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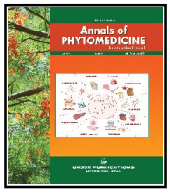

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Development and evaluation of quercetin proliposomal gel: A comprehensive study on anti-inflammatory activity

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Abstract

The purpose of the research was to develop and characterise quercetin proliposomes using the thin-film deposition technique with carriers such as mannitol, cholesterol, and soya lecithin. The proliposome formulations were characterised in terms of particle size analysis, surface charge, polydispersity index, drug content, entrapment efficiency, SEM, FT-IR, and stability studies. In this study, the proliposome formulation was mixed into a gel matrix, and then its rheological properties and behaviour were studied by *in vitro* methods. Quercetin proliposome was prepared by using different formulations like QPLF1, QPLF2, QPLF3, and QPLF4. However, the findings from the QPLF3 formulations revealed that the greatest entrapment efficiency (92.96%), which was influenced by the concentrations of phospholipid and cholesterol. The *in vitro* drug permeation study results showed a cumulative release rate of the QPLF3 as 86.28%, which further confirm this gel can sustain drug retention over an extended period of time. The *in vivo* paw edema anti-inflammatory model on proliposome gel (QPLF3) was found to be significantly more, with a maximum percentage of inhibition at 64.80%, compared to a commercially available gel that showed 54.51%. A stability study confirmed that the product remains stable when stored at refrigerator temperature. This quercetin-based proliposome gel has shown sustained release and improved anti-inflammatory effects, indicating its promise for effective topical treatment of inflammation.

1. Introduction

Inflammation is the body's innate protective reaction to damage tissues or injury (Michels da Silva *et al*., 2019). It is characterised by symptoms such as redness, pain, heat, swelling, and functional impairment in the affected area. Infections, burns, trauma, and various immune reactions are common triggers of inflammation (Fan *et al*., 2003). During an acute inflammatory response, cellular and molecular events reduce the threat of injury or infection (Rang *et al*., 2008).

Proliposomes stand as an innovative category within drug delivery systems that offer significant benefits over traditional liposomes (Noh, 1984). They are more stable and can be easily sterilised on a large scale, avoiding problems such as drug overload. Due to their ability to encapsulate a larger number of drugs, proliposomes facilitate penetration of drugs and ensure sustained release at the target site (Mallesh *et al*., 2012).

Quercetin, a flavonoid naturally occurring in fruits and vegetables, possesses distinctive biological properties that could enhance both mental and physical function while reducing the risk of infection (Mallesh and Diwan, 2011). Quercetin is extensively employed as a therapeutic remedy for various diseases (Rice-Evans *et al*., 2006). Vesicular carriers serve a crucial function in improving the solubility and bioavailability of the active compound. These benefits enable vesicular carriers to achieve a high therapeutic potential (Park *et al*., 2013).

Additionally, vesicular carriers aid in achieving sustained drug release, making them a focus of extensive research for topical drug delivery due to their distinctive phospholipid complex (Rahman *et al.,* 2010). The current research was to prepare and characterise the proliposome gel containing quercetin for effective topical drug therapy in overcoming the problems associated with the oral administration or in the treatment of inflammation by the technique of thin film deposition with a vacuum rotary evaporator.

2. Materials and Methods

2.1 Sample collection

Quercetin was obtained from Himedia laboratories, and soya lecithin was purchased from Glenmark Generic Limited, Mumbai. Mannitol, cholesterol, and carbopol 934 were procured from Nice Chemicals Pvt. Ltd., India, Mumbai. All remaining chemicals and materials employed for the investigation are of the analytical grade.

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2.2 Formulation of quercetin proliposome

Quercetin proliposomes (QPL) were developed utilising the thin film deposition technique employing a rotovap (SUPEFIT Rotatory Vacuum, India) (Ahniah *et al*., 1995). Various proliposome formulations were prepared using different concentrations of mannitol, soya lecithin, and cholesterol. Initially, 1 g of powdered mannitol (passed through a 100-mesh sieve) was added in a round-bottom flask and dried under vacuum at 60-70°C and 115 rpm for a period of 30 min. Quercetin (100 mg), lecithin, and cholesterol were mixed in chloroform and methanol (in a proportion of 8:2 v/v) for different formulations as outlined in Table 1. Subsequently, a 5 ml mixture of organic solvents was added to the round-bottom flask and stirred at 37°C. After the solution had completely dried, an additional 5 ml aliquot was added, and this procedure was repeated until the entire 10 ml of solution was utilized. The resulting developed proliposome is stored in a flask, dried overnight in a vacuum desiccator before being sieved through a 100-mm sieve (Mallesh *et al*., 2012).

Table 1: Composition of quercetin proliposome

Formulation	Quercetin (g)	Mannitol (g)	Soya lecithin (g)	Cholesterol (g)
QPLF1	0.1	1	0.05	0.03
QPLF2	0.1	1	0.1	0.03
QPLF3	0.1	1	0.2	0.05
QPLF4	0.1	1	0.3	0.05

2.3 Characterization of proliposomes

2.3.1 Compatibility studies (FT-IR)

FT-IR spectra analysis was to confirm the composition and interactions of the drug and excipient molecules within the quercetin proliposome in the solid state. The analysis was performed using a Shimadzu FT-IR-8400 spectrophotometer with a wavelength range of 400-4000 cm^{-1}. The KBr pellet method was utilised to investigate functional groups of molecules, with a scan range set at 5 cm/min and a resolution of 4 cm^{-1}.

