Int. J. Pharm. Investigation, 2024; 14(3):749-758.
https://www.jpionline.org

Original Article

Formulation and Evaluation of Quercetin Ethosomal Hydrogel for Topical Delivery System

Prashant Halagali, Vishal I Wannur, Abishek Kumar A Patil, Vaibhavi D. Torgal, Shrikrishna M Naik, Santosh A Marennavar, Sakshi Shahapurmath, Pankaj Patil*

Department of Pharmaceutics, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.

ABSTRACT

Introduction: Ethosomes are non-invasive drug delivery systems that enable drugs to enter the bloodstream or deep layers of the skin and these are phospholipid-based elastic Nano vesicles with a high ethanol concentration (20-45%). In vesicular systems, ethanol is a well-known permeability enhancer that has been utilized to produce elastic Nano vesicles. Quercetin (QT) is one of the most prevalent polyphenolic flavonoids found in fruits and vegetables, and it has several biological and health-promoting effects in a variety of disorders. The current study sought to formulate ethosomal gel and hydrogel as topical drug delivery. **Materials and Methods:** The ethosomes formulations were made by hot method using phospholipid and ethanol (20% to 40%) and then tested for particle size, PDI, percentage entrapment efficiency and Scanning Electron Microscopy (SEM), in vitro drug release study, zeta potential, Then quercetin-loaded ethosomal gel and quercetin hydrogel were prepared and tested for pH, spreadability, viscosity, washability, drug content, visual observation, and extrudability. **Results and Discussion:** FT-IR investigations demonstrated that there was no interaction between the medication and the excipients. Compared to regular hydrogel, the results suggested that ethosomal gel might be a useful transdermal delivery system for quercetin in the treatment of inflammation.

Keywords: Quercetin, Ethosomes, Hydrogel, Dermal delivery, Penetration enhancer, Proniosomes, Topical drug delivery.

Correspondence:
Mr. Pankaj Patil
Department of Pharmaceutics, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.
Email: pankajpatil@klepharm.edu

Received: 15-12-2023;
Revised: 19-01-2024;
Accepted: 28-02-2024.

INTRODUCTION

When applied to intact skin, Transdermal Delivery Systems (TDS) are discrete, self-contained dosage forms designed to deliver a drug or drugs through the skin and into the systemic circulation. TDS operate through the process of diffusion, in which the medication enters the main circulation through the skin after diffusing from the drug reservoir either directly or through the rate-controlling membrane and/or contact adhesive[1] Dermal (topical) administration should be utilised solely to define a targeting to diseased areas inside the skin, with limited systemic absorption. This sort of drug localization is crucial in the cure of dermatological disorders such as eczema, microbial infection, skin cancer, psoriasis and eczema, because the disease's seat is found in the skin.[2]

Through increasing cycle time, decreasing enzyme degradation, increasing drug stability and water solubility, and increasing target cell or tissue uptake rate, a class of nanomaterials called Nano-Drug Delivery Systems (NDDSs) can improve drug safety and efficacy. NDDSs have a high bioavailability and can be delivered through inhalation, oral administration, or intravenous injection.[3]

Touitou *et al.* developed ethosomes, a kind of UDV, in 1997.20 they are also known as elastic Nano vesicles due to their small size (150-200 nm) and remarkable deformability. Ethosomal systems are vesicles composed mainly of water, phospholipids, and a significant quantity of ethanol. Phospholipids are obtained from natural, semisynthetic, and synthetic sources, including soybeans and eggs, and can be used in concentrations ranging from 0.5% to 10%. Phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, and hydrogenated phosphatidylcholine are a few examples of phospholipids. At amounts ranging from 20% to 45%, ethanol can be used as an effective skin promoter by interacting with the polar head group of SC lipid molecules, this substance minimizes their melting point and enhances the permeability of cell membranes and the fluidity of lipid bilayers.[4] Ethosomes are safe drug delivery vehicles that enable medications to penetrate underneath the skin layers as well as systemic circulation. Since ethanol is known to disrupt the organization of the skin's lipid bilayer, its high ethanol content sets the ethosomes apart. Consequently, it permits the vesicle to

DOI: 10.5530/ijpi.14.3.84

penetrate the stratum corneum when incorporated into a vesicle membrane. Additionally, because of the high ethanol content, the lipid membrane is enclosed a bit loosely than in typical vesicles, resulting in a more flexible structure and enhanced medication circulation capability in the stratum corneum lipids.[5-9] The structure of Ethosomes was depicted in Figure 1.

Quercetin is a plant flavonoid that is a member of the polyphenol flavonoid family. It can be found in many different fruits, vegetables, grains, seeds, and leaves. Common foods with significant amounts of it include red onions, kale, and capers. It has a bitter taste and is utilised in dietary supplements, drinks, and meals.[10]

It is most recognized for its anti-inflammatory and anti-allergy properties. It is frequently given for food and inhalant allergies, asthma, eczema, psoriasis, gout, and ulcerative colitis because it stabilizes mast cell membranes and limits the release of histamine and other inflammatory chemicals. Quercetin can also block inflammatory processes mediated by "leukotriene's" (inflammatory effects a thousand times stronger than histamines), hyaluronidase (collagen-destroying enzymes), and lysosomal enzymes owing to its antioxidant impact.[11,12]

MATERIALS AND METHODS

Pre-formulation studies

Determination of absorption Maxima (λ_{max}) by UV-visible spectroscopy and Standard calibration curve of Quercetin

UV spectrophotometers (UV-1900) with matched 10 mm quartz cuvettes were used. Ethanol was used in the analysis of Quercetin. The following procedure was applied to determine the λ_{max} of quercetin, after accurately weighing 10 mg of the drugs; it was added to a 10 mL volumetric flask. Next, the volumetric flask was filled with 5 mL of ethanol and placed in an ultrasonicator for 5 min. Once a clear solution was obtained remaining volume was made up to mark with Phosphate Buffer pH 6.8. This is the stock solution that was used for the preparation of the standard calibration curve.

