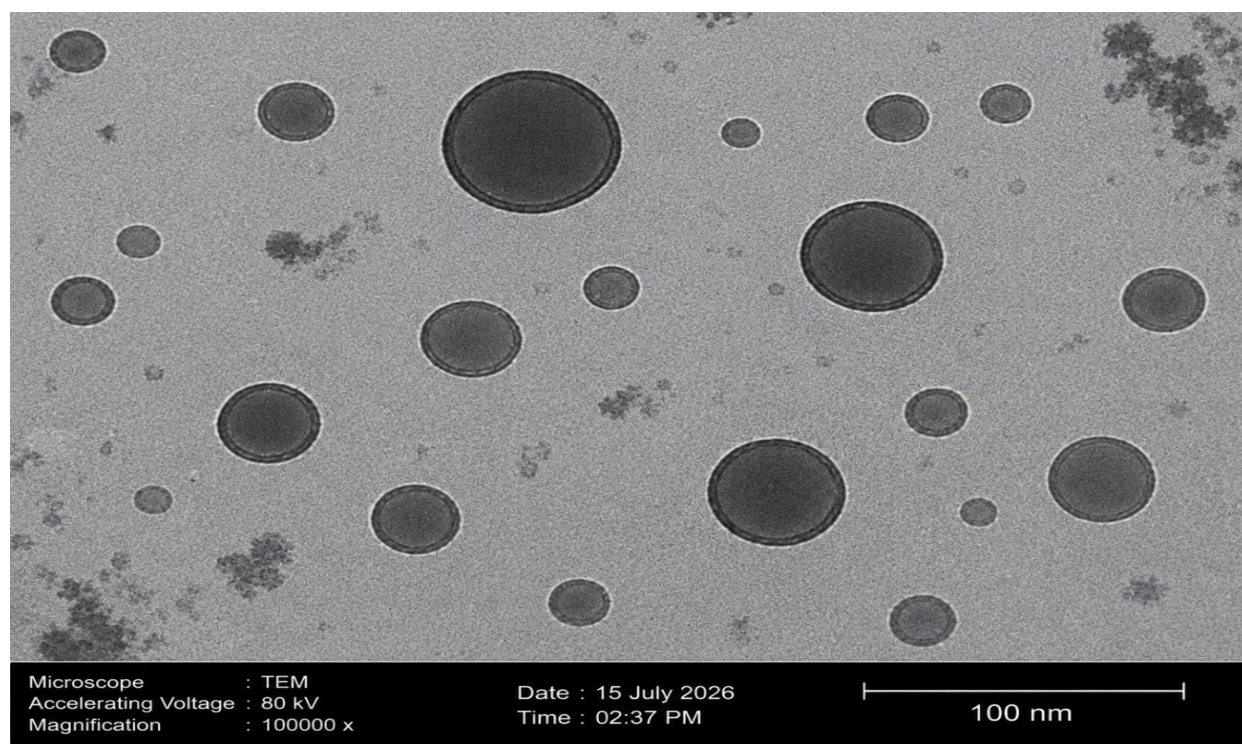


Figure 13: Supplied TEM micrograph of optimized liposomes (TF6).

Quality attribute	TF6 result	Source interpretation
Particle size	148.6 ± 3.2 nm	Nanosized vesicles
PDI	0.214 ± 0.01	Narrow distribution
Zeta potential	-31.8 ± 1.4 mV	Higher negative surface charge
Entrapment efficiency	$91.84 \pm 1.08\%$	High peptide encapsulation
Drug loading	$8.62 \pm 0.24\%$	Highest listed
Drug content	$99.12 \pm 0.84\%$	High content
Morphology	Spherical vesicles	Minimal aggregation

DLS and TEM provide complementary evidence: DLS describes hydrodynamic size in dispersion, while TEM provides direct visual morphology. Within the supplied experimental space, TF6 therefore showed a coherent nanoscale characterization profile.

11. Development and Optimization of the Liposomal Hydrogel

The optimized TF6 dispersion was incorporated into Carbopol® 940 hydrogel. Nine formulations varied Carbopol® 940 and propylene glycol.

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Optimized Liposomal Dispersion (TF6)	20	20	20	20	20	20	20	20	20
Carbopol® 940 (%)	0.50	0.75	1.00	0.75	1.00	1.25	1.25	1.50	1.50
Propylene Glycol (%)	5	7.5	10	7.5	10	12.5	10	12.5	15
Glycerin (%)	5	5	5	5	5	5	5	5	5
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Methyl Paraben (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl Paraben (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Purified Water	q.s. to 100 g	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

11.1 Preliminary observations

Sr. No.	Batch	Appearance	Homogeneity	Consistency	Observation
1	F1	Clear	Good	Low viscosity	Too fluid
2	F2	Clear	Good	Moderate	Acceptable
3	F3	Clear	Good	Moderate	Acceptable
4	F4	Transparent	Good	Moderate	Acceptable
5	F5	Transparent	Excellent	Good	Stable
6	F6	Transparent	Excellent	Excellent	Optimized
7	F7	Transparent	Good	High viscosity	Slightly stiff
8	F8	Transparent	Good	Very high viscosity	Difficult spreading
9	F9	Transparent	Good	Very high viscosity	Difficult extrusion

F1 was too fluid, whereas F7–F9 became increasingly stiff or difficult to spread/extrude. F5 and F6 had desirable consistency; F6 was described as transparent with excellent homogeneity and consistency.

11.2 Hydrogel design

Factor	Variable	Low (-1)	Medium (0)	High (+1)
X ₁	Carbopol® 940 (%)	0.50	1.00	1.50
X ₂	Propylene Glycol (%)	5	10	15

Dependent responses were viscosity, spreadability, drug content, cumulative release and pH. The source reports that increasing Carbopol® 940 increased viscosity while reducing spreadability and release.

12. Hydrogel Response Data

Batch	pH	Viscosity (cP)	Spreadability (g·cm/s)	Drug Content (%)	Drug Release (24 h) (%)
F1	6.21	16250	30.8	94.18	98.12
F2	6.28	17620	29.4	95.36	97.14
F3	6.35	18960	28.1	96.52	96.08
F4	6.30	18240	28.6	96.18	96.72
F5	6.42	19880	27.3	98.16	95.18
F6	6.48	20560	26.82	99.24	94.85
F7	6.55	22180	24.95	98.52	93.84
F8	6.62	23640	23.84	97.84	92.96
F9	6.70	24880	22.96	97.22	91.84