2.3.2 Vesicle size

The Quercetin proliposome vesicle size was analysed at 25°C using a disposable cell-equipped Malvern Zeta sizer. Version 6.01 of the dispersion technology program (Malvern) was used for data collection and analysis. This analysis provides insights into the particle size distribution and homogeneity of the quercetin proliposomes (Barupal *et al.,* 2010).

2.3.3 Zeta potential

The zeta potential is essential in characterisation studies as it shows the stability of proliposome formulations. In formulations stabilised by both electrostatic and steric stabilisers, a zeta potential below 20 mV is normally considered adequate for stability. The prepared proliposome formulation was performed with the Malvern Zetasizer from Malvern Instruments. Prior to measurement, the samples were diluted with deionised water, and the conductivity was adjusted by adding sodium chloride (Kurakula *et al.,* 2012).

2.3.4 Drug content

The quercetin concentration of the proliposome formulations was assessed using UV spectroscopy. The various batches of quercetin proliposomes, equal to 10 mg of quercetin proliposome, were precisely measured and dissolving in 10 ml of methanol. Subsequently, these solutions were diluted with phosphate buffer to the desired volume, ensuring thorough mixing, and were then subjected to UV spectroscopy analysis at a wavelength of 369 nm. This approachable, curated determination of the quercetin content in the proliposome formulations (Amsa *et al*., 2022).

Drug content = (Sample absorbance/Standard absorbance) × 100

2.3.5 Entrapment efficiency

The encapsulation efficiency of quercetin-loaded proliposomes was assessed through ultracentrifugation to separate the unencapsulated drug. The 5 ml of the prepared liposomal formulation was ultracentrifugation (using an Optima MAX-XP centrifuge by Beckman Coulter Inc., USA) at 6,000 rpm for 20 min, facilitating the isolation of unencapsulated drugs. Subsequently, the supernatant, containing the free drug, and the pellet, comprising the entrapped drug, were segregated through decantation. The pellet was then redispersed in 3 ml of pH 7.4, and this suspension was vigorously shaken for 10 min and then subjected to sonication for 5 min to ensure complete dispersion of the liposomes. The drug content was analysed in both the pellets and the supernatant using a UV spectrophotometer, with measurements obtained at 369 nm (Payne *et al.,* 1986).

% Entrapment efficiency = (Experimental drug content/Theoretical drug content) × 100

2.3.6 Scanning electron microscopy

Scanning electron photography of quercetin proliposomes was taken with a scanning electron microscope. The tested samples were mounted on to an aluminium stub with dual-sided adhesive tape. They were then coated with a platinum layer, approximately 10 Å thick, under an argon atmosphere using a gold sputtering module in a high vacuum evaporator. Once coated, the rod holding the samples was inserted into the SEM chamber for imaging (Rahul Jodh *et al*., 2022).

2.3.7 The yield of proliposomes

The proliposome powders had been thoroughly dried; they were carefully collected and weighed with precision. A calculation formula was employed to approximate the yield of proliposomes.

Percentage yield = [Total weight of proliposome/(Total weight of drug + Total weight of added content)] x 100

2.4 Preparation of carbopol gel

A 2 gm of Carbopol 934 was precisely measured using an electronic balance (Shimadzu). After that, it was slowly stirred into water and allowed to swell for 24 h to produce a 2% gel. After that, 1-2 ml of glycerol was added to achieve the desired gel consistency (Shah *et al*., 2020).

2.4.1 Preparation of proliposome gels

The 1 gm of the proliposome formulation was mixed with 10 ml of methanol and centrifuged at 6000 rpm for 20 min (REMI, India). After centrifugation, the supernatant containing the unentrapped drug was carefully removed. Proliposomes were incorporated into gels by slow mechanical mixing using a REMI stirrer type BS at 25 rpm for 10 min. 2% w/v concentrations were used to integrate all optimised formulations into gels (Shah *et al*., 2020).

2.5 Characterization of gel

The prepared proliposome gel was assessed for a number of factors, including rheological profile, drug content percentage, uniformity, and droplet size distribution of the quercetin proliposome after incorporation into gel (Gupta *et al*., 2008).

2.5.1 Physical examination

The appearance, colour, and homogeneity of the quercetin formulation were visually assessed (Malviya and Pande, 2021).

2.5.2 pH and rheological measurements

The topical gel should be non-irritating and compatible with the skin pH to avoid the risk of allergic reactions or irritation. Therefore, measuring the pH of the gel formulation is imperative. The pH of the hydrogel was determined by utilising pH paper due to the formulation's consistency. A rheological study was performed using the Brookfield viscometer. All measurements were performed in triplicate with a fixed rotation speed using the Spindle No. 64.

2.5.3 Drug content

To assess the amount of drug in the prepared gel, a portion of the formulation was diluted with pH 7.4 buffers. The resultant liquid was filtered through Whatman filter paper (No. 41) and appropriately diluted. Subsequently, this diluted solution was subjected to analysis with a UV spectrophotometer, measuring absorbance at 369 nm (Byeon *et al*., 2020).

2.6 *In vitro* permeation study

The *in vitro* release study was conducted using Franz diffusion cells. Each cell consisted of a semipermeable membrane separating the donor and receptor chambers with 30 ml of fresh phosphate buffer (pH 7.4) in the receptor chamber. An amount of 1 gm of proliposome gel was added to the semipermeable membrane (Vure *et al*., 2014). The Franz diffusion cells were then put over a magnet stirrer set at a constant temperature of 37°C. To ensure sink conditions, 5 ml samples were withdrawn from the receptor chamber at regular intervals and replaced with an equal volume of fresh buffer. The withdrawn samples were diluted and subsequently assessed with a UV spectrophotometer (Lab India 3200) at an absorbance of 369 nm (Gupta *et al*., 2010).