From the above stock solution, 1 mL was pippetted and diluted up to 10 mL by PBS to give a concentration of 100 µg/mL. At 200-400 nm, this solution was tested. To confirm the maximum absorbance of the drugs, the maximum absorbance was measured using a UV-vis spectrophotometer (UV-1900, Shimadzu, Japan).[13-19]

Fourier Transform Infrared Spectroscopy (FTIR)

The interaction and compatibility of pure drug, lipid, and surfactant used in TE preparation, as well as the physical mixture of drug and excipients, were studied using FTIR spectroscopic analysis. FTIR spectrometer was used for analyzing solid and liquid samples respectively. (SHIMADZU IR Affinity 1/8000, Japan and Bruker alpha II, USA). Inspecting was carried out using the transmission method, with a wave number range of 4000 to 400 cm^1.

For the physical mixture, the samples were taken in a 1:1 ratio and kept at room temperature. FTIR spectra were obtained for pure drug (QUERCITIN), lipid, and an actual combination of drug and lipid after a particular quantity of solid sample was combined with Potassium bromide (KBr).

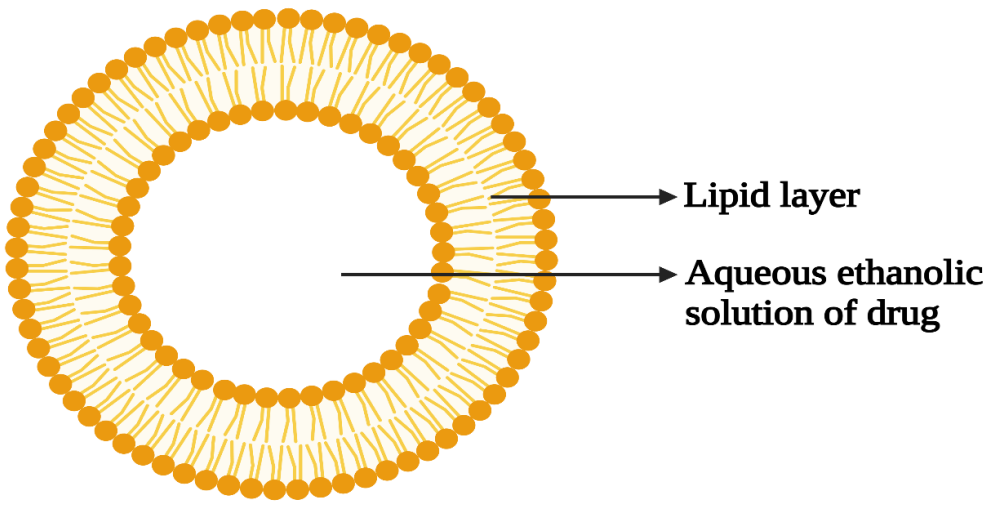

Figure 1: The structure of ethosomes.

For liquid samples, (Surfactant, mixture of drug and surfactant, and physical mixture of drug, lipid, and surfactant) specified amount is directly placed on the ATR crystal and analyzed.[20]

Differential Scanning Calorimeter (DSC)

DSC, a thermal analytical process, was employed to get the thermogram of quercetin by using aluminum pans. In this technique, an accurate weight of the sample was taken in the aluminum pans and sealed tightly. To determine the thermal behavior of the quercetin, the aluminum pans containing the sample were heated at a temperature range of 40-300°C, with a scanning rate of 20°C/min. To provide an inert atmosphere nitrogen gas was purged continuously at a 40 mL/min flow rate.

Preparation of quercetin loaded Ethosomes by cold method

Most likely the technique most frequently employed to prepare ethosomal formulation. This method uses a mixer and vigorous stirring to disperse drugs, phospholipids, and other types of lipid materials in ethanol in a wrapped vessel at room temperature. Propylene glycol or another polyol is added while stirring. The mixture is heated to 300°C in a water bath. After adding the water that has been heated to 300°C in a separate vessel, the mixture is stirred for 5 min in a covered vessel. The ethosomal formulation's vesicle size can be reduced to the appropriate degree by extrusion or sonication. Finally, the mixture is stored in a refrigerator.It was given in Tables 1 and 2.

Table 1: Materials and Suppliers.

Materials	Suppliers
Quercetin	Biomed Ingredients, Goa.
Soya lecithin	Himedia Laboratories Pvt. Ltd.,
Cholesterol	Loba Chemie Laboratory Reagents.
Propylparaben	Himedia Laboratories Pvt. Ltd.,
Carbopol 940	SD Fine Chem Limited.

Preparation of quercetin loaded Ethosomes gel

The drug-loaded ethosomes (which are equal to 1%) were added to gels by slow mechanical mixing for 30 min at 600 revolutions per minute (REMI-type BS stirrer). The perfect mixture was added to a 1% w/w concentration of carbopol, and 0.001% of propyl paraben was used as a preservative.

Preparation of quercetin Hydrogel

The preparation of the quercetin hydrogel was achieved by gradual addition of gelling agent 1% Carbopol 940 by mechanical mixing at 600 rpm (REMI type BS stirrer) for 30 min.[21]

Evaluation parameter of quercetin loaded Ethosomes

Particle size and PDI

Using a particle size analyzer (Nanopartica SZ-100, HORIBA Scientific, USA), the prepared quercetin-loaded TE formulations' particle size and PDI were measured using the malvan zeta sizer technique. The nanovesicles were diluted with distilled water and then analyzed at room temperature. The particle size and PDI of the diluted samples were measured in triplicate, and the results were reported as an average±standard

Percentage Entrapment Efficiency (%EE)

It's the efficiency of the drug to entrap the drug, can be measured using the centrifugation method. The unentrapped drug is separated by centrifugation method, at the end of the method supernatant liquid is obtained. The amount of drug entrapped is calculated.

Determination of Zeta Potential of optimized batch

Zeta potential is the measurement of the electric charge on a nanoparticle's surface that represents the system's physical stability. The Zeta potential of an optimized batch of transethosome was determined by the electrophoretic light scattering technique using a particle size analyzer (Nanopartica

Table 2: Formulation of quercetin ethosomes.

Formulation	Drug (mg)	Lipid (mg)	PEG
F1	30 mg	200 mg	2.6 mg/mL
F2	30 mg	200 mg	2.8 mg/mL
F3	30 mg	200 mg	3.0 mg/mL
F4	30 mg	300 mg	2.6 mg/mL
F5	30 mg	300 mg	2.8 mg/mL
F6	30 mg	300 mg	3.0 mg/mL
F7	30 mg	400 mg	2.6 mg/mL
F8	30 mg	400 mg	2.8 mg/mL
F9	30 mg	400 mg	3.0 mg/mL

SZ-100, HORIBA Scientific, USA Before analysis, the samples were diluted with double distilled water. The findings were presented as mean ± standard deviation, and the analysis was done in triplicate.