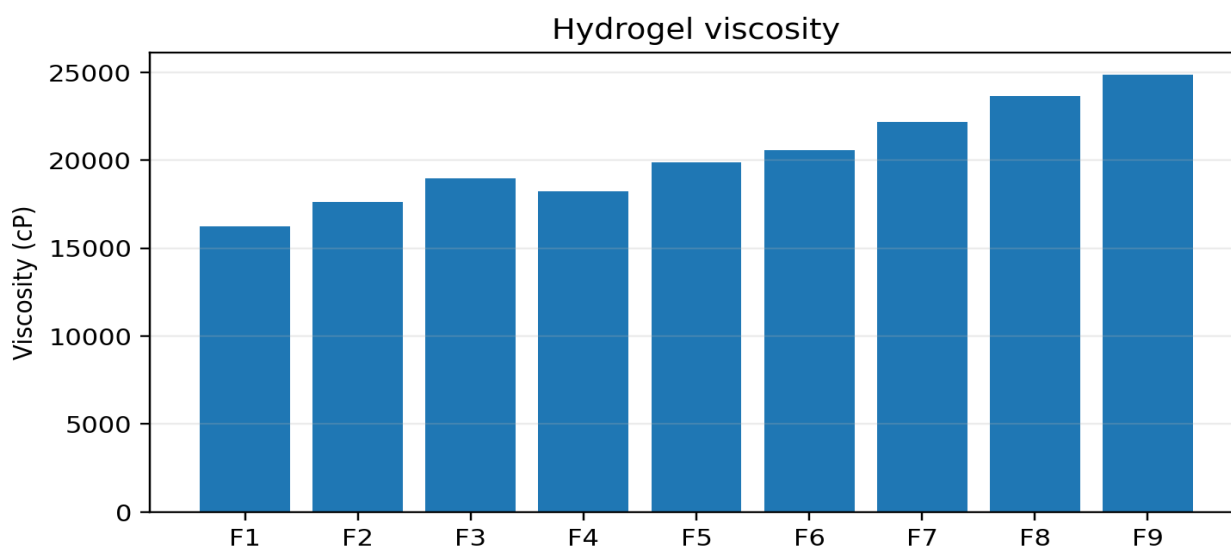


Figure 14: Viscosity of F1–F9.

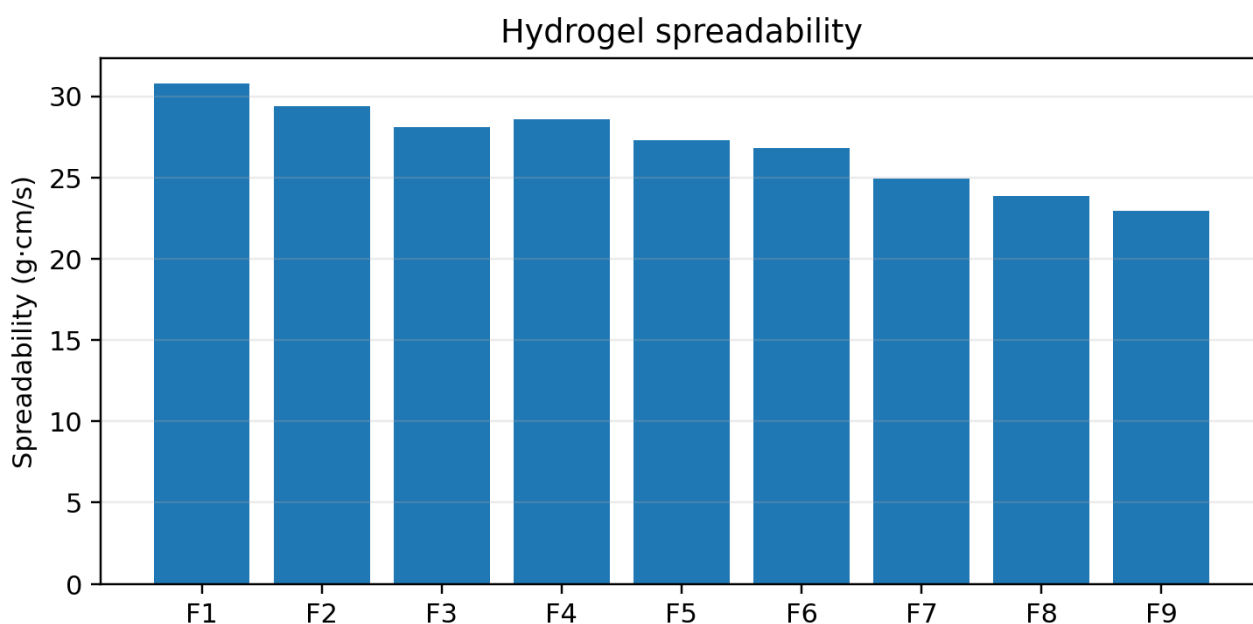


Figure 15: Spreadability of F1–F9.

Viscosity increased from 16,250 cP in F1 to 24,880 cP in F9, while spreadability decreased from 30.8 to 22.96 g·cm/s. This inverse relationship supports the source interpretation that increasing polymer concentration produces a denser matrix and greater resistance to spreading. The pH values increased from 6.21 to 6.70 across the listed batches.

13. Optimized Hydrogel F6: Quality Evaluation

F6 contained TF6 dispersion equivalent to 20 mg marine peptide, 1.25% Carbopol® 940, 12.5% propylene glycol, 5% glycerin, triethanolamine q.s., 0.18% methyl paraben, 0.02% propyl paraben and purified water q.s. to 100 g.

Sr. No.	Ingredient	Quantity
1	Optimized Liposomal Dispersion (TF6)	Equivalent to 20 mg Marine Peptide
2	Carbopol® 940	1.25% w/w
3	Propylene Glycol	12.5% w/w
4	Glycerin	5% w/w
5	Triethanolamine	q.s. (pH 6.4–6.6)
6	Methyl Paraben	0.18% w/w
7	Propyl Paraben	0.02% w/w
8	Purified Water	q.s. to 100 g
Sr. No.	Evaluation Parameter	Observation/Result
1	Physical Appearance	Transparent, smooth, glossy gel
2	Colour	Off-white
3	Homogeneity	Excellent; no grittiness or phase separation
4	pH	6.48 ± 0.03
5	Viscosity (cP)	20,560 ± 245
6	Spreadability (g·cm/s)	26.82 ± 0.54
7	Extrudability	Excellent (485 ± 12 g)
8	Gel Strength (sec)	52.4 ± 1.6
9	Drug Content (%)	99.24 ± 0.78
10	Content Uniformity (%)	98.96 ± 0.84