2.7 Skin sensitization study

The experiment followed guidelines from the institutional animal ethics committee, specifically adhering to OECD Guidelines 406 for the guinea pig maximisation test (GPMT). This test assesses skin sensitisation through a sensitisation and challenge process commonly used to predict human skin reactions (Namdeo and Jain, 2002). Before the experiment, guinea pig dorsal hair was removed by clipping with a 0.1 mm animal hair clipper. The animals were categorised into three groups: Group I (control, receiving plain gel), Group II (test, receiving QPLF3 gel at 2%), and Group III (standard, receiving diclofenac gel at 2%). Skin changes were monitored daily for a period of seven days, and application sites were assessed visually using a consistent scoring system by the same investigator. A: No reaction; B: Slight, patchy erythema; C: moderate but patchy erythema; D: moderate erythema; E: without edema.

2.8 *In vivo* anti-inflammatory activity

Wistar rats of both genders, weighing between 150 and 180 g, were procured from the Kerala Veterinary and Animal Science University in Thiruvazhamkunnu, Kerala. On arriving, the animals were acclimated to the animal house environment at Nandha College of Pharmacy, Erode, for 14 days by placing them in suitable cages with proper bedding materials. The rats were appropriately grouped and marked with picric acid for identification purposes. They were housed under controlled environmental conditions, including a relative humidity of 50 ± 10% and a 12 h light-dark cycle throughout the duration of the experiment.

During the acclimatisation period, the rats were given a standard diet of rat pellets (Hindustan Lever Pvt Ltd., Bangalore) and clean drinking water. All methods of experimentation conducted were in adherence with the guidelines outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethical Committee (Proposal number: NCP/IAEC/No: 2023-24/13).

There are five groups of animals, each with six individuals. Inflammation is induced by a single subcutaneous injection of 0.1 ml 0.1 M carrageenan into the left hind paw of the rats, followed by light anesthesia. Group I serves as the control, *i.e*., distilled water; Group II serves as the negative control, which received 0.1 mL of 0.1 M of carrageenan. Group III served as the standard (0.1 ml of 0.1 M carrageenan + diclofenac gel 2%), Group IV as the test control (0.1 ml of 0.1 M carrageenan + quercetin gel), and Group V as the test control (0.1 ml of 0.1 M carrageenan + quercetin proliposome gel, 2%).

The anti-inflammatory effect was assessed by using the method of carrageenan-induced paw oedema in rats (Bhatia *et al*., 2004; Ansel *et al*., 1995). 1 h after the carrageenan injection, the quercetin gel and the 2% topical proliposome quercetin gel formulation were gently rubbed into the plantar surface of the paw with the index finger for one minute. The control group received the gel base of the formulation in a similar manner. The standard group was treated with 2% diclofenac gel (Mohan Kumar *et al*., 2023).

The anti-inflammatory activity was examined by measuring paw volume before (0 h) and at 1, 2, 3, and 4 h post-carrageenan injection using a vernier caliper. The paw circumference of the non-inflamed right hind paw of the rats was used as a normal reference, and the paw circumferences were compared with the standard group to assess the anti-inflammatory activity (Sameemabegum *et al*., 2022).

The following formula was used to calculate the inhibition of anti-inflammatory action.

% Inhibition = (1 – Mean paw volume of standard/Mean paw volume of treatment) x 100

2.9 Stability studies

A physical stability study was conducted to examine the optimal QPLF3, containing 2% quercetin proliposome gel. The formulations were kept in amber-coloured glass containers that were tightly sealed and kept at different temperatures: 4 ± 2°C and 25 ± 2°C/60 ± 5% RH for the duration of 3 months (Rahul Jodh and Mukund Tawar, 2022). Stability chambers from Thermolab were utilised for this purpose. Regular assessments were performed to monitor changes in drug content, pH, and release of drug properties throughout the storage period (Sri Bhuvaneswari *et al*., 2021).

3. Results

3.1 Characterization of proliposomes

Quercetin proliposomes were made by the thin-film deposition technique, which was proven to be effective in producing liposomes without agglomeration. The proportions of mannitol, phospholipid, and cholesterol were revealed to be essential for the formation and stability of these proliposomes. For optimal proliposome function, vesicle size and liposome size distribution are the important characteristics to be monitored during proliposome synthesis. Numerous investigations have indicated the impact of liposome size on drug release and skin deposition.

3.2 Vesicle size and polydispersity index

The size and distribution of liposomes are crucial factors that significantly influence the fate of proliposomes *in vivo*. The vesicle sizes of different formulations (F1: 240.7 nm, F2: 341.8 nm, F3: 199.7 nm, and F4: 477.7 nm) were investigated, with formulation F3 showing a remarkably narrow and uniform size distribution, indicating improved homogeneity. Figure 1 shows the vesicle size of the quercetin proliposomes. The PDI values for formulations F1 to F4 range from 0.348 to 0.616. Although F3 shows the lowest PDI of 0.348, indicating excellent uniformity in particle size distribution.

