ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-8 | Issue-5 | Nov-Dec 2026 | **DOI: https://doi.org/10.36346/sarjps.2026.v08i05.004**

Original Research Article

Quercetin-Loaded Ethosomes Prepared by the Cold Method: Effect of Lipid and Ethanol Content on Vesicle Size, Entrapment, Elasticity and Skin Flux

Raghvendra Chaubey[1]*, I. K. Yadav[1], Jitendra Kumar Malik[1]

[1]Faculty of Pharmacy, P.K. University, Shivpuri, M.P. India

***Corresponding Author:** Raghvendra Chaubey
Faculty of Pharmacy, P.K. University, Shivpuri, M.P. India

Article History
Received: 05.09.2026
Accepted: 10.11.2026
Published: 16.11.2026

Abstract: Quercetin is a plant flavonol with useful antioxidant and anti-inflammatory activity, but it dissolves poorly in water and is absorbed poorly by mouth. Applying it to the skin in a vesicular carrier is one way around these problems. In this study we prepared eight quercetin-loaded ethosomal formulations (E1–E8) by the cold method, varying the amounts of soya lecithin, cholesterol and ethanol, and followed with probe sonication. The vesicles were assessed for entrapment efficiency, size, polydispersity index (PDI), elasticity and flux across excised rat skin, and the drug was checked for compatibility with the lipids. Entrapment efficiency ranged from 78.62 to 88.12%, mean vesicle size from 95.88 to 151.27 nm, PDI from 0.118 to 0.179, and flux from 836.51 to 958.14 µg/cm²/h. Batch QEF-5 combined a small size (97.45 nm), a PDI of 0.158, an entrapment efficiency of 84.08%, the highest elasticity (90.9) and the highest flux, and it was taken forward for gel preparation. TEM and SEM images showed rounded, closed vesicles. Because lipid and ethanol content were changed together in these batches, their separate contributions cannot be told apart, several batches were not statistically distinguishable, and one representative light-scattering record showed a second, larger population and a near-neutral zeta potential. The data show that quercetin can be loaded into ethosomes with good efficiency, but the size distribution and the very high flux values need to be confirmed by repeat work.

Keywords: Quercetin, Ethosomes, Cold Method, Entrapment Efficiency, Vesicle Elasticity, Ex-Vivo Skin Permeation, Transdermal Delivery.

1. INTRODUCTION

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most common flavonoids in the human diet. It scavenges free radicals, chelates metals and dampens inflammatory signalling, and these properties have made it an attractive candidate for skin conditions in which oxidative stress and inflammation play a part (Boots *et al*., 2008; Nijveldt *et al*., 2001; D'Andrea, 2015; Li *et al*., 2016). The difficulty lies in getting it to where it is needed. The compound is practically insoluble in water, it is absorbed poorly after oral dosing, and it is rapidly conjugated in the intestine and liver (Manach *et al*., 2005).

The skin avoids some of these problems, but it has a barrier of its own. The stratum corneum, made of flattened cells embedded in ordered lipid lamellae, keeps most molecules out, and a lipophilic, crystalline compound like quercetin does not cross it easily (Bouwstra & Ponec, 2006; Barry, 2001; Prausnitz & Langer, 2008). Vesicular carriers were developed to deal with this. Ordinary liposomes tend to stay on the surface of the skin, while more deformable vesicles can reach deeper layers (Cevc & Blume, 1992).

Ethosomes, introduced by Touitou and co-workers, are phospholipid vesicles that contain a fairly high proportion of ethanol (Touitou *et al*., 2000; Godin & Touitou, 2004). Ethanol makes the vesicle bilayer more flexible and also fluidises the lipids of the stratum corneum, and the two effects together seem to improve delivery beyond what ethanol or

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

phospholipid achieve alone (Verma & Fahr, 2004; Mbah *et al*., 2014). Ethosomes have been tried with many drugs, including diclofenac and anthralin (Ghanbarzadeh & Arami, 2013; Fathalla *et al*., 2020), and ethosomes and transethosomes have been described for quercetin on melanoma cells (Ferrara *et al*., 2022).

In an ethosomal formulation, the lipid and ethanol content strongly influence vesicle size, entrapment and flexibility, so a rational starting point is to test a range of compositions. This paper describes eight quercetin ethosome batches made by the cold method. Our aims were to see how entrapment, size, PDI, elasticity and flux across excised rat skin varied with composition, to check that quercetin was compatible with the lipids used, and to choose a batch for incorporation into a gel, which is described in a companion paper.

2. MATERIALS AND METHODS

2.1 Materials

Quercetin was used as the model drug. Soya lecithin (soya phosphatidylcholine) served as the vesicle-forming lipid, cholesterol as the membrane stabiliser, and ethanol and propylene glycol as the penetration enhancers. Distilled water formed the aqueous phase.

