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FORMULATION AND EVALUATION OF LIPOSOME CONTAINING QUERCETIN HAVING ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

This study focuses on the formulation and evaluation of a liposomal drug delivery system containing Quercetin to enhance its anti-inflammatory properties. Quercetin is a flavonoid with potent anti-inflammatory, antioxidant, and immunomodulatory effects, but its use is limited by poor aqueous solubility, low bioavailability, and rapid metabolism. Liposomes, which are spherical phospholipid bilayer vesicles, were used to address these issues by improving the stability, solubility, and pharmacokinetics of Quercetin. The liposomes were prepared using the thin film method with Quercetin, soy lecithin, cholesterol, and chloroform-methanol. The final formulation was evaluated for several parameters, including pH, particle size, zeta potential, and solubility. The optimized liposome batch had a pH of 6.40, a particle size of 168.1 nm, and a zeta potential of -14.9 mV. The particle size suggests a nanoscale formulation suitable for enhanced skin absorption, while the zeta potential indicates moderate colloidal stability. In vitro studies demonstrated the formulation's therapeutic potential. The anti-inflammatory activity was assessed by measuring the inhibition of protein denaturation, with the liposomal formulation showing 61.7% inhibition at 100 µg/mL, which is comparable to the 72.29% inhibition by the standard drug, Diclofenac sodium. The in vitro dissolution study confirmed a sustained release profile, and stability studies showed the formulation remained stable for one month under accelerated conditions, adhering to ICH guidelines. These results indicate that the Quercetin-loaded liposomal formulation is a promising strategy for delivering the compound with improved therapeutic efficacy and stability.

KEYWORDS: Quercetin, Liposome, Anti-inflammatory activity, In-vitro evaluation, Stability studies.

INTRODUCTION

Quercetin, a bioactive flavonoid present in numerous fruits, vegetables, and medicinal plants, has garnered significant attention for its potent anti-inflammatory, antioxidant, and immunomodulatory properties.[1] Its ability to inhibit pro-inflammatory mediators such as cyclooxygenase, lipoxygenase, and cytokines positions quercetin as a promising candidate for managing chronic inflammatory conditions.[2] However, its therapeutic potential is often limited by inherent challenges such as poor aqueous solubility, low bioavailability, and rapid systemic metabolism, which collectively reduce its efficacy when administered in traditional formulations.[3] To address these limitations, the incorporation of quercetin into liposomal drug delivery systems has emerged as a promising strategy. Liposomes are spherical vesicles composed of phospholipid bilayers that can encapsulate both hydrophilic and lipophilic drugs, thereby improving their stability, solubility, and pharmacokinetics.[3] By encapsulating quercetin, liposomes protect the active compound from enzymatic degradation, facilitate its targeted delivery to inflamed tissues, and provide sustained release, thereby enhancing its anti-inflammatory effects.[4] Recent studies have demonstrated that quercetin-loaded liposomes significantly reduce inflammatory markers in various models of inflammation, highlighting their potential as an effective therapeutic intervention for inflammatory disorders.[5]

This innovative approach not only addresses the pharmacokinetic drawbacks of quercetin but also ensures

its controlled release at the site of action, minimizing systemic side effects and optimizing therapeutic outcomes. Therefore, quercetin-loaded liposomes represent a novel and efficient method for exploiting the anti-inflammatory benefits of quercetin in clinical settings.

MATERIALS AND METHODS

Materials

Quercetin was purchased from Yucca Phytochemicals, Mumbai.

Soy lecithin, Cholesterol, Chloroform, methanol these excipients purchased from Loba chemie, Mumbai.

Formulation of liposome

Liposomes were prepared by the thin-film hydration method with slight modifications, as described in earlier studies.[13,4] Briefly, varying concentrations of soya lecithin (100–300 mg) and cholesterol (100–300 mg) were dissolved in a chloroform–methanol mixture (1:1, v/v). Quercetin (200 mg) was incorporated into this organic phase to ensure uniform drug dispersion.[11]

The solvent was evaporated under reduced pressure using a rotary evaporator to obtain a thin lipid film on the wall of the round-bottom flask.[4] The dried film was then hydrated with 10 mL of phosphate-buffered saline (PBS, pH 7.4) at room temperature, resulting in the formation of multilamellar vesicles (MLVs).[13]