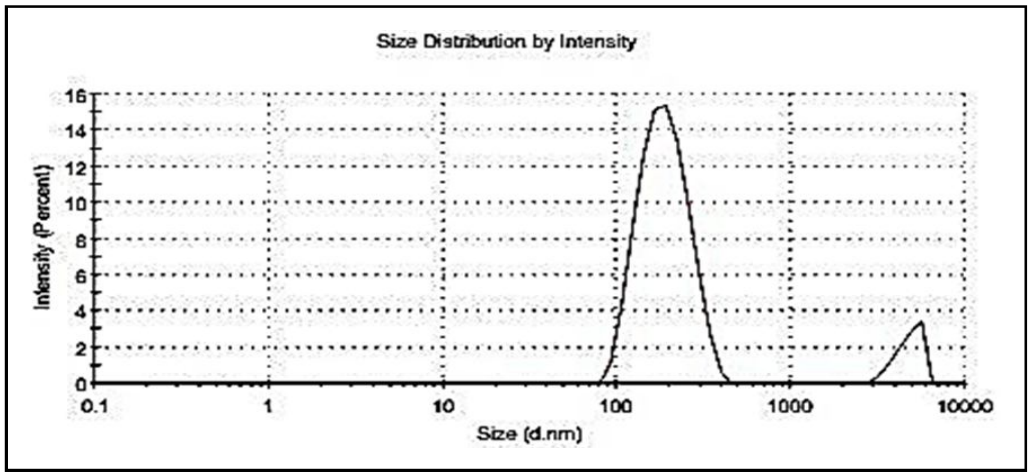


Figure 1: Particle size and poly dispersity index of quercetin proliposome.

3.3 Zeta potential analysis

The determination of the zeta potential is essential for the assessment of the electrical double layer on colloidal particle surfaces, especially for the evaluation of the physical stability of proliposomes. The study of the surface charge showed that several proliposome formulations exhibited a negative potential between -16.5 and -21.4 mV, which successfully prevented the aggregation of the vesicles. Figure 2 represents the Zeta potential graph of the quercetin proliposome.

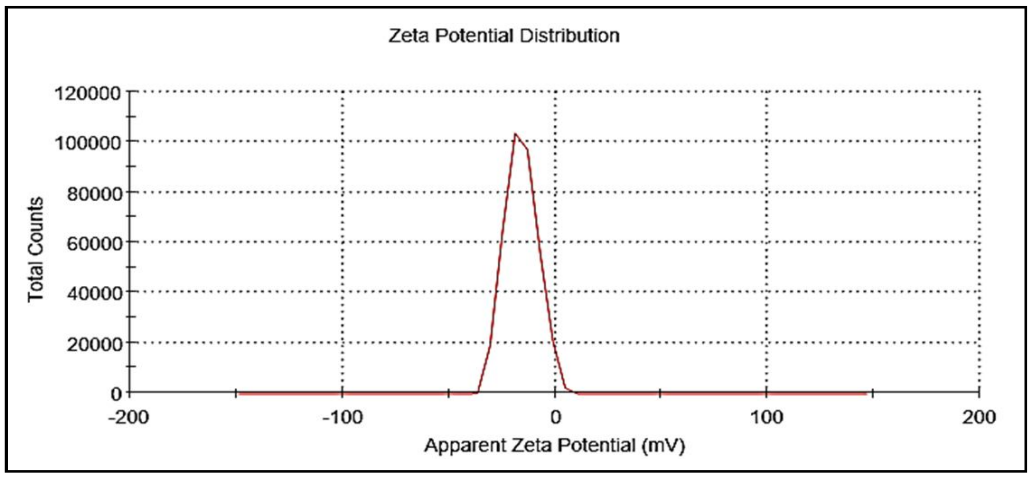


Figure 2: Zetapotential of quercetin proliposome.

3.4 Drug content, entrapment efficiency and yield of proliposome

The concentration of quercetin proliposomes varied between 83.61% and 94.61% depending on drug-to-phospholipid ratios. The results indicate that formulation F3 had the highest drug content of all formulations tested. The entrapment efficiency of the quercetin proliposome formulation ranged from 83.38% to 92.96%. The formulation QPLF3 showed the highest efficiency of 92.96%. Results show that the efficiency of phospholipid and cholesterol entrapment increases with increasing concentrations. The ratio of drug to phospholipid in the proliposomes gave different percentage yields for the different formulations, ranging from 71.61 ± 0.63% to 78.61 ± 0.32%. The percentage yield of formulations increased as lecithin concentration increased.

Table 2: Drug content, entrapment efficiency, and yield of proliposome

S.No.	Formulationcode	Drug content (%)	Entrapment efficiency (%)	Yield of proliposome (%)
1	QPLF1	83.61 ± 0.63	81.38 ± 0.23	81.61 ± 0.63
2	QPLF2	85.89 ± 0.12	83.98 ± 0.86	83.89 ± 0.12
3	QPLF3	94.61 ± 0.32	92.96 ± 0.62	95.61 ± 0.32
4	QPLF4	92.75 ± 0.57	90.92 ± 0.52	93.75 ± 0.57

All values are presented as mean ± SEM, n = 3.

3.5 Drug excipient compatibility studies

The Fourier transform spectrometer records infrared spectra from (400-4000 cm^{-1}). In molecules, IR absorption by functional groups can vary greatly due to the complex interactions of the atoms. However, many structural groups exhibit distinct IR absorption within certain narrow frequency ranges. Stretching and bending vibrations in molecules can be observed and change after formulation, due to changes in molecular structure or interactions within the formulation matrix. By examining the entire spectrum, including the positions, intensities and shapes of the absorption bands, insight can be gained into the molecular composition, structure and changes caused by the formulation process.

The Fourier transform infrared (FT-IR) spectra confirm the successful conjugation between quercetin, mannitol, choesterol, and soya lecithin in the quercetin-proliposome formulation. Characteristic peaks around ~2718.40 cm^{-1} and ~1657.27 cm^{-1} correspond to C=O carbonyl stretching in quercetin-proliposome. Free quercetin showed the characteristic bands due to the presence of several functional groups. The distinctive bands observed in the infrared spectrum of free quercetin correspond to specific functional groups. The band at approximately ~2726.40 cm^{-1} is belonging to C-H asymmetric stretching vibrations, while the peak around ~1650.60 cm^{-1} is associated with C=O stretching.