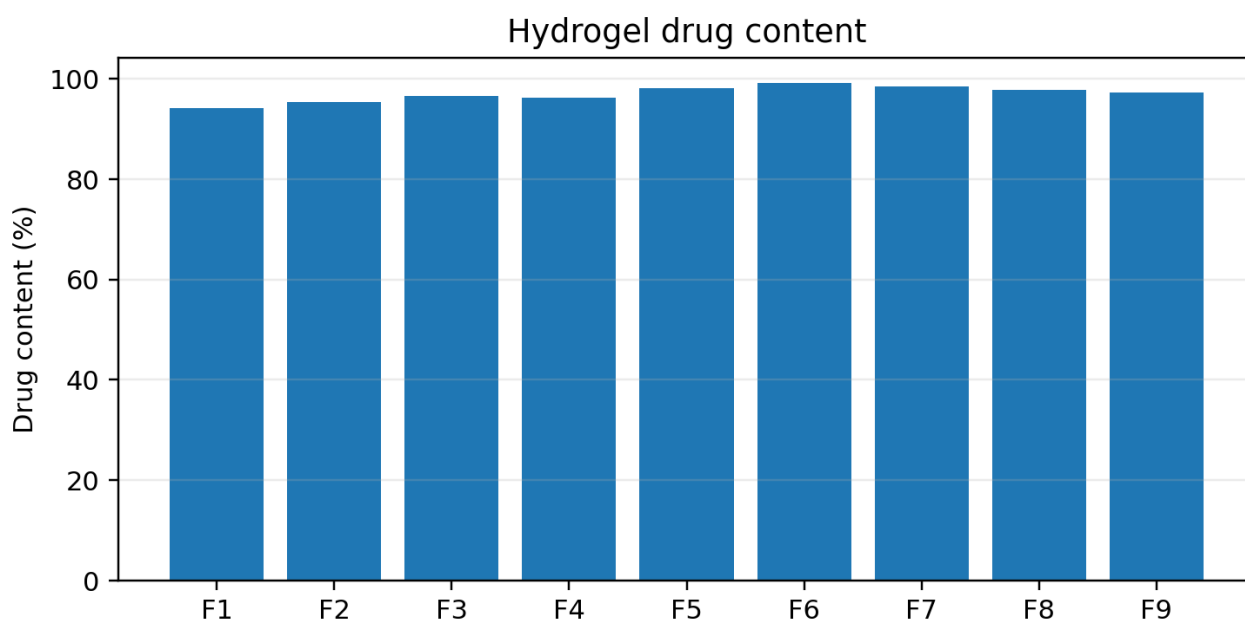


Figure 16: Drug content across hydrogel formulations.

F6 was transparent, smooth and glossy, with excellent homogeneity and no visible grittiness or phase separation. pH was 6.48 ± 0.03 ; viscosity $20,560 \pm 245$ cP; spreadability 26.82 ± 0.54 g·cm/s; extrudability 485 ± 12 g; gel strength 52.4 ± 1.6 s; drug content $99.24 \pm 0.78\%$; content uniformity $98.96 \pm 0.84\%$.

14. Comparative Interpretation of Hydrogel Formulations.

Batch	Viscosity (cP)	Spreadability	Drug content (%)	24 h release (%)
F1	16250	30.8	94.18	98.12
F2	17620	29.4	95.36	97.14
F3	18960	28.1	96.52	96.08
F4	18240	28.6	96.18	96.72
F5	19880	27.3	98.16	95.18
F6	20560	26.82	99.24	94.85
F7	22180	24.95	98.52	93.84
F8	23640	23.84	97.84	92.96
F9	24880	22.96	97.22	91.84

From F1 to F9, viscosity increased by approximately 53.1%, while 24-hour release decreased by approximately 6.4 percentage points. Drug content remained high across the series (94.18–99.24%), indicating that release differences were not simply attributable to gross drug loss during formulation.

14.1 Selection of F6

F6 provided a near-neutral pH, moderate-to-high viscosity, intermediate spreadability, high drug content and sustained 24-hour release of 94.85%. The supplied source states that experimental responses were close to predicted values generated during optimization.

The complete Design-Expert® ANOVA coefficients and predicted-response matrix were not included in the supplied result tables, so they are not reconstructed here.

15. In Vitro Drug Release: Complete Dataset

Franz Diffusion Cell testing was performed for 24 h using phosphate buffer pH 7.4. All formulations showed progressive release.

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.0	18.42	16.84	15.62	16.21	14.84	13.62	12.54	11.86	11.24
1.0	30.16	28.54	26.84	27.92	25.64	24.18	22.96	21.84	20.76
2.0	45.62	43.18	40.84	41.56	39.22	37.84	36.08	34.62	33.24
4.0	63.84	60.96	58.42	59.36	56.84	54.92	52.84	50.68	49.36
6.0	74.58	72.36	69.84	70.42	67.94	66.28	64.16	62.42	60.84
8.0	83.96	81.52	79.36	80.14	77.84	76.48	74.62	72.54	71.18
10.0	90.42	88.64	86.58	87.16	84.96	83.62	81.94	80.16	78.84
12.0	95.24	93.62	91.48	92.14	89.82	88.56	87.12	85.42	84.16
24.0	98.12	97.14	96.08	96.72	95.18	94.85	93.84	92.96	91.84

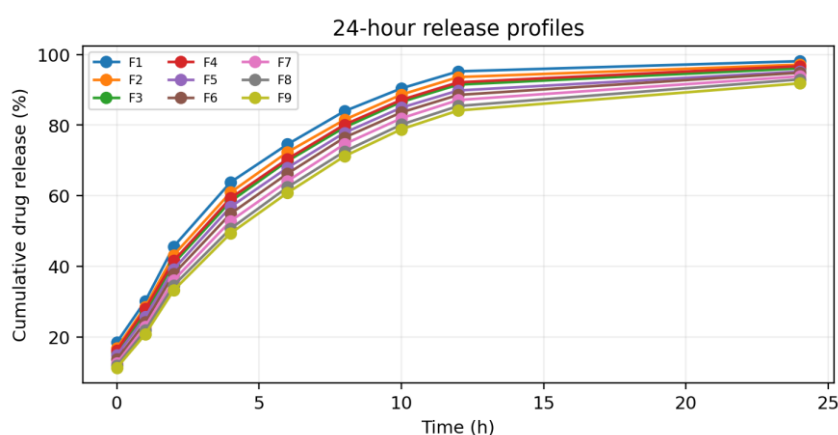


Figure 17: Complete cumulative release profiles of F1–F9.

At 24 h, release ranged from 91.84% in F9 to 98.12% in F1. F6 released 94.85%, which the source identifies as the optimized sustained-release profile.

16. Release Profile Discussion

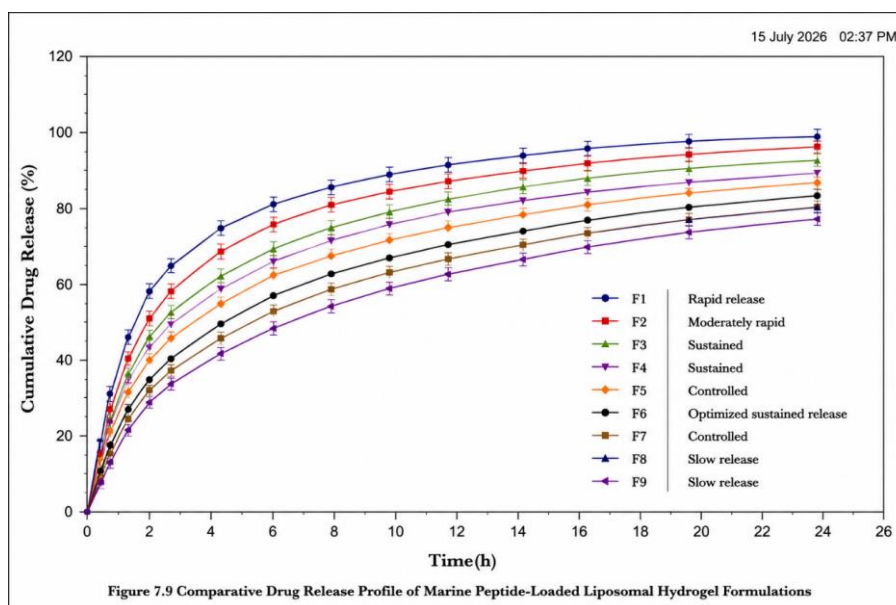


Figure 18: Supplied comparative release plot of F1–F9.