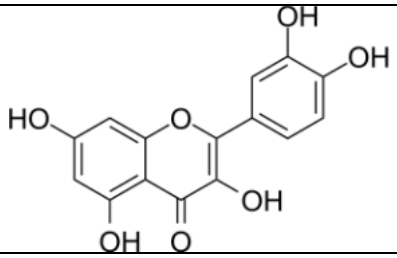
The suspension was vortexed for 30 minutes to enhance detachment of lipid layers[14], followed by probe sonication for 10–60 minutes to reduce the vesicle size and obtain small unilamellar vesicles (SUVs).[4]

Table 1: Composition of liposome formulation.

Ingredients	L1	L2	L3	L4
Drug	200 mg	200 mg	200 mg	200 mg
Soy lecithin	300 mg	250 mg	300 mg	250 mg
Cholesterol	100 mg	150 mg	150 mg	100 mg
Chloroform - methanol	10 ml	10 ml	10 ml	10 ml
PBS (7.4)	10 ml	10 ml	10 ml	10 ml
Sonication time	30 min	30 min	30 min	30 min

Docking

Table 2: Docking.

Quercetin	
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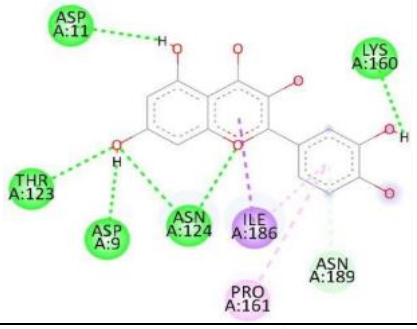
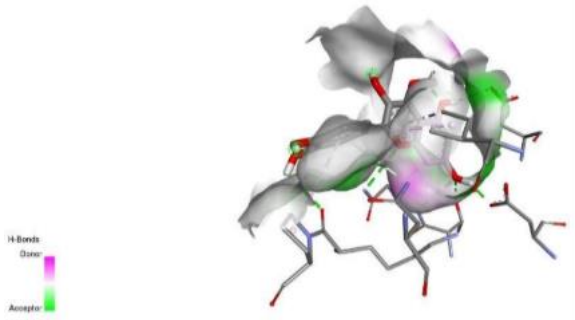
2D	
3D	

Energy	-8.9					
Bond	A:THR	A:ASN	A:ASN	A:LYS	A:ASP	A:ASP
Bond length	3.1372	3.1364	3.3057	2.7457	2.2038	1.8523

Evaluation parameter of liposome formulation

1. Calibration curve

1) Standard calibration curve of drug optimized liposome. An accurately weighed 100 mg of liposome is dissolved in pH 6.8 Phosphate buffer and make up the volume up to 100 ml in a volumetric flask (Stock solution: I mg/ml).
2) From this 10 ml of solution was pipette out and make up the volume up to 100 ml (Stock solution: II), 100 µg/ml).
3) The aliquots were prepared by taking 0.5ml, 1ml, 1,5ml, 2ml, 2.5ml and 3ml and dilute to 10ml whose concentration ranging from 5-30 µg/ml and the absorbance was measured at 252 nm by using UV spectrophotometer, against the blank.

2. FTIR (Fourier Transform Infrared Spectroscopy)

FTIR spectroscopy was performed on FTIR spectrophotometer and it is used to study and find out whether there are interactions between the drug and Excipients used in the formulation. The infrared (IR) spectra were recorded using an FTIR spectrophotometer.

3. Particle size

The particle size is one of the most important parameters for the characterization of liposome. The size of optimized liposome was measured using Horiba SZ-100 instrument.

4. pH

The pH of liposome preparation has been done using Digital pH meter.

5. Solubility

The liposome demonstrated good solubility in a range of solvents including water, ethanol, methanol, and phosphate-buffered saline (PBS), indicating its favourable compatibility with both aqueous and organic media. This solubility profile suggests that the formulation may facilitate efficient drug dispersion, enhance bioavailability of the active herbal constituents, and support consistent therapeutic release under physiological conditions.

6. Zeta Potential

The zeta potential of liposome preparation has been done using Horiba SZ-100 instrument.