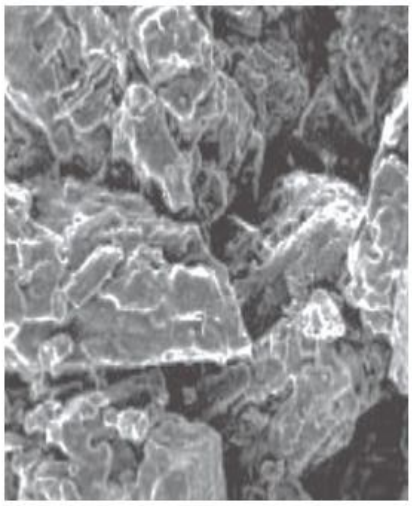
Table 3: Interpretation of IR spectrum of different quercetin proliposome formulation

Transition	IR range (cm^{-1})	QPL F1	QPL F2	QPL F3	QPL F4
O-H stretching alcohols, phenols	3500-3200	3396.41, 3357.84, 3290.33	3399.30, 3351.09, 3291.30	3398.34, 3350.12, 3289.37	3398.34, 3338.55, 3290.33
O-H stretching carboxylic acid	3300-2500	3083.96. 3009.71, 2924.85.	3010.67, 2983.67, 2924.85.	3036.71, 3009.71, 2924.85.	3009.71, 2949.92, 2924.85.
C-H stretching alkane	3000-2850	2741.62	2739.69	2726.19	2725.23
HC°CH stretching alkynes	2260-2100	2199.66	2141.80, 2055.01	2154.64	2142.77, 2119.62
C=O stretching carbonyl	1760-1665	1745.46, 1733.89, 1684.70.	1745.46, 1735.81, 1650.95	1735.81, 1717.49, 1650.95.	1738.71, 1701.10, 1651.92
C=C stretching alkenes	1680-1640	1650.95	1650.95	1650.95	1651.92
C-O stretching alcohol carboxylic acid	1320-1000	1314.58, 1081.03.	1313.43, 1081.03	1314.43, 1082.03	1314.43, 1081.03
C=C stretching heterocyclic aromatic	1550-1475	1557.41, 1520.09	1557.41, 1520.77.	1557.41, 1520.78	1557.45, 1503.25.
C-O stretching alcohol carboxylic acid	1320-1000	1168.78, 1260.39	1169.45, 1280.45.	1156.86, 1310.14.	1164.54, 1301.14.
=C-H bending alkene	1000-650	700.11, 772.44	701.12, 774.54.	772.44, 698.22.	773.40, 698.18.

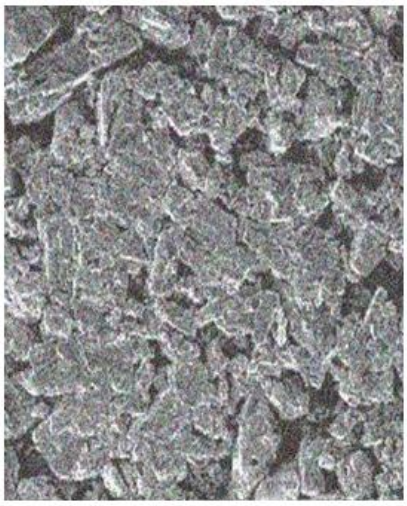
3.6 Scanning electron microscopy

The surface morphology of the QPLF was assessed by scanning electron microscopy (SEM). The Figure 3 displays the SEM images of the formulation (QPLF). The SEM images (Figure 3) showed that pure quercetin had a crystalline structure with irregularly shaped particles of different sizes. The proliposome formulation consisted mainly of small particles that were uniform in both size and shape.

A. SEM image of Quercetin

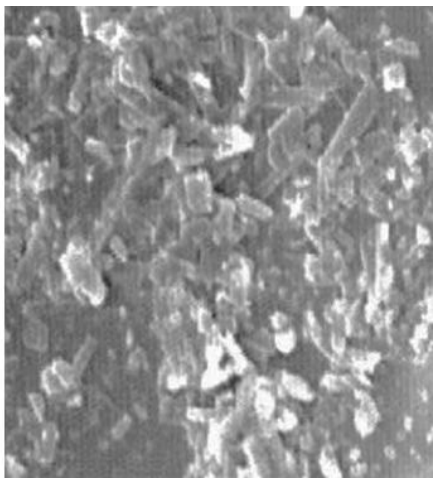


B. SEM image of QPLF1



C. SEM image of QPLF2

C. SEM image of QPLF3



D. SEM image of QPLF4

Figure 3: SEM images of the formulations (QPLFs).

3.7 Characterization of proliposomal gels

3.7.1 Physical examination

The physical properties of the QPLF were evaluated and revealed a translucent, yellowish appearance with a shiny and smooth texture. The gel was found to be non-greasy on application.

3.7.2 pH and rheological measurements

The pH of the QPLF corresponded to that of human skin, making them suitable for topical application. The pH values of the gels ranged between 5.5 and 6.5, ensuring they are compatible with the pH of the skin.

3.7.3 Rheological studies

The gel's viscosity has been assessed with a Brookfield viscometer. The results indicated that the formulation exhibited a viscosity of 11247 cps when measured at 10 rpm.