Sr. No.	Formulation	Drug Release (%)	Release Behavior
1	F1	98.12 ± 1.36	Rapid release
2	F2	97.14 ± 1.28	Moderately rapid
3	F3	96.08 ± 1.22	Sustained
4	F4	96.72 ± 1.26	Sustained
5	F5	95.18 ± 1.18	Controlled
6	F6	94.85 ± 1.24	Optimized sustained release
7	F7	93.84 ± 1.16	Controlled
8	F8	92.96 ± 1.12	Slow release
9	F9	91.84 ± 1.08	Slow release

The release curves show a faster early phase followed by a slower approach to the 24-hour values. The supplied discussion attributes slower release at higher Carbopol® 940 levels to increased gel viscosity and matrix density. F1–F3 released relatively rapidly, whereas F7–F9 were slower.

F6 was selected as a multi-response optimum rather than as the formulation with the highest or lowest release. Its release remained below F1–F4 and above F7–F9, while its viscosity, spreadability and drug content were favorable. The source therefore describes F6 as providing an appropriate balance between drug retention, application properties and prolonged release.

17. Drug Release Kinetics

Model	Reported equation	R ²	Source interpretation
Zero-order	$y = 3.566x + 28.42$	0.973	Good linearity
First-order	$y = -0.064x + 2.004$	0.952	Lower fit
Higuchi	$y = 20.84x + 14.62$	0.992	Highest reported fit; diffusion control
Korsmeyer–Peppas	$y = 0.680x + 1.238$	0.986	$n = 0.68$; anomalous diffusion

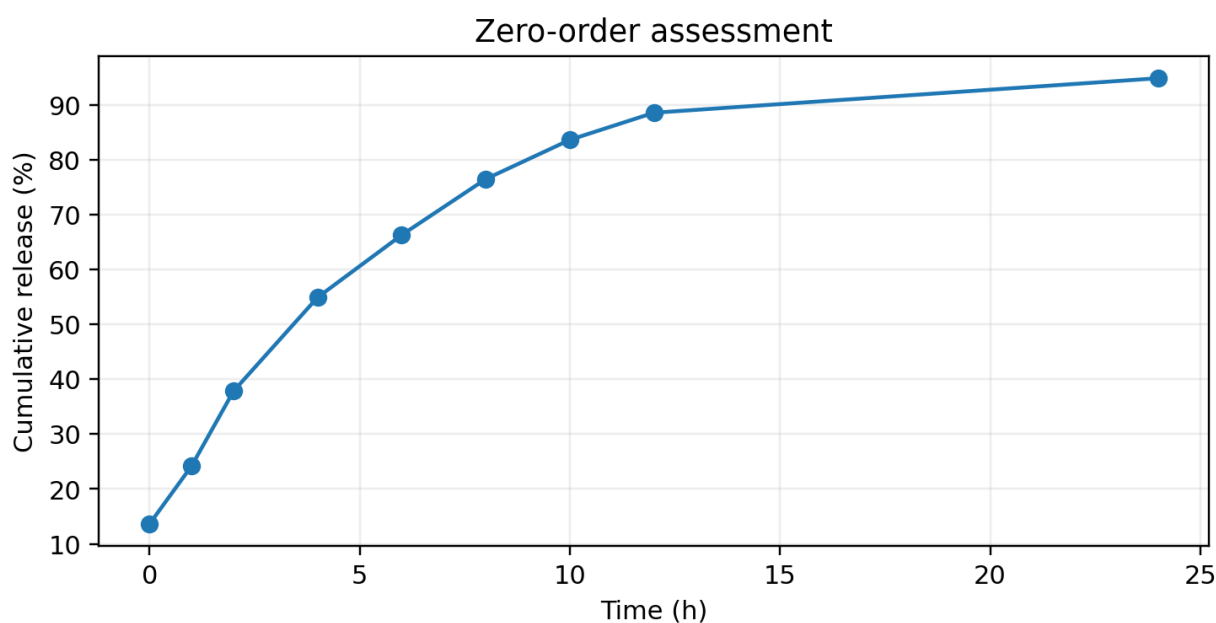


Figure 19: F6 Zero-order assessment.

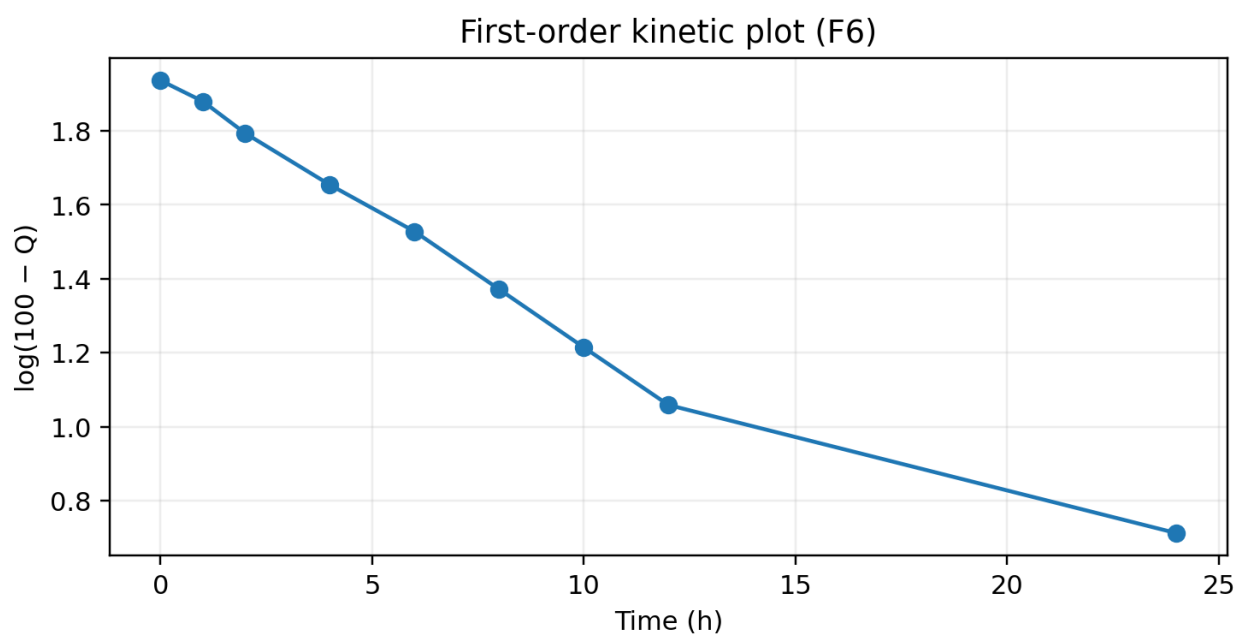


Figure 20: F6 First-order transformed plot.