2.2 Preformulation

Quercetin was scanned from 200 to 400 nm on a Shimadzu UV-1800 spectrophotometer in methanol, water and phosphate buffer pH 7.4 (10 µg/mL). Calibration curves were prepared in methanol (5–30 µg/mL) and in pH 7.4 phosphate buffer (5–25 µg/mL). FTIR spectra (KBr) of the drug were recorded on a Shimadzu FTIR-8400S. Compatibility with the excipients was examined in two ways. First, 1:1 blends of quercetin with soya lecithin, cholesterol, Carbopol and propylene glycol were stored at 2–8 °C and at 25 °C for four weeks and inspected weekly for colour, texture or other change. Second, FTIR spectra were recorded for the pure drug and for physical mixtures with phospholipid, phospholipid plus cholesterol, and phospholipid, cholesterol and Carbopol 934.

2.3 Preparation of Ethosomes

Ethosomes were made by the cold method. Soya lecithin and cholesterol were dissolved in ethanol in a closed vessel with vigorous magnetic stirring at room temperature. Propylene glycol was added slowly with continuous stirring, and quercetin (20 mg) was then dissolved in the lipid solution. Distilled water was added up to 30 mL, the dispersion was stirred for 5 min in a closed vessel, and the vesicles were reduced in size by probe sonication (Vibra-Cell, 13 mm probe; five cycles of 2 min, 2 s pulses, 60% amplitude). The dispersion was viewed under an optical microscope (40× and 100×) and stored in a refrigerator. The compositions of the eight batches are shown in Table 1.

Table 1: Composition of the quercetin ethosome batches (final volume 30 mL)

Batch	Quercetin (mg)	Soya lecithin (mg)	Cholesterol (mg)	Ethanol (mL)	Ethanol (% v/v)	Propylene glycol (mL)
E1	20	15	15	6	20	3
E2	20	15	30	6	20	3
E3	20	15	45	6	20	3
E4	20	30	15	9	30	3
E5	20	30	30	9	30	3
E6	20	30	45	12	40	3
E7	20	45	15	12	40	3
E8	20	60	45	15	50	3

Ethanol percentage calculated from the ethanol volume and the 30 mL final volume.

2.4 Characterisation

Entrapment efficiency. One millilitre of ethosome dispersion was diluted to 10 mL with phosphate buffer and centrifuged at 14,000 rpm for 1 h. The supernatant was filtered (0.2 µm) and the free drug measured by UV spectrophotometry. Entrapment efficiency was calculated as the initial drug minus the free drug, divided by the initial drug, multiplied by 100. *Size, PDI and zeta potential.* These were measured by dynamic light scattering with a Malvern Zetasizer after dilution with distilled water. *Elasticity.* The dispersion was extruded through a 100 nm polycarbonate membrane (50 mm stainless steel holder) at 2.5 bar, and the volume extruded in 5 min was used to calculate an elasticity value. *Morphology.* Vesicles were viewed by TEM (positive staining) and SEM. *Ex-vivo skin permeation.* Permeation was studied on excised rat skin mounted in a Franz diffusion cell with the stratum corneum facing the donor compartment. Receptor samples were withdrawn at set intervals, replaced with fresh medium and assayed by UV spectrophotometry. Flux (µg/cm²/h) was taken from the slope of the linear part of the cumulative amount permeated per unit area against time. Results are given as mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Preformulation and Compatibility

Both calibration curves were highly linear. Regression on the calibration data gave R^2 of 0.999 in methanol (5–30 µg/mL) and 0.998 in pH 7.4 buffer (5–25 µg/mL), so UV spectrophotometry was suitable for the assays in this work. In the four-week compatibility test the drug alone and its 1:1 blends with soya lecithin, cholesterol, Carbopol and propylene glycol showed no change in appearance at either 2–8 °C or 25 °C. The FTIR spectra of the physical mixtures kept the characteristic bands of quercetin (hydroxyl, carbonyl and aromatic C=C), with no obvious shifts and no new bands, which suggests that no chemical interaction took place and that the excipients are suitable for the formulation.

3.2 Entrapment Efficiency

Entrapment efficiency (EE) of all eight batches was high, from 78.62 ± 2.21% for E1 to 88.12 ± 2.41% for E8 (Table 2). It rose in the order E1 to E8 as the lipid and ethanol content increased. That trend is understandable. Quercetin is lipophilic and sits in the phospholipid bilayer, so more lipid gives it more room, and ethanol raises the solubility of the drug in the vesicle-forming mixture. However, the standard deviations (0.7 to 3.0 points) are larger than the differences between neighbouring batches, so only the gap between the extremes is likely to be real. In addition, lipid and ethanol content went up together in these batches, so we cannot say how much of the increase is due to one and how much to the other. It is generally reported that entrapment in ethosomes passes through an optimum with increasing ethanol, because too much ethanol makes the bilayer leaky (Touitou *et al*., 2000). We saw no such fall within the range studied, and 50% ethanol may not have been high enough to show it.