3.7.4 Drug content

The quercetin containing proliposome gel, upon preparation, exhibited a consistent drug content of 78.56%, suggesting that the drug was evenly distributed in the formulation. Moreover, there were no significant differences in the percentage of drug content between the formulations, indicating that the approach for dispersing the liposomal dispersion in the gel base is effective. Table 4 shows the drug content and the rheological studies of the proliposome gel of quercetin.

Table 4: Characterization of quercetin proliposomal gels

Formulations	Viscosity (cps)	Drug content (%)
QPL F1	10456.58	75.09 ± 0.843
QPL F2	10846.15	79.74 ± 0.774
QPL F3	11247.13	88.56 ± 0.362
QPL F4	11189.56	83.26 ± 0.315

All values are presented as mean ± SEM, n = 3.

3.8 *In vitro* drug permeation study

An *in vitro* permeation release investigation was performed with different formulations (F1-F4) in pH 7.4 phosphate buffer. The cumulative release rate (CDR) of the QPLF3 was recorded to be 86.28%. Initially, the gels showed moderate drug release, but as gelation progressed, the release rate slowed down, followed by a second phase of increased release. These findings strongly suggest that proliposomal gels retain the drug in the skin to a significantly greater extent compared to non-liposomal formulations. This suggests that the liposomes not only enhance drug penetration but also facilitate the drug's localisation within the skin. Figure 4 shows an *in vitro* study of the proliposomal gel formulation of quercetin. However, the results indicate that these gels can sustain drug retention over an extended period.

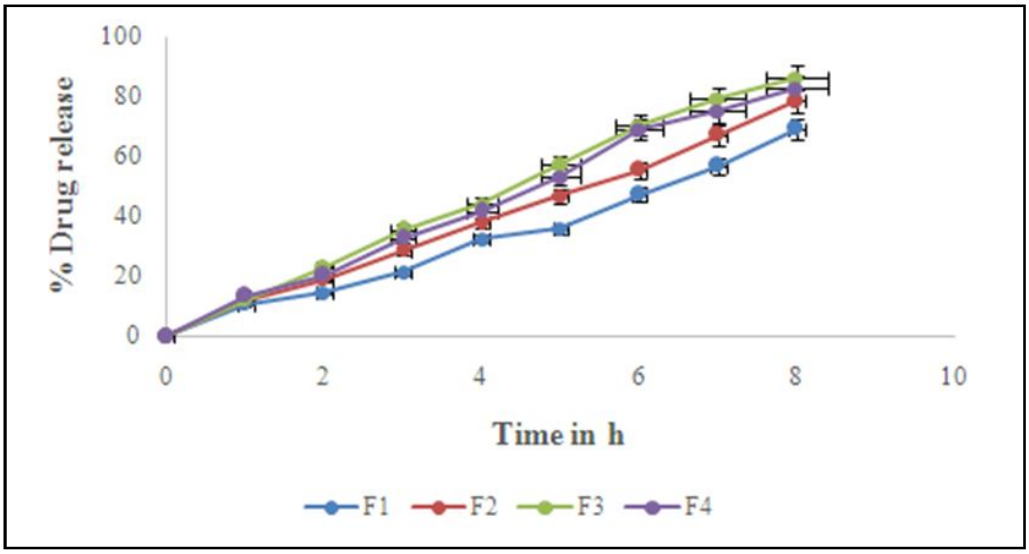


Figure 4: *In vitro* drug release of quercetin proliposomal formulation (F1-F4).

Table 5: Skin sensitization for erythema

S.No.	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Control	A	A	A	A	A	A	A
2	Test (F3)	A	A	A	A	A	A	A
3	Standard (Diclofenac gel)	A	A	A	A	A	A	A

3.9 Skin sensitization test

In the skin irritation study with the proliposome gel QPLF3, no signs of sensitisation were observed in guinea pigs exposed to this formulation. The average primary irritancy index for the QPLF3 formulation was assessed over a 7-day observation period. The results showed no signs of irritation or erythema (redness of the skin), indicating that proliposome gel QPLF3 is a safe formulation for topical application.

A - No reaction, B - Slight, patchy erythema, C - Slight but confluent

D - Moderate erythema, E - without edema

The results from the skin irritation study with the proliposomal gel QPLF3 indicate that it is a safe formulation for topical application.

3.10 *In vivo* anti-inflammatory study

The *in vivo* efficacy of the optimised proliposome quercetin gel was investigated using carrageenan treated with paw oedema model. Figure 5 demonstrates the anti-inflammatory effects of the topical application of the gel formulations. The QPLF3 formulation not only significantly reduced inflammation but also maintained this reduction over a prolonged period. Both QPLF3 and the conventional gel formulations show a significant ($p<0.05$) decrease in paw volume compared to the control formulations. The QPLF3 achieved a higher

inhibition rate of 64.80% at 4 h, compared to the 54.51% inhibition observed with the standard diclofenac gel, as shown in Figure 6. This improved performance is likely due to the prolonged localisation of the drug at the application site, resulting in sustained release. Mallesh *et al*., 2012, that discusses proliposome gel and its suitability for topical skin application compared to traditional liposomes. The gel form of the proliposome can potentially enhance the penetration of drugs into the skin. This is due to the ability of proliposome gels to form liposomes *in situ* upon contact with the skin. The percentages of swelling and inhibition were computed.