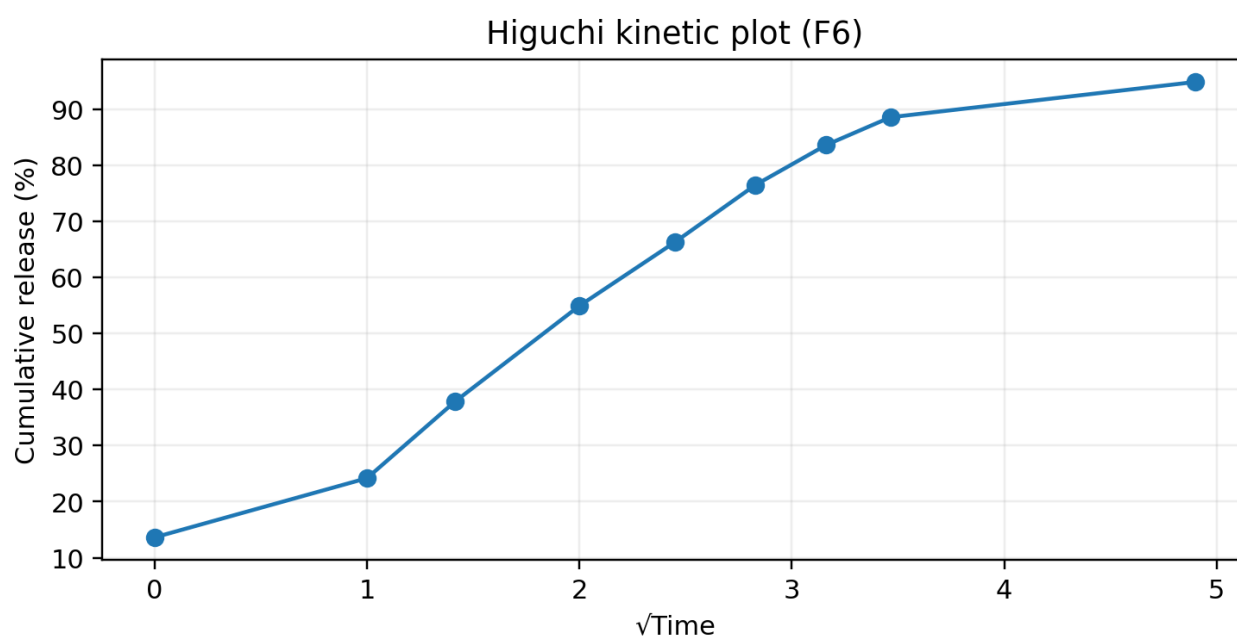


Figure 21: F6 Higuchi plot.

The source identifies Higuchi as the best-fit model because it had the highest reported R^2 (0.992). The Korsmeyer–Peppas exponent $n = 0.68$ was interpreted as non-Fickian/anomalous transport, involving diffusion and polymer relaxation.

18. Korsmeyer–Peppas Interpretation and RP-HPLC Development

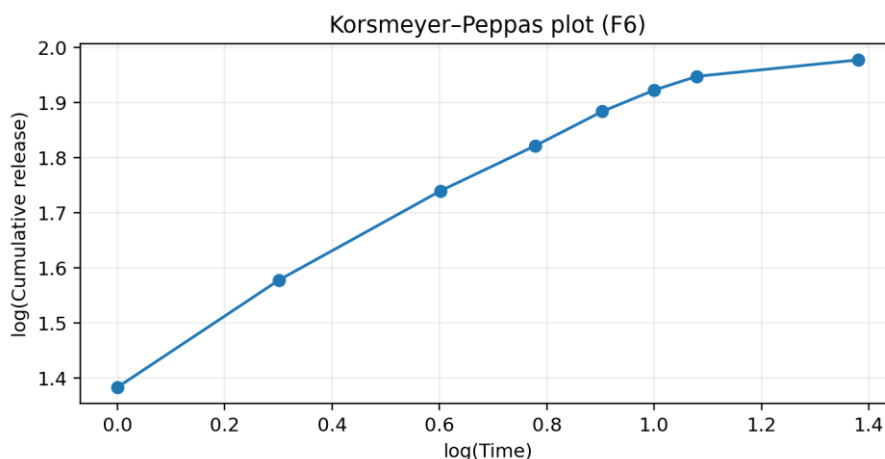


Figure 22: F6 Korsmeyer–Peppas transformed plot.

The release-model results are mechanistic descriptors of the experimental profile. The high Higuchi R^2 indicates a major diffusion-associated contribution, while the reported n value indicates a contribution from polymer relaxation.

18.1 RP-HPLC method development

The developed RP-HPLC method used a C18 column (250×4.6 mm, $5 \mu\text{m}$), acetonitrile:phosphate buffer 60:40 v/v, flow rate 1.0 mL/min, detection at 220 nm, injection volume 20 μL , ambient temperature and 10-min run time. Retention time was 4.82 ± 0.05 min.

Sr. No.	Parameter	Optimized Condition
1	Instrument	RP-HPLC System
2	Column	C18 Column (250×4.6 mm, $5 \mu\text{m}$)
3	Mobile Phase	Acetonitrile: Phosphate Buffer (60:40, v/v)
4	Flow Rate	1.0 mL/min
5	Detection Wavelength	220 nm
6	Injection Volume	20 μL
7	Column Temperature	Ambient ($25 \pm 2^\circ\text{C}$)
8	Run Time	10 min
9	Retention Time	4.82 ± 0.05 min

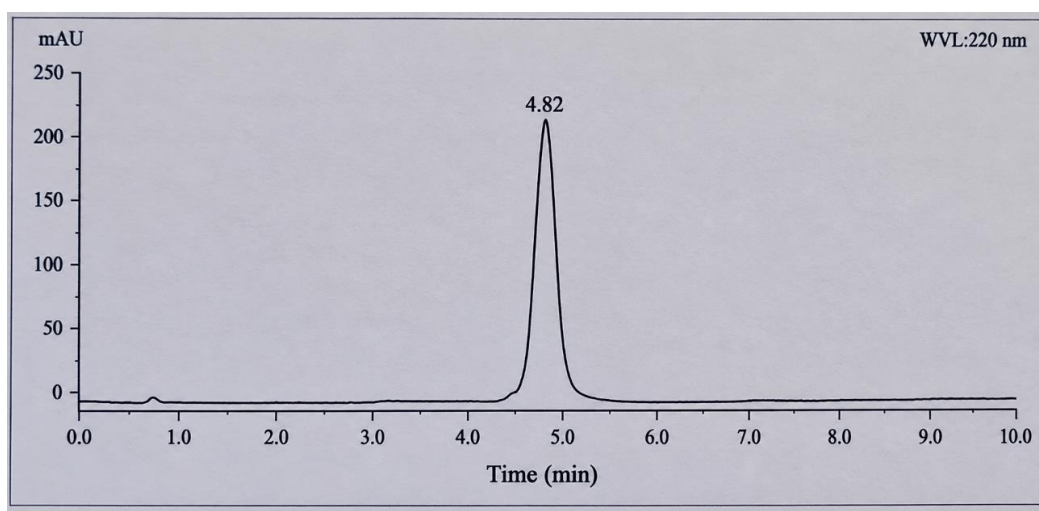


Figure 23: Supplied RP-HPLC chromatogram showing peak at 4.82 min.