Scanning Electron Microscopy (SEM)

A scanning electron microscope was used to study the morphology of the optimum ethosomes.

In vitro Drug release study

Samples of nanoparticles were put into dialysis bags, sealed, and submerged in a dissolution medium. The USP Dissolve Test Apparatus, Type II, was used to conduct a 2 hr drug release study at 37±0.5° and 100 rpm. 5 mL of sample were taken at each interval and replaced with brand-new buffers. Following suitable dilutions, spectrophotometric evaluation was performed on the samples.[22]

Evaluation of Quercetin-Loaded Ethosomal hydrogel

pH

The digital pH meter was used to determine the gel's pH. After agitating 1 g of ethosomal gel in distilled water until a homogenous suspension was achieved, the pH of the mixture was determined.

Spreadability

To compress the sample to a consistent thickness, an excess of gel (2 g) was sandwiched between petri dishes, and 50 g of weight was placed on slides for 5 sec. The tin (sec) required to separate the two slides, was taken as a measure of spreadability.

It was calculated using the formula,

$$S=M.\ L/T$$

Where, S=spreadability

M=weight tied to upper slide

L=length of glass slide

T=time taken in min

Viscosity

The gel's viscosity was measured with a Brookfield viscometer (LV) of the Di type. As spindle number four is non-Newtonian in nature, it is utilized. Viscosity was measured at 0.3 rpm for a predetermined duration of 2 min.[23]

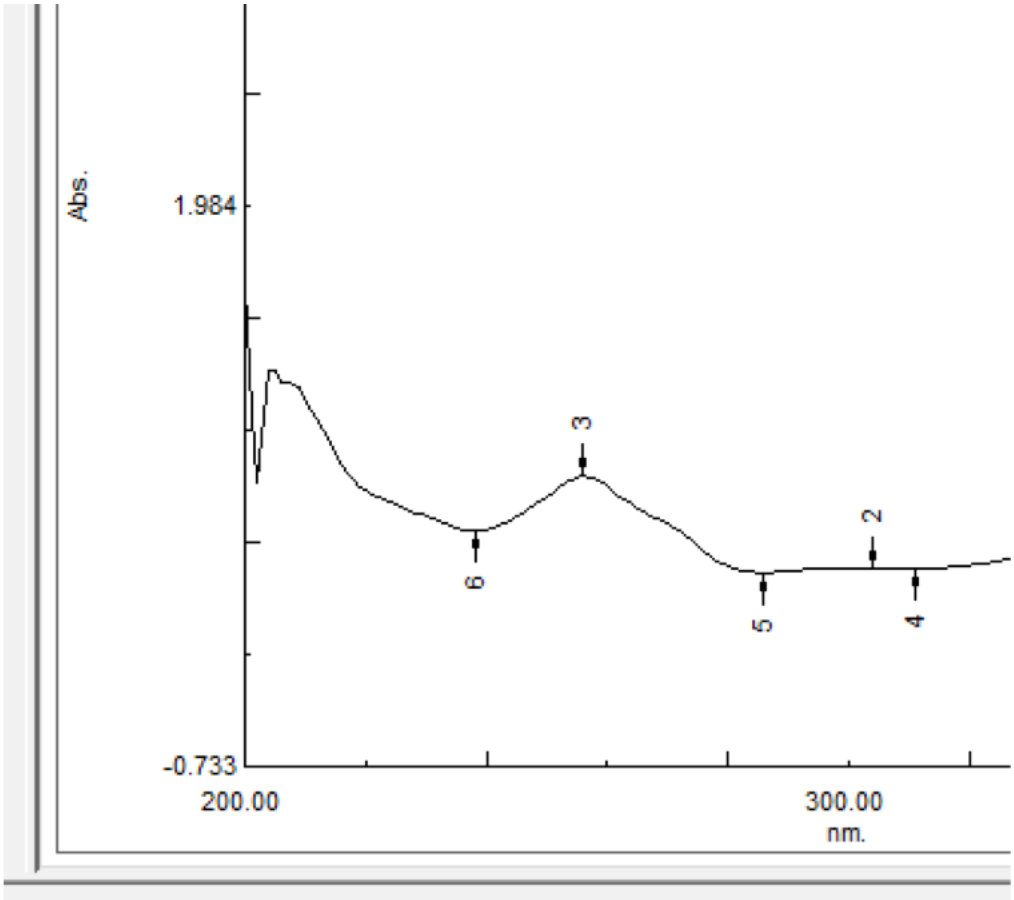

Figure 2: Determination of λ_{max}.

Washability

Formulas were applied to the skin before being manually assessed for the ease and extent of water cleansing.[24]

Visual Observation

Color matters when it comes to patient compliance. We visually examined the prepared gels to ensure they were stable, uniform, and had a pleasing texture.[25]

RESULTS

Pre-formulation Studies

Determination of absorption Maxima (λ_{max}) by UV-visible spectroscopy and Standard calibration curve of Quercetin

Absorption maxima of Quercetin was carried out using wavelength from 800-200nm. At 373nm, the highest absorption was recorded and employed. λ_{max} was depicted in Figure 2. The Quercetin standard calibration curve was estimated by plotting concentration v/s Absorption. The standard calibration curve was found to be linear at a maximum distance of 373nm in the beer's range. Since y=0.073-0.0048, the obtained correlation coefficient R_2=0.9993.

The drug shows a linear relationship between absorbance and drug concentration level. The synthesized impurity shows maximum absorbance at 373 nm. The equation of the straight line for quercetin is y=0.073-0.0048 and the regression coefficient is R_2= 0.9997 which is given in Figure 3**.**

FTIR study

The FTIR spectra of pure quercetin, physical nature of quercetin, carbopol, HPMC, is shown in Figure 4. The quercetin FTIR spectra clearly showed all of the characteristic peaks. Thus, all the drug's properties remained largely unchanged, and quercetin was effectively incorporated into the gel formulation.