7. In-vitro Dissolution study

The in-vitro studies of formulated liposome were evaluated using Franz diffusion cell with a semi - permeable membrane. The diffusion cell included an upper cylindrical compartment that was open from above, a diffusion membrane at its base, and a diameter of 1.5 cm with a capacity of 20 ml. The donor part contained 1g of drug, whereas the receptor section had 20 ml of 6.8 pH phosphate buffer.

During the experiment, the temperature was kept at 37°C ± 0.5 °C, and a magnetic stirrer was used to agitate the buffer at 100 rpm. After then, for a maximum of 1 hours, 5 ml samples were taken out of the receiving chamber every hour and replaced with a fresh buffer solution of the same volume. The extracted samples were observed for absorbance using UV spectrophotometer at 245 nm.

8. Anti-inflammatory activity

1) The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of plant extract so that final concentrations become 20, 40, 60, 80 and 100µg/ml.
2) Similar volume of double- distilled water served as control. Then the mixtures were incubated at (37±2) °C in Incubator (Universal) for 15min and then heated at 70 °C for 5min.
3) After cooling, their absorbance was measured at 660 nm (Shimadzu 1700) by using vehicle as blank.
4) Diclofenac sodium at the final concentration of (20-100µg/ml) was used as reference drug and treated similarly for determination of absorbance and viscosity.
5) The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 × (Vt / Vc -1)

Where,

Vt = absorbance of test sample,

Vc = absorbance of control

9. Stability studies

The loaded liposome formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing (250C±20C and 60±5% RH) and (400C±20C and 70±5% RH) for 1 months. The formulation was checked for evaluation parameter viscosity and pH at the interval of 30 days. The formulation was tested for stability under accelerated storage condition for 1 months in accordance with International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in evaluation parameter viscosity and pH. All Results were compared against final formulation of 0 days as the reference.

RESULT AND DISCUSSION

1. Standard Calibration Curve

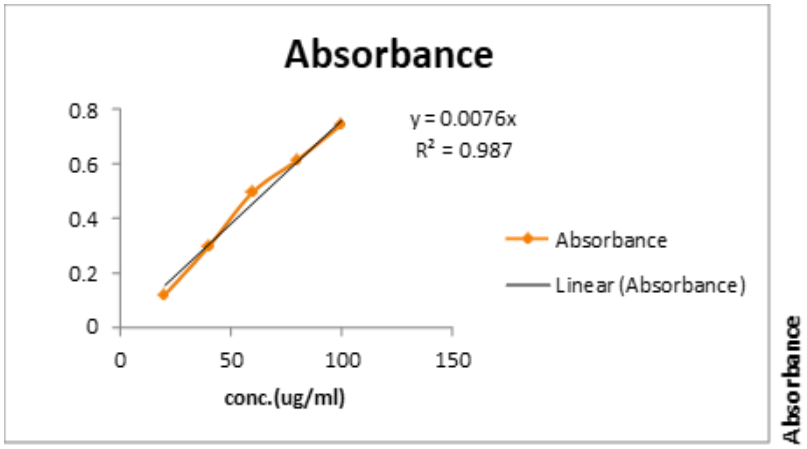


Fig 1. Calibration curve.

Table 3: Absorbance.