19. RP-HPLC Calibration and Specificity

Sr. No.	Concentration (µg/mL)	Mean Peak Area
1	2	124,862
2	4	248,514
3	6	372,964
4	8	497,182
5	10	621,458
6	12	744,925
7	14	868,744
8	16	992,615
9	18	1,116,382
10	20	1,240,846

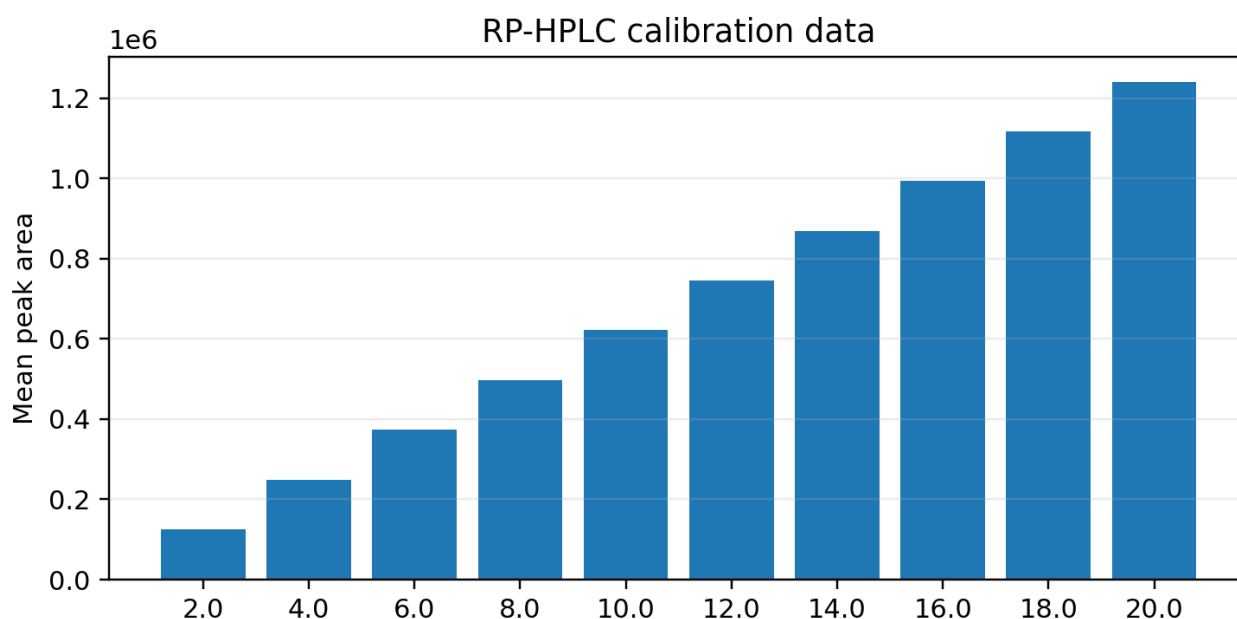


Figure 24: RP-HPLC calibration data (2–20 µg/mL).

Sr. No.	Parameter	Result
1	Concentration Range	2–20 µg/mL
2	Regression Equation	$y = 61989x + 980.6$
3	Correlation Coefficient (R^2)	0.9998

The method showed a linear relationship over 2–20 µg/mL with regression equation $y = 61989x + 980.6$ and $R^2 = 0.9998$.

19.1 Specificity

Sr. No.	Sample	Observation
1	Blank	No interfering peak observed
2	Placebo	No interfering peak observed
3	Standard	Sharp and symmetrical peak at 4.82 min
4	Sample	No interference from formulation excipients

No interfering peak was reported at the marine peptide retention time in blank, placebo or formulation samples. The standard produced a sharp, symmetrical peak at approximately 4.82 min.

20. RP-HPLC Validation

Sr. No.	Recovery Level	% Recovery	%RSD
1	80%	99.18	0.72
2	100%	99.84	0.56
3	120%	100.42	0.48

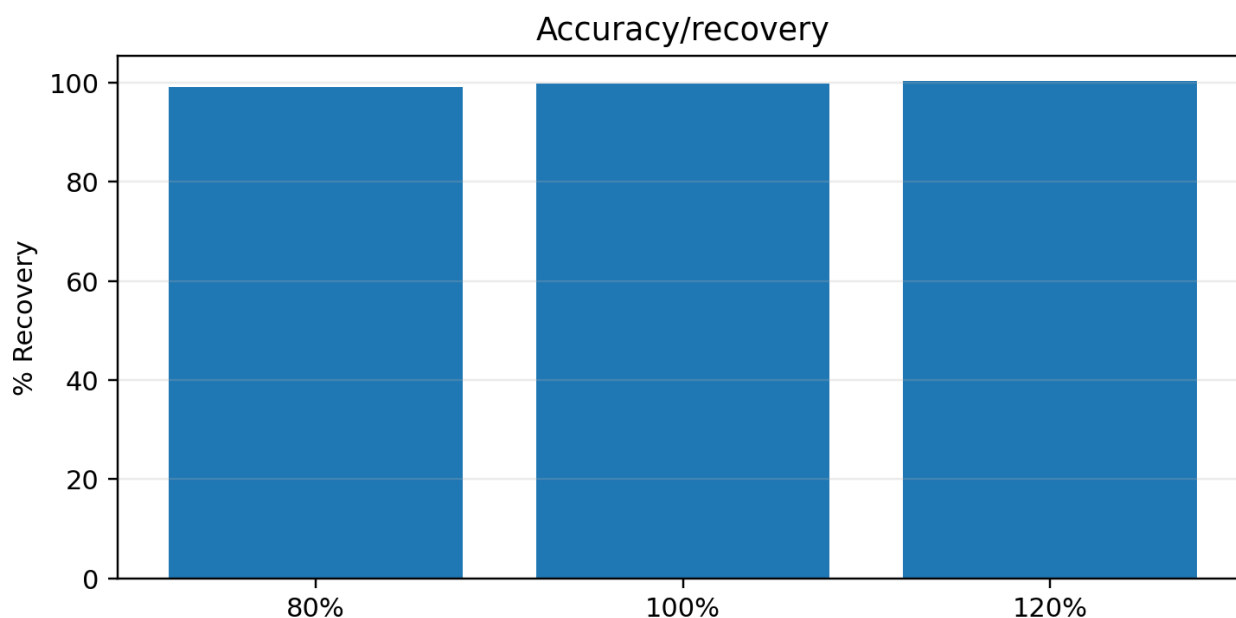


Figure 25: Recovery at 80%, 100% and 120% levels.