Table 2: Entrapment efficiency, size, PDI, elasticity and flux of the ethosome batches (mean ± SD)

Batch	EE (%)	Size (nm)	PDI	Elasticity	Flux (µg/cm²/h)
QEF-1	78.62 ± 2.21	96.12 ± 0.52	0.118	86.72 ± 5.46	955.05 ± 43.56
QEF-2	80.15 ± 2.98	110.84 ± 0.49	0.136	81.93 ± 7.32	895.70 ± 51.27
QEF-3	81.32 ± 1.12	151.27 ± 0.44	0.162	84.07 ± 5.20	877.27 ± 39.45
QEF-4	82.74 ± 1.35	147.96 ± 0.28	0.179	89.40 ± 4.67	956.87 ± 41.36
QEF-5	84.08 ± 0.71	97.45 ± 0.71	0.158	90.90 ± 5.77	958.14 ± 43.54
QEF-6	85.92 ± 2.87	115.63 ± 0.55	0.139	86.68 ± 7.44	836.51 ± 21.56
QEF-7	86.48 ± 2.19	95.88 ± 0.68	0.144	82.33 ± 3.22	951.67 ± 39.53
QEF-8	88.12 ± 2.41	102.37 ± 0.92	0.169	80.91 ± 3.21	896.51 ± 34.58

EE, entrapment efficiency; PDI, polydispersity index. Batches QEF-1 to QEF-8 correspond to E1 to E8 in Table 1.

3.3 Vesicle Size, PDI and Zeta Potential

Mean vesicle size ranged from 95.88 nm (QEF-7) to 151.27 nm (QEF-3), and PDI from 0.118 to 0.179. Values in this region would normally be taken to describe small, fairly uniform vesicles, a size range that suits skin delivery and the low PDI of the table is in line with that (Danaei *et al*., 2018). The two largest batches, QEF-3 and QEF-4, did not share an obvious feature in composition (QEF-3 had low lecithin and high cholesterol, whereas QEF-4 had moderate lecithin and low cholesterol), so we do not offer a simple explanation for their size.

There is, however, an important qualification. The representative light-scattering record for the formulation labelled F5 showed two populations in the intensity distribution, one in the range of a few tens of nanometres and a second at several hundred nanometres, and the zeta potential distribution was centred close to 0 mV. A single mean size of about 100 nm with a PDI below 0.2 does not fit well with a bimodal trace, and a near-neutral surface would be consistent with a sterically stabilised vesicle but also with a system that is not strongly protected against aggregation. The size and zeta potential data should therefore be repeated, preferably with intensity, volume and number distributions reported together, before these values are relied on.

3.4 Elasticity

Elasticity values lay between 80.91 and 90.90 (Table 2), with QEF-5 the highest. Deformability is considered one of the properties that allow ethosomes to pass through the narrow channels of the stratum corneum (Cevc & Blume, 1992; Touitou *et al*., 2000). The standard deviations (3 to 7) were of the same order as the differences between batches, so only QEF-5 against QEF-8 or QEF-2 can be called a clear difference, and even that is a modest one. No simple relationship with composition was visible.

3.5 Skin Flux

Flux across excised rat skin ranged from 836.51 to 958.14 µg/cm²/h. Four batches (QEF-1, QEF-4, QEF-5 and QEF-7) had practically the same flux, between 951.67 and 958.14, with differences far smaller than the standard deviations of about 40. The lower fluxes belonged to QEF-3 and QEF-6 (877.27 and 836.51). Combined ethanol and phospholipid are thought to enhance skin penetration synergistically, partly through the action of ethanol on the intercellular lipids and partly through the deformability of the vesicle (Verma & Fahr, 2004). Our data are consistent with such an effect but cannot

separate it from the effect of the drug in solution, because no control with drug in an ethanolic or aqueous solution was run alongside the vesicles in this experiment.

The size of the flux values also deserves a comment. They are large for a flavonoid, and with 20 mg of quercetin per batch, a flux of this order would use up a considerable part of the dose over the course of an experiment. We recommend that the permeation result be checked by a mass balance of the applied dose and by measuring the drug retained in the skin.