DSC study

DSC thermograms of pure quercetin, physical mixture of quercetin, carbopol. Sharp endothermic peaks at 270.67°C were visible on the thermal graph of pure quercetin, signifying the compound's melting point (Figure 5).

Evaluation of quercetin loaded Ethosomes

Particle size and PDI

Particle size was found to be 57.4 nm and PDI was found to be 1 which was depicted in Figure 6.

Entrapment efficiency

Entrapment efficiency was found to be 92%.

Zeta potential

The optimized formulation's zeta potential was found to be 0.38 after the analysis was conducted in triplicate.

Scanning Electron Microscopy (SEM)

A scanning electron microscope was used to study the morphology of the optimum ethosomes. The particles was found to be sphere shape which was given in Figure 7.

In vitro Drug release study

The graph of the cumulative percentage of the drug released over time was plotted to obtain the *in vitro* drug release profiles of quercetin from optimized batch at pH 7.4 is shown in figure. The drug release study indicates that the optimized batch of quercetin ethosomes releases with an initial burst release of roughly 21.48%, respectively, in one hour, followed by maximum amount of drug released was observed up to 24 hr (Figure 8).

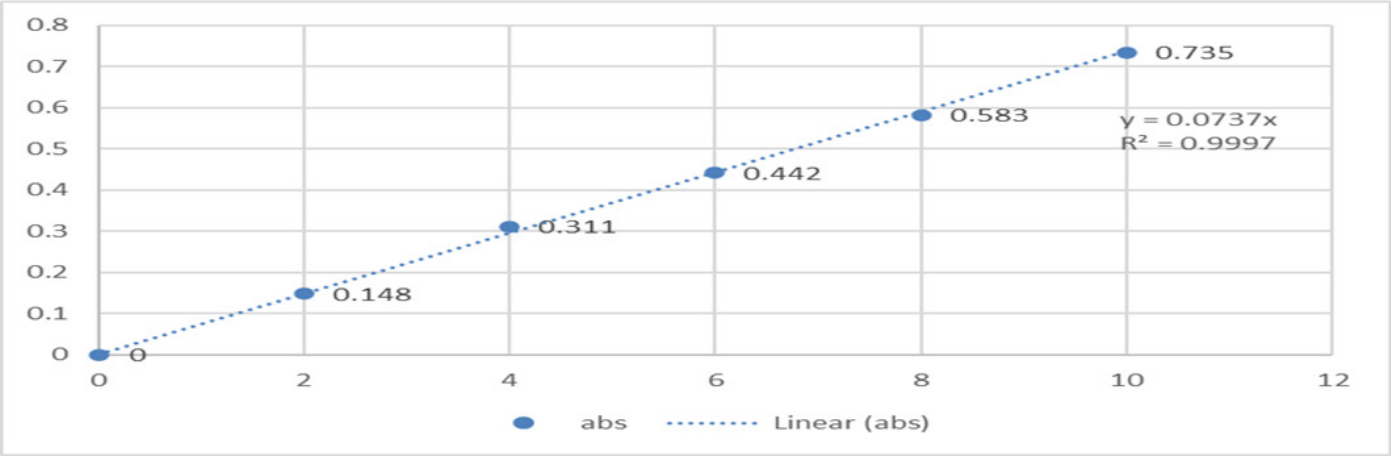

Figure 3: Standard calibration curve of Quercetin.

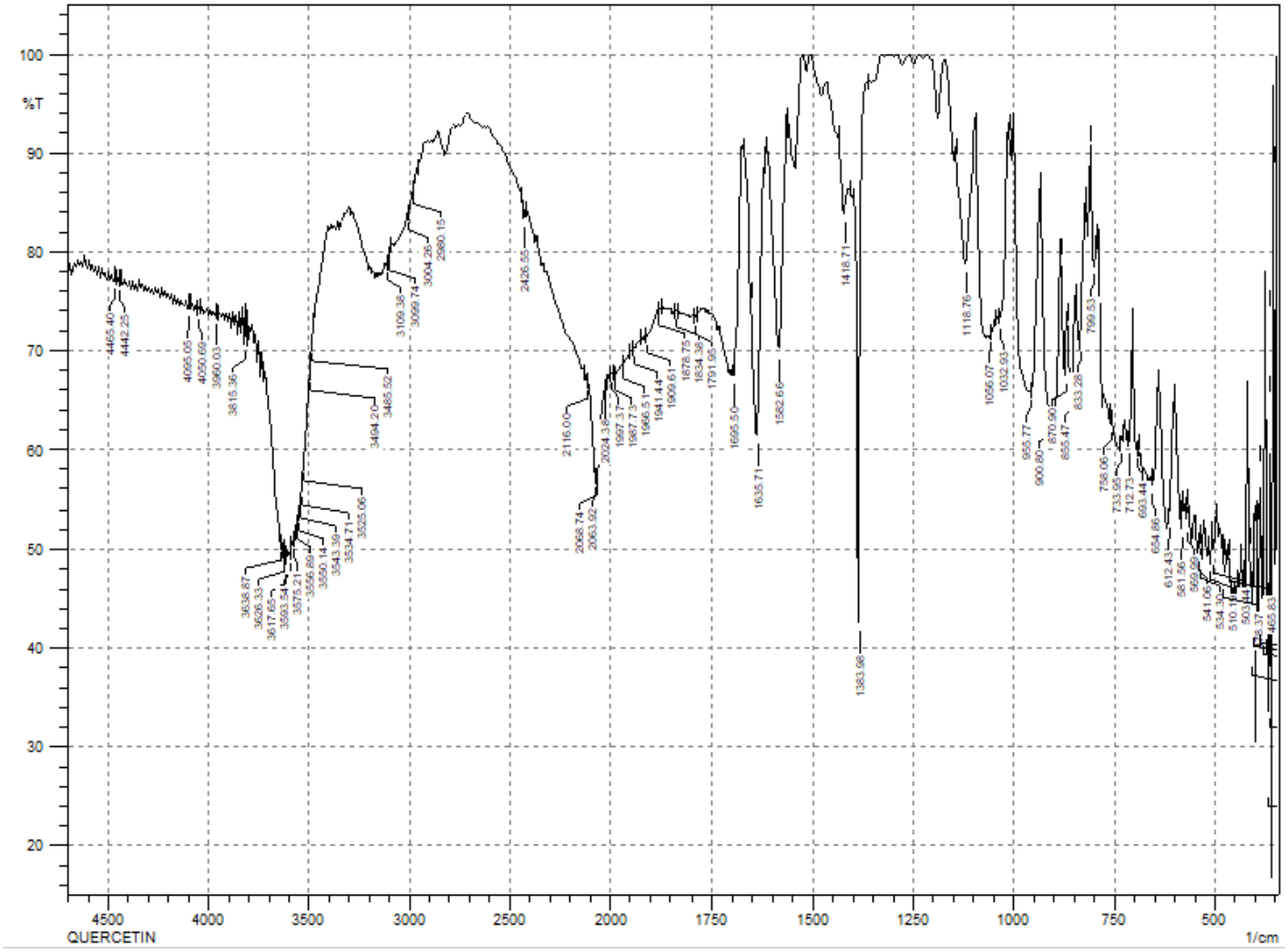

Figure 4: FTIR Spectra of quercetin.