Sr. No.	Precision Parameter	%RSD
1	Intra-day Precision	0.84
2	Inter-day Precision	1.12

Recovery ranged from 99.18% to 100.42%, with %RSD values of 0.48–0.72%. Intra-day precision was 0.84% RSD and inter-day precision was 1.12% RSD.

Sr. No.		Parameter		Result	
1		LOD		0.18 µg/mL	
Sr. No.		Parameter		Result	
1		LOQ		0.56 µg/mL	
Sr. No.	Parameter Varied		Variation	%RSD	Observation
1	Flow Rate		±0.1 mL/min	0.96	No significant effect
2	Detection Wavelength		±2 nm	1.08	No significant effect
3	Mobile Phase Composition		±2% Organic Phase	1.14	No significant effect

The reported LOD was 0.18 µg/mL and LOQ 0.56 µg/mL. Robustness testing produced %RSD values of 0.96–1.14% for the tested deliberate changes, with no significant effect reported.

21. Stability Study: Long-Term Condition

Time	Appearance	pH	Viscosity (cP)	Drug content (%)	Release (%)
0 month	Transparent; smooth; no separation	6.48	20,560	99.24	94.85
1 month	No change	6.46	20,482	99.02	94.62
2 months	No change	6.44	20,395	98.74	94.28
3 months	No change	6.42	20,268	98.42	93.96

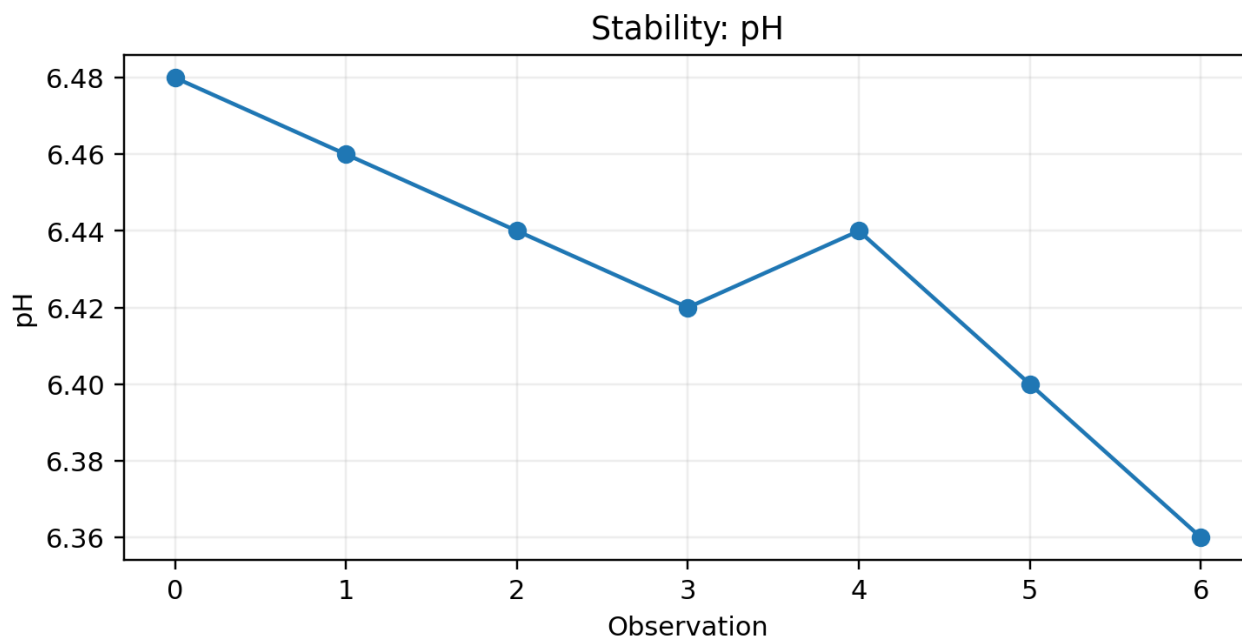


Figure 26: pH trend across the reported stability observations.



Figure 27: Viscosity trend across the reported stability observations.

Under long-term storage, pH declined from 6.48 to 6.42 and viscosity from 20,560 to 20,268 cP. Drug content remained above 98%, and release remained above 93%. The source reports no visible phase separation or other major physical change.

22. Stability Study: Accelerated Condition.

Time	Appearance	pH	Viscosity (cP)	Drug content (%)	Release (%)
1 month	No significant change	6.44	20,336	98.78	94.18
2 months	Slight decrease in viscosity	6.40	20,145	98.36	93.74
3 months	No phase separation; slight viscosity reduction	6.36	19,984	97.92	93.26

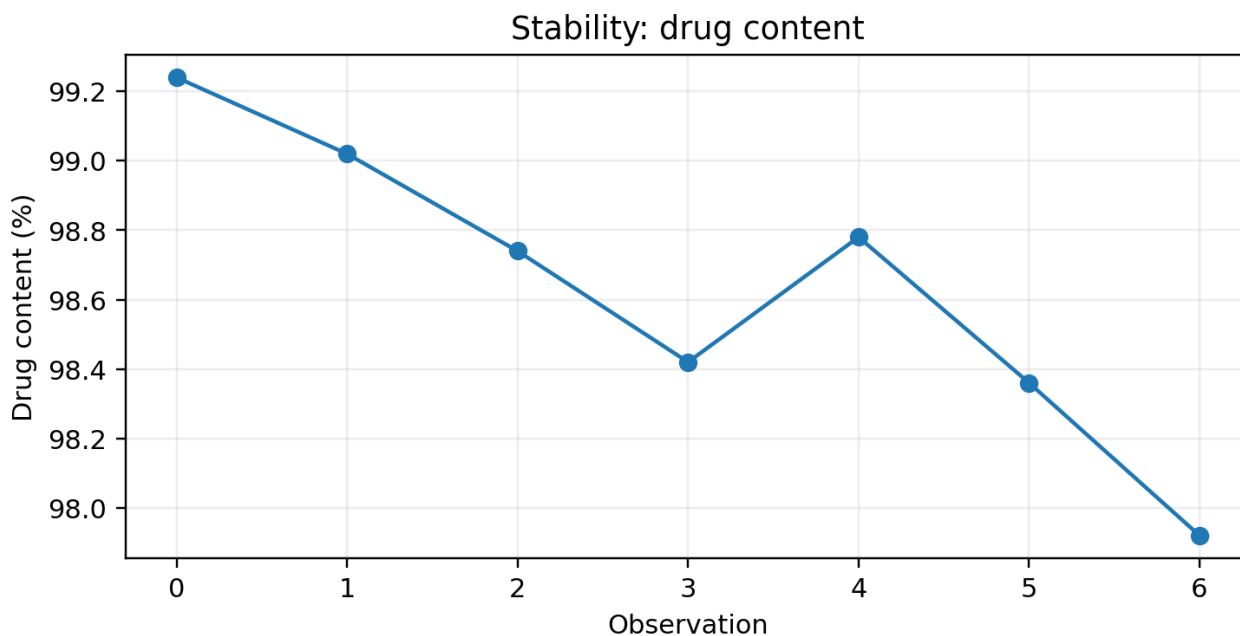


Figure 28: Drug-content trend across the complete stability dataset.