Conc.(ug/ml)	Absorbance
20	0.115
40	0.294
60	0.494
80	0.612
100	0.742

2. FTIR

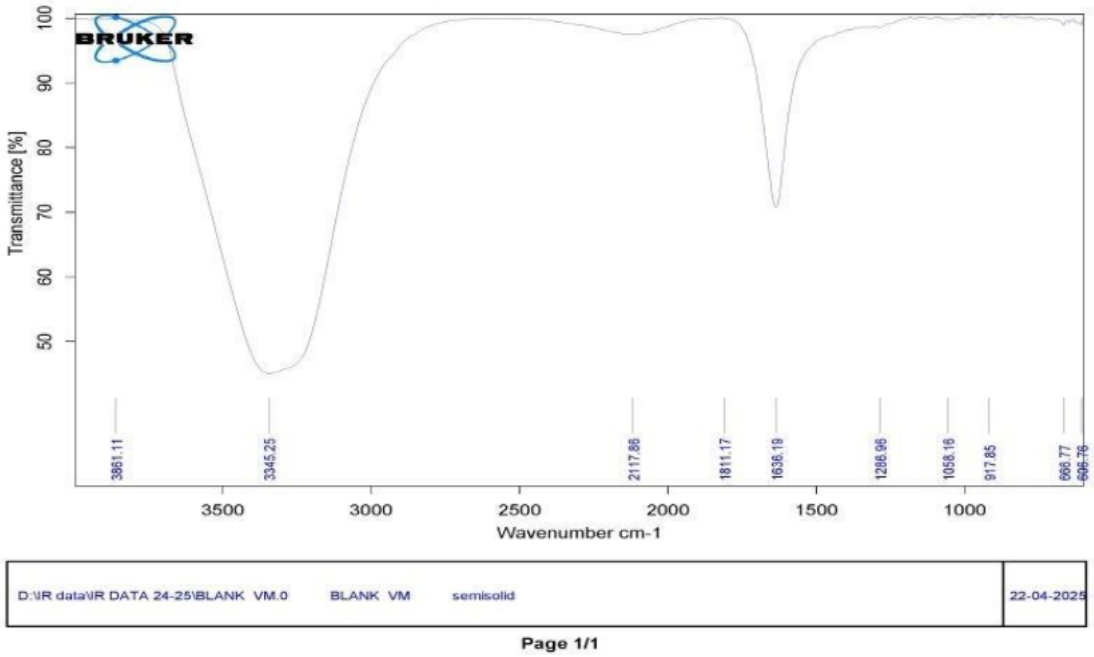


Fig. 2: FTIR (without quercetin).

Table 4: Interpretation of FTIR (without quercetin).

Sr.No	Frequency Range	Functional Group
1	1266.96	Alkyl Ketone
2	1636.19	Ketones
3	1811.17	Carbonyl group
4	2117.86	C=C stretching bond of alkynes molecule
5	3345.25	Bonded N-H/C-H/O-H stretching of amines and amides
6	3861.11	O-H stretching vibration

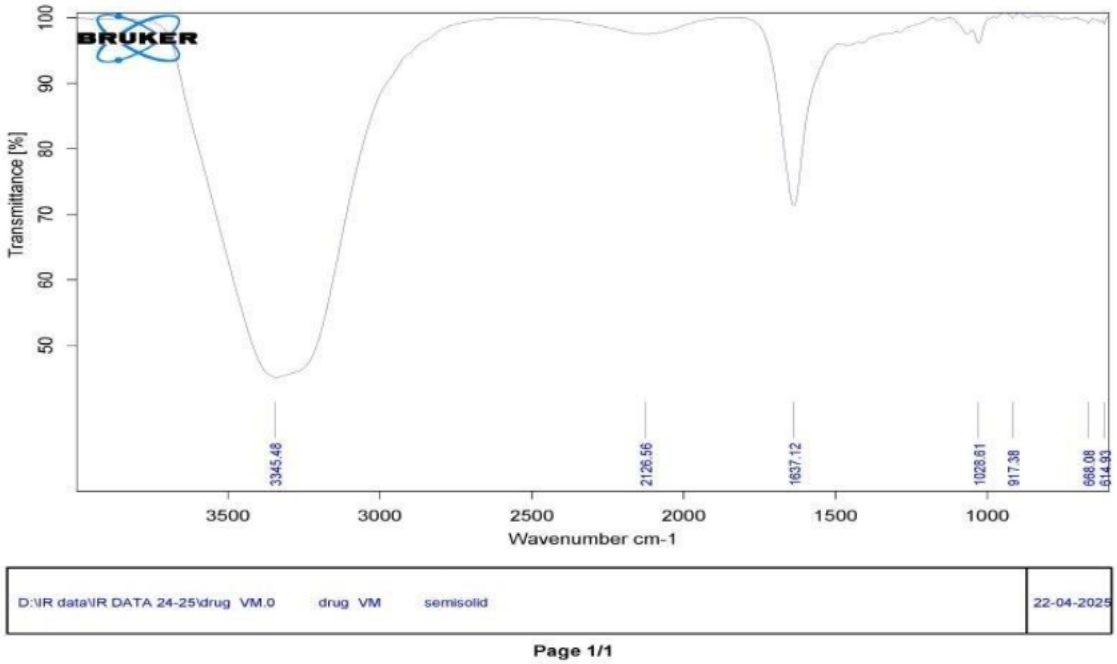


Fig. 3: FTIR with quercetin.