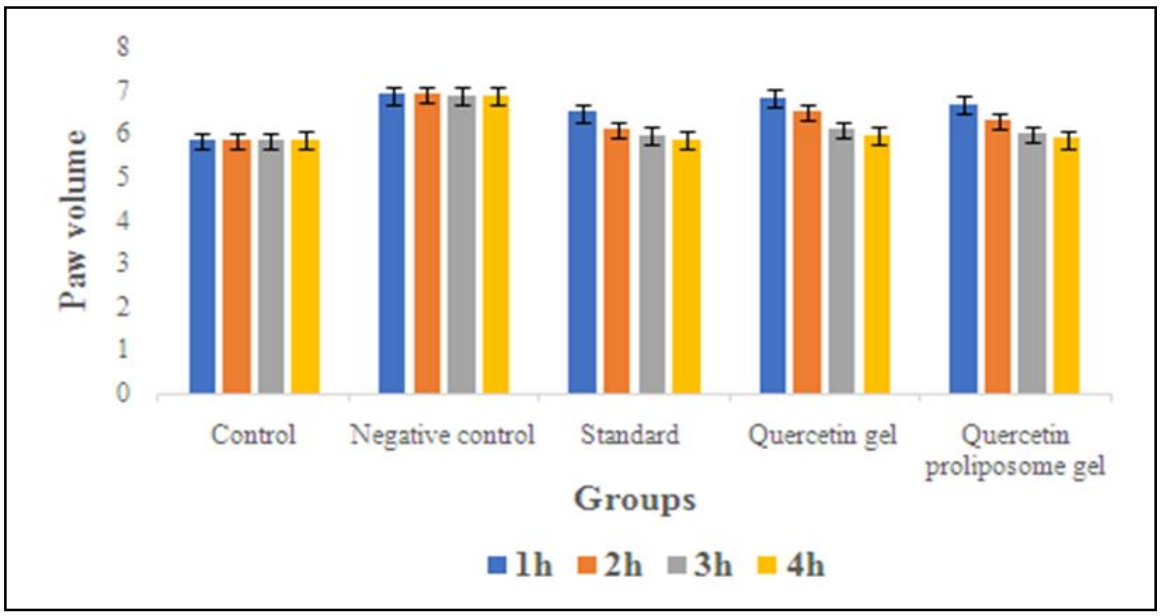


Figure 5: Different in paw edema volume in mm.

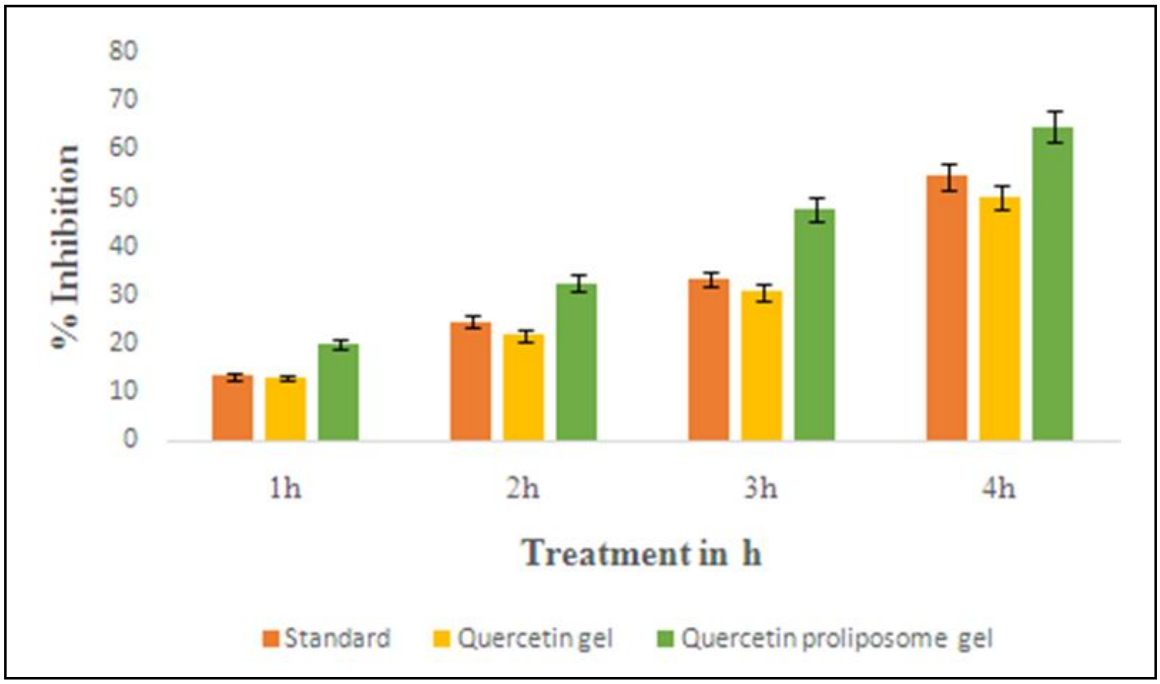


Figure 6: Different in paw edema volume (% inhibition)

3.11 Stability studies

The stability studies performed with the proliposome gel formulation QPLF3 showed minimal changes in deviation in pH, drug content, and drug release, confirming that the formulations remain stable during storage. In particular, the stability studies showed that proliposomes exhibit higher stability at freezing temperatures than at room conditions, suggesting that storage of the product at lower temperatures is advisable. In addition to its stability profile, the proliposome gel exhibited a higher percentage of oedema inhibition in comparison to alternative formulations. These results emphasise the potential of proliposome gel formulations with quercetin for

topical anti-inflammatory therapy. The enhanced stability of proliposomes compared to conventional liposomes increases their potential utility in transdermal delivery systems. This property contributes to extended shelf-life and improved efficacy of pharmaceutical products utilising proliposome technology**.** These findings exhibit the benefits of utilising proliposome gel formulations, especially those incorporating quercetin, for topical inflammation treatment. Figure 7 represents stability studies of formulation QPLF3.