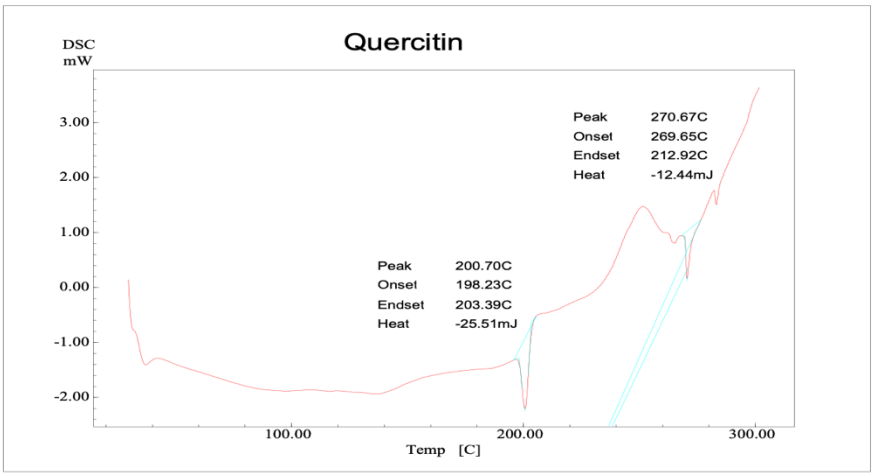

Figure 5: DSC thermograms of quercetin.

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	57.4	-	-	57.4	57.4
Polydispersity Index (PI)	1	-	-	1	1
Derived Mean Count Rate (kcps)	1196	-	-	1196	1196
Fit Error	0.02172	-	-	0.02172	0.02172
In Range (%)	90.44	-	-	90.44	90.44

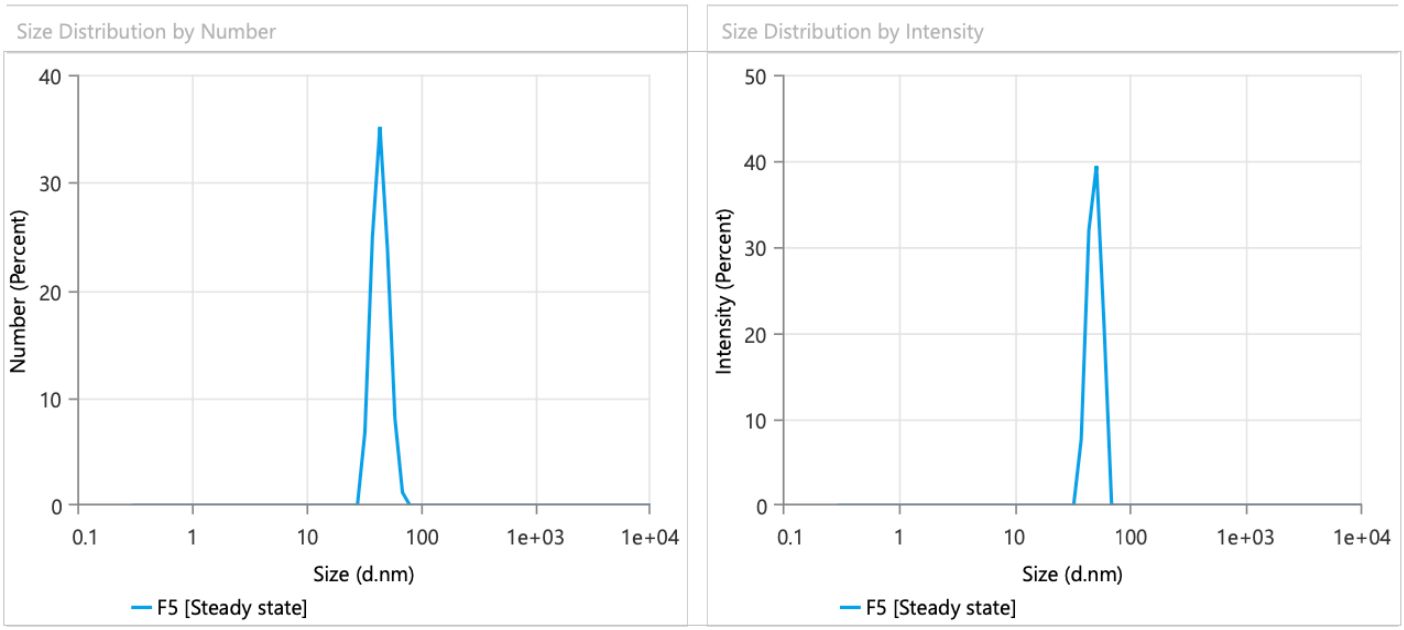

Figure 6: Determination of particle size and PDI.