Table 5: Interpretation of FTIR with quercetin.

Sr.No	Frequency Range	Functional Group
1	1028.61	Alkyl amines
2	1637.12	Ketones
3	2126.56	C-N bond
4	3345.48	Bonded N-H/C-H/O-H stretching of amines and amides.

3. Particle Size

Table 6: Particle size.

Peak no.	S.P.Area ratio	Mean	S.D.	Mode
1	1.00	168.1 nm	45.7 nm	160.4 nm

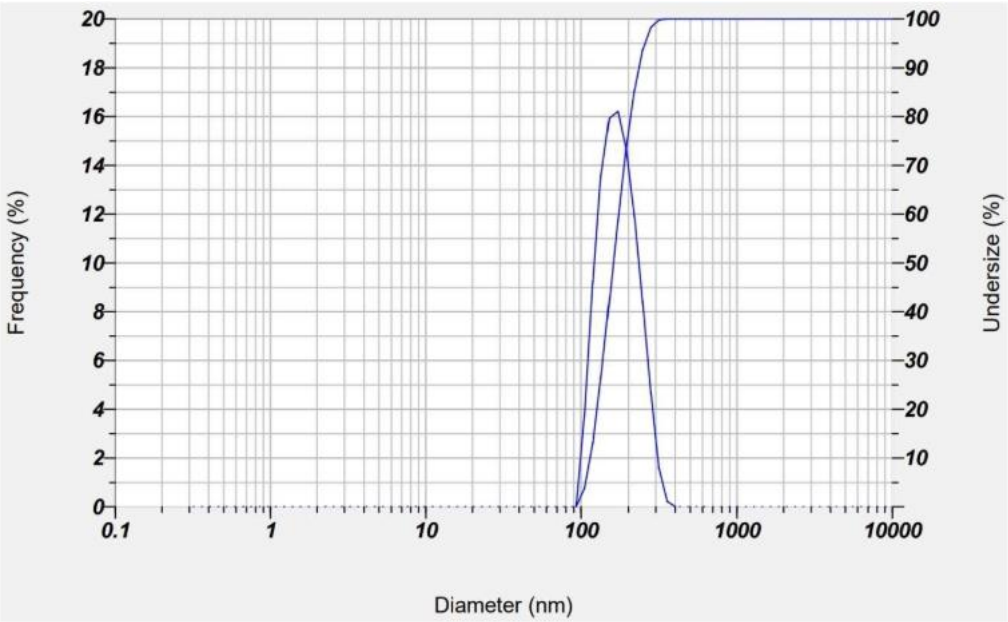


Fig. 4. Particle size.

4. PH

The pH of optimized liposome batch is 6.40.

Fig. 5: pH meter.

5. Zeta Potential

Table 7: Zeta Potential.

Peak no.	Zeta Potential	Electrophoretic Mobility
1	-14.9 mV	-0.000115 cm2/Vs

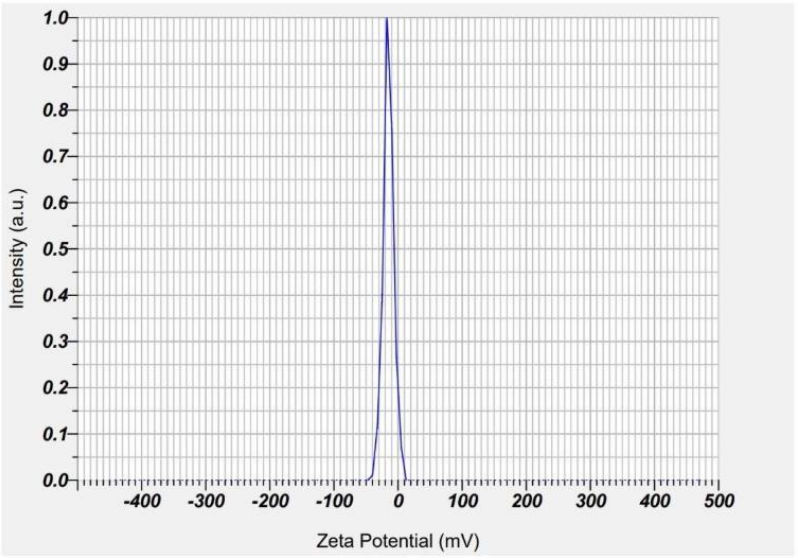


Fig. 6: Zeta potential.