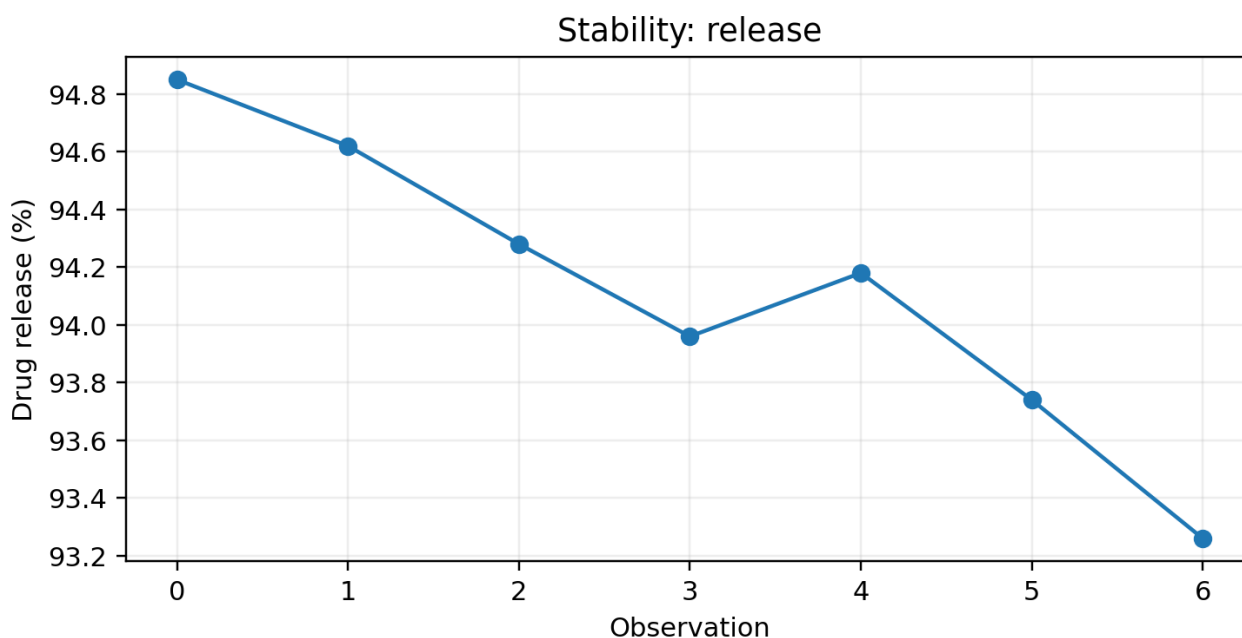


Figure 29: Release trend across the complete stability dataset.

22.1 Comparative stability summary.

Parameter	Initial	Long-Term (3 Months)	Accelerated (3 Months)	Observation
Physical Appearance	Transparent	No Change	No Significant Change	Stable
pH	6.48	6.42	6.36	Within acceptable range
Viscosity (cP)	20,560	20,268	19,984	Slight decrease
Drug Content (%)	99.24	98.42	97.92	Within acceptable limits
In Vitro Drug Release (%)	94.85	93.96	93.26	Sustained release maintained

At three months, accelerated storage produced somewhat larger numerical changes than long-term storage, particularly in pH, viscosity and drug content. Nevertheless, the source reports retained transparent appearance, no phase separation and sustained release above 93%. Because the study covers three months, these data support the tested short-term stability period rather than establishing a formal commercial shelf life.

23. Integrated Results and Discussion

The study shows a sequential optimization strategy. Preformulation established water/phosphate-buffer compatibility, near-neutral pH and a UV maximum at 220 nm. FTIR and DSC supported preservation of characteristic peptide features in the presence of the selected excipients.

At the liposome stage, TF6 combined the smallest particle size (148.6 nm), lowest PDI (0.214), most negative zeta potential (−31.8 mV), highest entrapment efficiency (91.84%), highest drug loading (8.62%) and highest drug content (99.12%) among the listed batches. TEM provided visual confirmation of spherical vesicles.

At the hydrogel stage, increasing Carbopol® 940 raised viscosity while reducing spreadability and release. F6 provided a balance: pH 6.48, viscosity 20,560 cP, spreadability 26.82 g·cm/s, drug content 99.24% and 24-hour release 94.85%. This balance was the basis reported for optimization.

Release testing demonstrated that matrix composition influenced peptide transport. F1 released 98.12% by 24 h, whereas F9 released 91.84%; F6 released 94.85%. Higuchi had the highest reported kinetic fit (R^2 0.992), while $n = 0.68$ supported the reported anomalous/non-Fickian interpretation.

The RP-HPLC method showed R^2 0.9998 across 2–20 µg/mL, recovery of 99.18–100.42%, low precision variability and robustness %RSD below 1.2%. Stability testing showed modest changes over three months, with drug content remaining 98.42% under long-term storage and 97.92% under accelerated storage.

23.1 Study limitations

- The complete Design-Expert® ANOVA tables and polynomial coefficients were not included in the supplied result file and are therefore not reconstructed.
- The supplied material describes marine peptide at a class level rather than providing a single fully specified peptide sequence.
- The reported work is formulation, analytical and in vitro in scope; the dataset does not provide clinical outcome measurements.

24. CONCLUSION

The supplied experimental dataset demonstrates successful development and optimization of a marine peptide-loaded liposomal hydrogel for topical burn-wound delivery. The optimized liposome TF6 showed nanosized vesicles, narrow distribution, negative surface charge, high entrapment, drug loading and drug content. The optimized hydrogel F6 retained these features while providing suitable pH, viscosity, spreadability, extrudability, gel strength and drug content.

The 24-hour release study demonstrated controlled release, with F6 reaching $94.85 \pm 1.24\%$ cumulative release. Higuchi kinetics produced the highest reported fit ($R^2 = 0.992$), while the Korsmeyer–Peppas exponent of 0.68 supported the reported non-Fickian/anomalous transport interpretation. The RP-HPLC method demonstrated specificity, linearity, accuracy, precision, sensitivity and robustness within the tested conditions. Three-month stability testing showed only minor changes in the evaluated quality attributes.

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