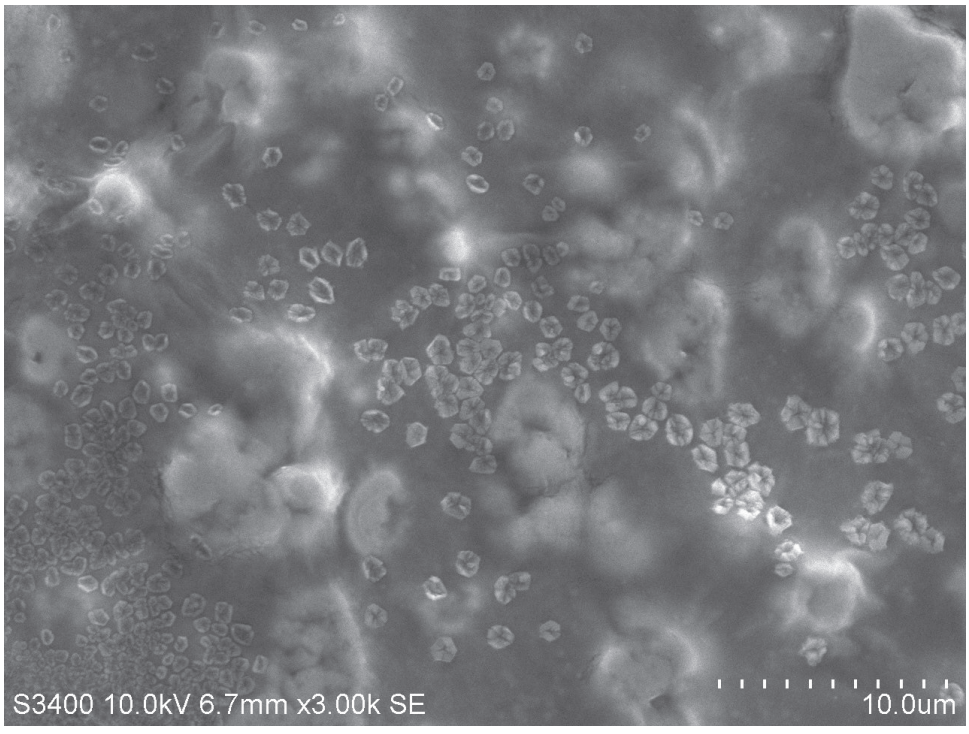

Figure 7: SEM of the quercetin loaded Ethosomes.

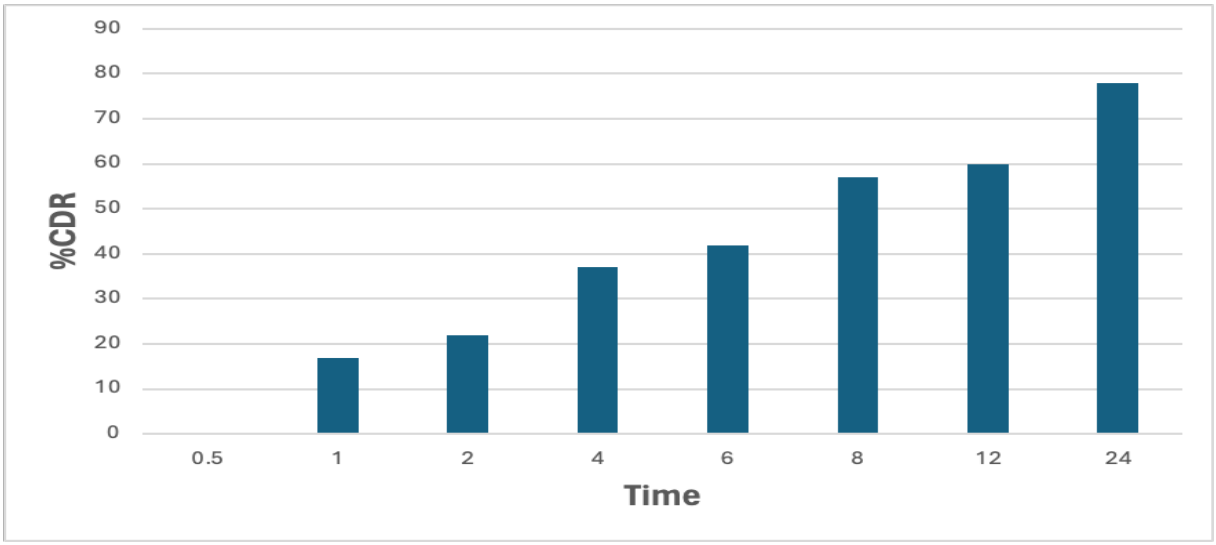

Figure 8: *In vitro* drug release study.