3.6 Selection of Batch QEF-5

QEF-5 was chosen for the gel because it gave the best combination of properties: the highest elasticity (90.90), the highest flux (958.14 µg/cm²/h), a small size (97.45 nm), a PDI of 0.158 and an entrapment efficiency of 84.08%. QEF-8 had a higher entrapment efficiency, but its elasticity and flux were lower. Since flux in four batches overlapped, the choice rests mainly on elasticity and on entrapment and size acting together, and it should be seen as a practical choice and not as a statistical optimum.

3.7 Morphology

TEM with positive staining showed rounded vesicles with a distinct boundary, which is what one expects for sealed, closed bilayer structures. SEM images likewise showed rounded vesicles with smooth surfaces. These observations were qualitative, and neither technique was used here to measure the size of vesicles. Sample preparation for electron microscopy can itself change vesicle shape, so the images support the presence of vesicles but do not by themselves prove the size obtained by light scattering.

4. Limitations

The study has several limits. Lipid and ethanol content were changed together across the batches, so their individual effects were not separated, and no statistical model or optimisation was fitted. For many responses the differences between batches were smaller than the standard deviations, and no statistical tests are reported. The light-scattering record shown for one batch indicated more than one population and a near-neutral zeta potential, which does not match the narrow distributions in the summary table. The flux values are high and no vesicle-free control (drug in ethanol–water or in plain buffer) was run in the same experiment. The elasticity index is a relative figure, and the skin source and receptor conditions should be reported in full. Stability of the vesicles on storage and cytotoxicity were not examined.

5. CONCLUSION

Quercetin was loaded into ethosomes by the cold method with entrapment efficiencies of 78.62–88.12%, and the vesicles were small and elastic, with high flux across rat skin. Batch QEF-5 (soya lecithin 30 mg, cholesterol 30 mg, ethanol 30% v/v in 30 mL) offered the best balance of size, elasticity, entrapment and flux and was selected for incorporation into a Carbopol gel. The size distribution, the zeta potential and the magnitude of the flux need to be verified by repeat experiments, and the separate effects of lipid and ethanol content remain to be sorted out with a factorial design.

DECLARATIONS
Conflict of Interest: None
Funding: None

REFERENCES

- Barry, B. W. (2001). Novel mechanisms to enable successful transdermal drug delivery. *European Journal of Pharmaceutical Sciences, 14*(2), 101–114.
- Boots, A. W., Haenen, G. R. M. M., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology, 585*(2–3), 325–337.
- Bouwstra, J. A., & Ponec, M. (2006). The skin barrier in healthy and diseased state. *Biochimica et Biophysica Acta, 1758*(12), 2080–2095.
- Cevc, G., & Blume, G. (1992). Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. *Biochimica et Biophysica Acta, 1104*(1), 226–232.
- D'Andrea, G. (2015). Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia, 106*, 256–271.
- Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics, 10*(2), 57.
- Fathalla, D., Youssef, E. M., & Soliman, G. M. (2020). Liposomal and ethosomal gels for the topical delivery of anthralin: Preparation, comparative evaluation, and clinical assessment in psoriatic patients. *Pharmaceutics, 12*(5), 446.
- Ferrara, F., Benedusi, M., Sguizzato, M., Cortesi, R., Baldisserotto, A., Buzzi, R., Valacchi, G., & Esposito, E. (2022). Ethosomes and transethosomes as cutaneous delivery systems for quercetin: A preliminary study on melanoma cells. *Pharmaceutics, 14*(5), 1038.

- Ghanbarzadeh, S., & Arami, S. (2013). Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes. *BioMed Research International, 2013*, 616810.
- Godin, B., & Touitou, E. (2004). Ethosomes: New prospects in transdermal delivery. *Critical Reviews in Therapeutic Drug Carrier Systems, 21*(1), 63–102.
- Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M. T., Wang, S., Liu, H., & Yin, Y. (2016). Quercetin, inflammation and immunity. *Nutrients, 8*(3), 167.
- Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition, 81*(1), 230S–242S.
- Mbah, C. C., Builders, P. F., & Attama, A. A. (2014). Nanovesicular carriers as alternative drug delivery systems: Ethosomes in focus. *Expert Opinion on Drug Delivery, 11*(1), 45–59.
- Nijveldt, R. J., van Nood, E., van Hoorn, D. E., Boelens, P. G., van Norren, K., & van Leeuwen, P. A. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition, 74*(4), 418–425.
- Prausnitz, M. R., & Langer, R. (2008). Transdermal drug delivery. *Nature Biotechnology, 26*(11), 1261–1268.
- Touitou, E., Dayan, N., Bergelson, L., Godin, B., & Eliaz, M. (2000). Ethosomes—novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. *Journal of Controlled Release, 65*(3), 403–418.
- Verma, D. D., & Fahr, A. (2004). Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. *Journal of Controlled Release, 97*(1), 55–66.