The value -14.9 mV indicates the surface charge of the particles in liposomes. The zeta potential value in this range typically suggests moderate stability; the particles are likely to aggregate over time but may remain stable under control conditions.

6. In-Vitro Dissolution Study of Optimized Batch

Fig. 7: In-vitro dissolution Study.

Table 8: Absorbance of optimized batch.

Sample	Absorbance
Sample 1	0.071
Sample 2	0.138
Sample 3	0.201
Sample 4	0.268
Sample 5	0.324

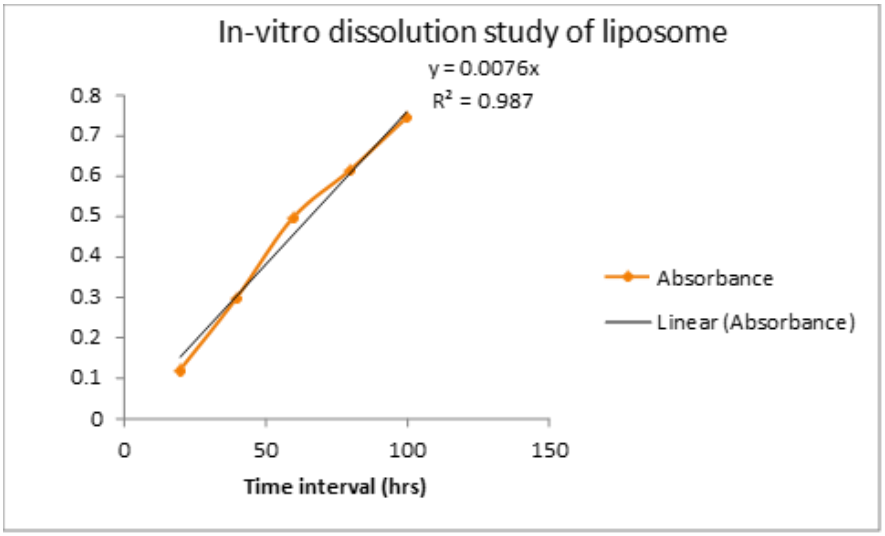


Fig. 8: In-vitro dissolution study of optimized batch.

7. In-Vitro Anti-inflammatory Study

Table 9: Effect of Diclofenac sodium (Standard) on protein denaturation.

Sr.no	Concentration of extract (µg/ml)	Absorbance at 660 nm	% Inhibition
1	Control	1.101	-
2	20	0.733	33.42
3	40	0.616	44.05
4	60	0.533	51.58
5	80	0.425	61.39
6	100	0.305	72.29

Table 10: Effect of Optimized batch on protein denaturation.

Sr.no	Concentration of extract (µg/ml)	Absorbance at 660 nm	% Inhibition
1	Control	1.021	-
2	20	0.016	1.56
3	40	0.360	35.2
4	60	0.494	48.3
5	80	0.605	59.2
6	100	0.630	61.7

CONCLUSION

The present study successfully formulated and evaluated a quercetin-loaded liposomal system for its anti-inflammatory potential. The optimized formulation exhibited favorable characteristics, including a pH of 6.40 (skin-compatible), nanoscale particle size of 168.1 nm (enhanced absorption), and a zeta potential of −14.9 mV (moderate stability). FTIR confirmed no major drug–excipient interactions, while in-vitro anti-inflammatory testing showed 61.7% inhibition of protein denaturation at 100 µg/mL, approaching the activity of diclofenac sodium (72.29%). The formulation also demonstrated sustained drug release and remained stable under accelerated storage conditions for one month, complying with ICH guidelines.

Overall, the liposomal system effectively enhanced the therapeutic potential of quercetin by improving its solubility, stability, and permeability. These findings suggest that quercetin liposomes represent a promising approach for topical management of inflammatory

conditions, warranting further preclinical and clinical investigation.

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