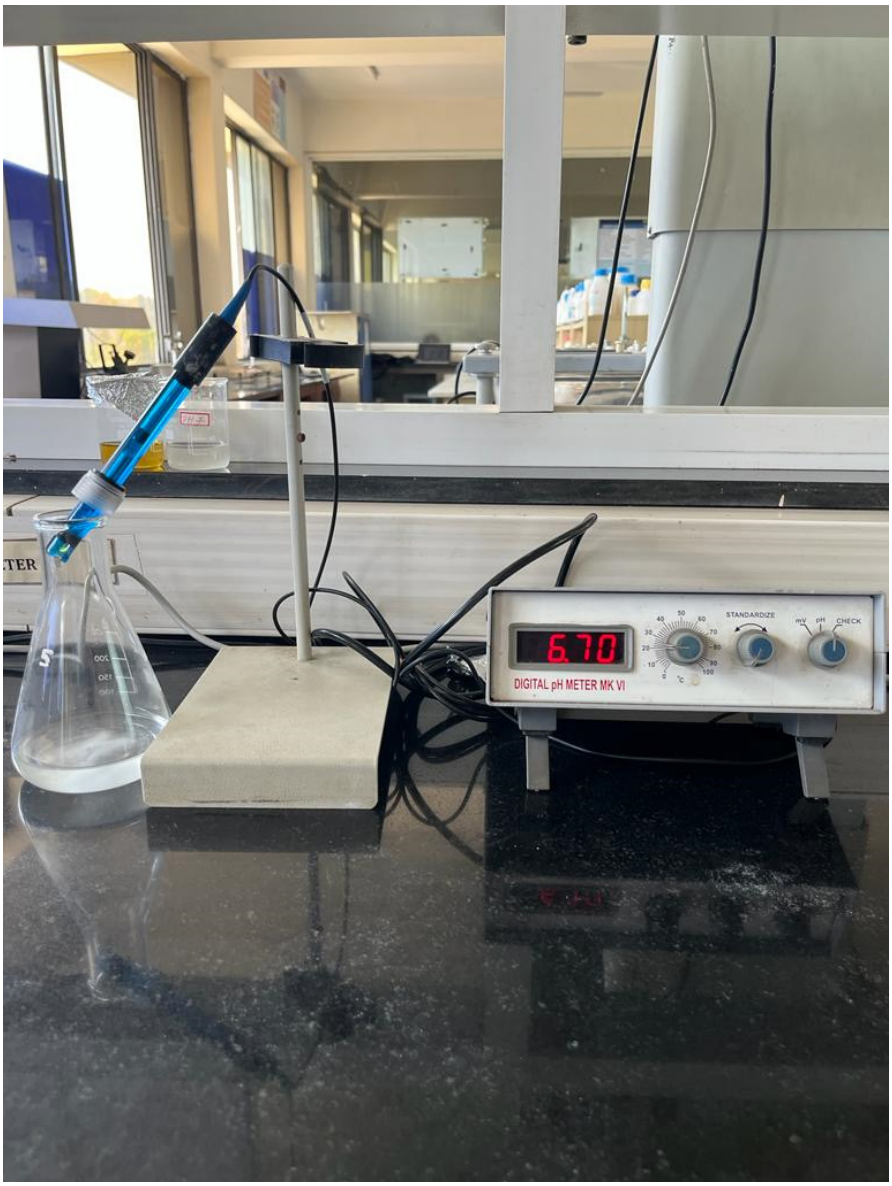

Figure 9: Determination of pH.

Evaluation of quercetin loaded ethosomal hydrogel

pH

The pH of Quercetin loaded ethosomal gel was found to be 6.4 (Figure 9) and pH of hydrogel was found to be 6.8. The pure gel was found to be 7 which may avoid any skin irritation.

Spreadability

The therapeutic effect of the Quercetin loaded ethosomal gel depends on the spreading value. The spreadibility of Quercetin loaded ethosomal gel was 2.31 g cm/sec and of hydrogel was 1.85 g cm/sec (Figure 10).

Viscosity

The viscosity of Quercetin loaded ethosomal gel was found to be 665 CPE and viscosity of hydrogel was found to be 748 CPE (Figure 11).

Washability

The washability of Quercetin loaded ethosomal gel was found to be 5.70 sec and that of hydrogel was 8.23 sec (Figure 12).

Visual observation

The colour of Quercetin loaded ethosomal gel and hydrogel was pale yellow.

DISCUSSION

Hydrogel showed better physical stability and ease of preparation compared to the ethosomal gel. Hydrogels are also biocompatible and is readily taken away from the skin without leaving any residue. However, the hydrogel showed lower skin permeation and retention of quercetin that can be confirmed through better spreadability, viscosity and washability of ethosomal gel when compared to quercetin hydrogel.

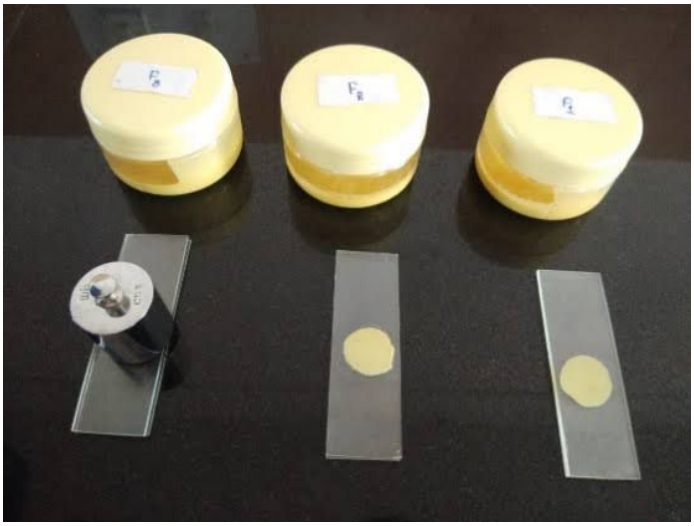

Figure 10: Spreadability studies.

Figure 11: Determination of viscosity of the quercetin.

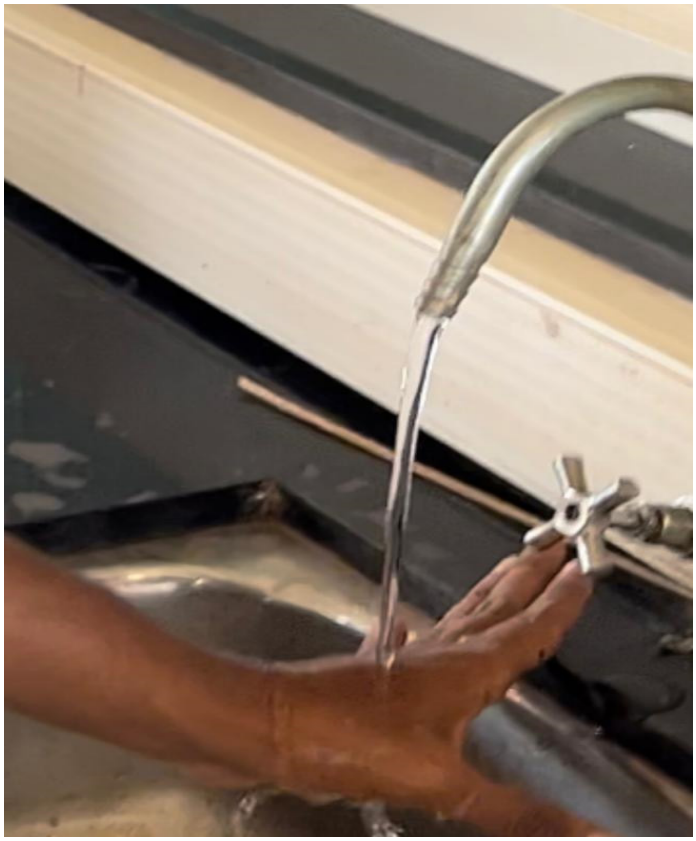

Figure 12: Determination of washability of quercetin.

Therefore, the choice of formulation depends on the specific needs and requirements of the intended application. Ethosomal gel may be preferred when high skin permeation and retention of quercetin are desired, whereas hydrogel may be preferred for ease of preparation and removal, and better physical stability.