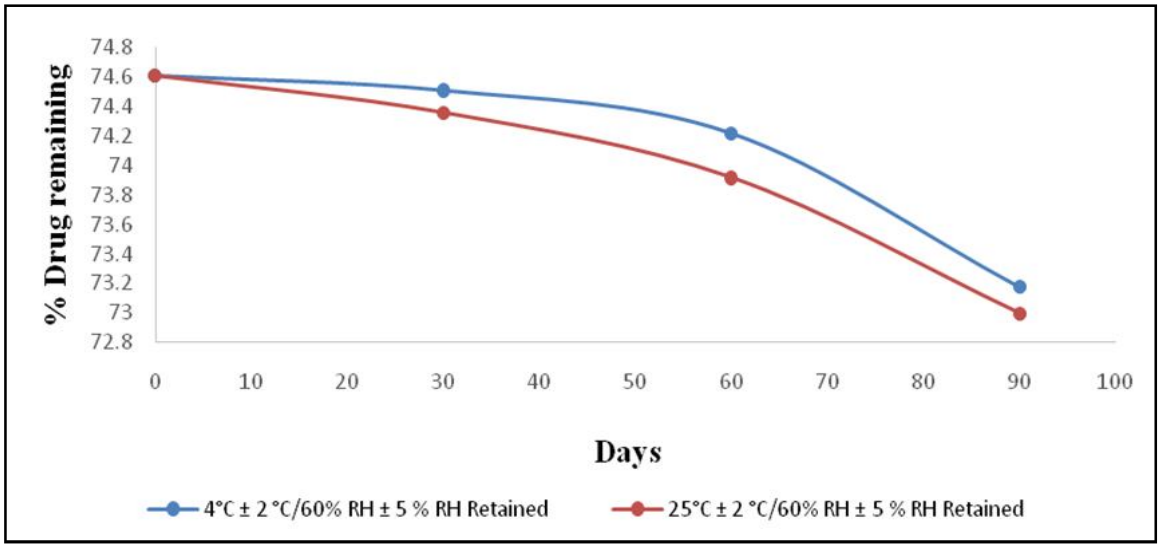


Figure 7: Stability studies of formulation QPLF3.

4. Discussion

The most important part that needs to be monitored in the production of liposomes to achieve the best performance is the size and size distribution of the vesicle. The formation of vesicles within the liposomal dispersion has been observed. Research indicates that the size of the liposome influences both drug release and skin deposition. There is a positive correlation between the size of liposome vesicles and the presence of phospholipids and cholesterol.

The negative zeta potential found in all formulations signifies a negative sign on the polar head of the phospholipid, which could be due to the presence of free fatty acids in the stabilizer. The benefits of this negative charge include reduced vesicle aggregation and increased stability of the colloidal system. Kumari *et al*., 2010 reported the entrapment efficiency % is influenced by its solubility in the matrix. All proliposome formulations exhibited high drug content, indicating minimal loss of drug during the preparation process and uniform dispersion within the quercetin formulation. The results show that the efficiency of phospholipid and cholesterol entrapment increases with increasing concentration. The phospholipid content was found to increase the percentage yield of the formulations. The porous structure seen in the pictures confirms the production of proliposomes. The crystalline structure of mannitol has changed, which suggests successful incorporation of the lipids.

The FTIR spectroscopy analysis of the quercetin proliposome formulations shows the absence of new peaks and disappearance of existing peaks compared to the pure quercetin. This suggests that it does not chemically interact with the pure drug and the carrier. A rheological study of proliposome gels containing 2% w/w carbopol provided a clear idea of what was required through the preparation of steady-state proliposome gel formulation.

The pH values of the proliposome gel prepared were within acceptable limits. The results show that the efficiency of inclusion of phospholipids and cholesterol increases with increasing concentration. The phospholipid content was found to increase the percentage yield and *in vitro* studies concluded that enhance skin permeation of the formulations. Vure prasad *et al*. (2014) reported proliposomes which helped to produce the depot effect; Hence, proliposomes drug delivery system was better choice for sustained release of drug through topical drug delivery.

The *in vivo* study revealed a significantly higher percentage of oedema inhibition with the proliposome gel formulation as compared to both the marketed diclofenac gel and quercetin gel. The results indicate the potential therapeutic value of the proliposome gel formulation with anti-inflammatory agents in topical treatments for inflammation. The stability study showed that the optimised formulation QPLF3 does not exhibit significant changes in physical parameters such as pH, drug content, and drug release and is considered stable. Therefore, the proliposome drug delivery system proves to be a superior choice for sustained drug release through topical administration, which improves patient compliance and is particularly convenient for patients who cannot take the drug orally.

5. Conclusion

The quercetin proliposome was successfully prepared by using the thin film deposition technique with carriers like mannitol, cholesterol, and soya lecithin. Spectroscopic analyses confirmed successful encapsulation of quercetin without interaction between the drug and carriers. Particle size analysis indicated nano-sized particles with sufficient zeta potential to prevent aggregation. High drug encapsulation efficiency was observed, especially in formulation QPLF3. The changed crystalline structure of mannitol was confirmed by SEM images confirming proliposome formation. Rheological studies of proliposomal gels with carbopol indicated stability without requiring pH adjustment. The proliposomal gel exhibited superior *in vivo* anti-inflammatory activity compared to commercial and

quercetin gels. It concluded that quercetin proliposomal gel offers a promising approach for sustained topical drug delivery, enhancing patient compliance and efficacy.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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