CONCLUSION

Based on the comparative study of quercetin ethosomal gel and hydrogel, it can be concluded that both formulations have their own advantages and limitations. Quercetin ethosomal gel showed higher skin permeation and retention of quercetin compared to hydrogel. This can be attributed to the enhanced penetration ability of ethosomes due to its low particle size and high entrapment efficiency, which allows easy passage of drug through the stratum corneum and reach deeper skin layers. The study's optimal formulation was chosen to be the ethosomal formulation F5, which displayed lower particle size and higher entrapment efficiency. The ethosomal gel of optimized F5 formulation was prepared that exhibited better antioxidant and anti-inflammatory activity, which can be beneficial in treating various skin disorders.

ACKNOWLEDGEMENT

The authors express heartfelt gratitude towards the KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, for providing all the obligatory facilities for completion of this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

QT: Quercetin; **TDS:** Transdermal delivery systems.

REFERENCES

1. Ueda CT, Shah VP, Derdzinski K, Ewing G, Flynn G, Maibach H, *et al.* Topical and transdermal drug products. Dissolut Technol. 2010;17(4):12-25.
2. Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: Current and future prospects. Drug Delivery: Journal of Delivery and Targeting of Therapeutic Agents. 2006;13(3):175-87.
3. Deng Y, Zhang X, Shen H, He Q, Wu Z, Liao W, *et al.* Application of the Nano-Drug Delivery System in Treatment of Cardiovascular Diseases. Front Bioeng Biotechnol. 2020;7.
4. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praça FG, *et al.* Development, characterization, and skin delivery studies of related ultradeformable vesicles: Transfersomes, ethosomes, and transethosomes. Int J Nanomedicine. 2015;10:5837-51.
5. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. J Adv Pharm Technol Res. 2010;1(3):274-82.
6. Parashar T, Soniya, Sachan R, Singh V, Singh G, Tyagi S, *et al.* Review Article ETHOSOMES : A RECENT VESICLE OF TRANSDERMAL DRUG DELIVERY SYSTEM. International Journal of Research and Development in Pharmacy and Life Sciences. 2013;2(2):285-92.
7. Pandey N. Proniosomes and ethosomes: New prospect in transdermal and dermal drug delivery system. Int J Pharm Sci Res [Internet]. 2011;2(8):1988-96. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L368484060%5Cnhttp://vb3lk7eb4t.search.serialssolutions.com?sid=EMBASE&issn=23205148&id=doi:&atitle=Proniosomes+and+ethosomes%3A+New+prospect+in+transdermal+and+dermal+drug+delivery+
8. Sankar V, Wilson V, Siram K, Karuppaiah A, Hariharan S, Justin A. Topical delivery of drugs using ethosomes: A review. Indian Drugs. 2019;56(8):7-20.
9. Dubey A, Kumar N, Mishra A, Tiwari P. Ethosomes: A Novel Approach in Transdermal Drug Delivery System A REVIEW ON β-ESCIN View project Ethosomes: A Novel Approach in Transdermal Drug Delivery System. International Journal of Pharmacy & Life Sciences [Internet]. 2020;11(5):6598-608. Available from: https://www.researchgate.net/publication/344478082
10. Kelly G.S. Alternative Medicine Review (AMR). 2011;
11. Ebrahimpour S, Zakeri M, Esmaeili A. Crosstalk between obesity, diabetes, and alzheimer's disease: Introducing quercetin as an effective triple herbal medicine. Ageing Res Rev. 2020;62.
12. Ibrahim ESA, Hassan MA, El-Mahdy MM, Mohamed AS. Formulation and evaluation of quercetin in certain dermatological preparations. J Drug Deliv Sci Technol. 2007;17(6):431-6.
13. Chen C, Zhou J, Ji C. Quercetin: A potential drug to reverse multidrug resistance. Life Sci. 2010;87(11-12):333-8.
14. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H YY. Quercetin, inflammation and immunity.
15. Gupta V, Sanyogita KM, Manigauha A. Novel Formulation of *Aloe vera* and Quercetin in the Management of Dermal Disease: Eczema. Journal of Pharmaceutics and Drug Research JPDR [Internet]. 2021;4(2):480-7. Available from: www.scitcentral.com
16. Hatahet T, Morille M, Hommoss A, Devoisselle JM, Müller RH, Bégu S. Quercetin topical application, from conventional dosage forms to nanodosage forms. European Journal of Pharmaceutics and Biopharmaceutics. 2016;108:41-53.
17. Shi GJ, Li Y, Cao QH, Wu HX, Tang XY, Gao XH, *et al. In vitro* and *in vivo* evidence that quercetin protects against diabetes and its complications: A systematic review of the literature. Biomedicine and Pharmacotherapy. 2019;109:1085-99.
18. Madi, R., Hanafi, N. I., Abd-ElGawad, A. M., & Salem HA. Topical application of quercetin in wound healing reduces oxidative stress and inflammation.
19. Zhang, X., Zhang, S., Lu, L., Zhao, Y., Liu, X., Yan, Y., & Yang H. Ethosomal Quercetin Encapsulating Bio-Functional Gel for Effective Treatment of Psoriasis in Animal Model.
20. Touitou E., Dayan N., Bergelson L., Godin B. EM. Ethosomes: A Novel Vesicular Carrier for Enhanced Transdermal Delivery of Bioactive Molecules. In: Touitou E. (eds) Formulation and Delivery of Proteins and Peptides.
21. Saha, S., Roy, K., & Pal S. Ethosomes as a carrier system for transdermal drug delivery: An overview.
22. Ahmed M. Samy. Development and characterization of ethosomes-based hydrogel for enhanced transdermal delivery of diclofenac diethylamine.
23. K. K. Kavitha, K. J. Geetha, A. H. Kumar and NHS. "Preparation and Characterization of Ethosomes by Cold Method,."
24. Spoorthi Shetty S, Halagali P, Johnson AP, Spandana KMA, Gangadharappa H V. Oral insulin delivery: Barriers, strategies, and formulation approaches: A comprehensive review. Int J Biol Macromol. 2023;242.
25. Angolkar M, Paramshetti S, Halagali P, Jain V, Patil AB, Somanna P. Nanotechnological advancements in the brain tumor therapy: a novel approach. Ther Deliv. 2022;13(11):531-57.

Cite this article: Halagali P, Wannur VI, Patil AKA, Patil PP, Naik SM, Marennavar SA, *et al*. Formulation and Evaluation of Quercetin Ethosomal Hydrogel for Topical Delivery System. Int. J. Pharm. Investigation. 2024;14(3):749-58.