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Physicochemical Characterization, Release and Penetration Study of Nanostructured Lipid Carriers Quercetin Incorporated into Membrane-Type Patches

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ABSTRACT

Quercetin is a bioactive flavonoid that possesses anti-inflammatory, antioxidant, and osteoarthritic properties. Nevertheless, quercetin does possess several limitations, including poor solubility, degradation in the gastrointestinal tract, and inadequate transdermal absorption. To address this issue, quercetin is incorporated into a nanostructured lipid carrier (NLC). The NLC is then enclosed in a membrane patch, which acts as a reservoir to maintain the stability of the drug content. The aim of this study was to investigate the physicochemical properties, release kinetics, and penetration of quercetin when formulated as NLC and incorporated into a membrane-type patch. Quercetin-loaded NLC was manufactured utilizing high shear homogenization using stearic acid as the solid lipid and oleic acid as the liquid lipid in various ratios such as 6:4, 7:3, and 8:2. Furthermore, the NLC was poured over the drug reservoir in a 3.8 cm diameter backing layer mold and covered with membrane solution. The physicochemical characteristics evaluated include particle size, polydispersity index, pH, viscosity, zeta potential, entrapment efficiency, thickness, humidity, flatness, drug content, homogeneity, release study and penetration. The result showed that Formula 1 had good characteristics among other formulas with a particle size of 533 ± 50 nm, PDI of 0.343 ± 0.01, viscosity of 322 ± 12 cps, pH of 5.84 ± 0.07, thickness of 0.32 ± 0.02 mm and produced the highest release and flux penetration, namely 0.6517 ± 0.02 μg/cm^2/min and 0.0013 μg/cm^2/min, respectively. NLC as reservoir in membrane-type patch with higher concentration of liquid lipids could increase release and flux penetration.

Keywords: Nanostructured Lipid Carrier, Quercetin, Membrane-Type Patch, Solid Lipid, Liquid Lipid.

Introduction

A phenolic flavonoid known as quercetin is a substance that is present in variety of plants, including berries, apples, cauliflower, tea, beans, cabbage and onions. These substances have a variety of pharmacological actions, including antioxidant, antiviral, anticancer and anti-inflammatory properties.[1] Quercetin has anti-inflammatory actions by suppressing TNF-alpha and preventing the activation of inflammatory mediators such AP-1 and NF-kB.[2] Quercetin exhibits limited bioavailability, low solubility, and undergoes hepatic degradation during first metabolism.[3] In order to overcome this limitation, various alternate administration routes can be employed, including pulmonary, nasal, buccal, and injection. Nevertheless, several of these methods of delivery have certain drawbacks, including limited absorption through the nasal and buccal surfaces, discomfort associated with injections, and variability in dosing due to variations in pulmonary inhaler technique.[4] As a result, transdermal administration can serve as an alternative method to deal with this disadvantage.

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In spite of that, transdermal administration is restricted by several limitations, including the existence of a complicated barrier (*stratum corneum*, continuous exfoliation of the outermost layer, lipid bilayers encapsulating corneocytes). Nevertheless, the use of quercetin topically is hindered by its poor ability to penetrate the epidermis.[5] Therefore, in order to enhance skin penetration, it is necessary to implement a strategy, such as incorporating quercetin into the lipid nanoparticle system.

There are two lipid nanoparticle systems, namely solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLC). When compared to SLN, NLC provides benefits that can improve drug entrapment effectiveness, increase drug release, and decrease the leakage of drugs from the system.[6] Compared to the quercetin present in the SLN, which is 127 nm in size, the quercetin present in the NLC is smaller, measuring 34 nm.[7] Because of its ability to increase cellular absorption with immediate action, NLC intended for transdermal delivery has a particle size of 50-300 nm.[8] The ratio of solid to liquid lipids utilized can influence the particle size of NLC. According to Hendradi et al., when compared to other ratios, the ratio of solid to liquid lipids with a higher proportion of liquid lipids, namely 6:4, can produce a smaller particle size, namely 351.9 nm.[9] Additionally, NLC has a better entrapment efficiency (EE) than SLN. In the investigation by Pedzisai et al., NLC was able to create efarivenz, a medication used to treat the human immunodeficiency virus (HIV) EE at a greater level, at 99.93% as opposed to 96.77% for SLN.[10] Nevertheless, the efficacy of drug encapsulation in NLC can diminish both during storage at ambient temperature and storage at low temperatures.[11] In order to address this, NLC can be incorporated into a membrane-type patch thus can maintain the drug entrapment efficiency.[12] The aim of this study is to determine the physicochemical properties, release and

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penetration study of membrane-type patches containing quercetin loaded NLC.

Materials And Methods

Materials

Quercetin (Sigma Aldrich, China), stearic acid (PT. Sumi Asih Oleichemicals Industry, Indonesia), oleic acid (Marks & Nos Inc.), Tween 80 (PT. KAO), menthol and PEG 400 (PT. Bratachem, Indonesia), HPMC 606 (Wuhan Senwayer Century Chemical Co. Ltd, China), ethanol p.a (Merck, Germany) and aquadest.

Experimental Animals

This study received ethical approval from the Faculty of Veterinary Medicine Ethics Committee at Universitas Airlangga, Surabaya, Indonesia (No: 2.KEH.057.04.2023).

NLC Preparation

The emulsification process with high shear homogenization was used to create quercetin-loaded NLC. The formula incorporates stearic acid as a solid lipid and oleic acid as a liquid lipid in the ratios of 6:4, 7:3, and 8:2, as indicated in Table 1. Stearic acid and oleic acid were mixed and heated to 65^{o}C prior to the addition of quercetin. Tween 80 and phosphate buffer pH 6 were mixed together and heated to the same temperature as the water phase. The water phase was added to the oil phase all at once and mixed with an Ultra Turax at 5600 rpm for 5 minutes, then rested for 2 minutes before stirring at 18000 rpm for 8 minutes. The substance was cooled by subjecting it to rotation using a magnetic stirrer at a speed of 500 rpm until it reached the room temperature (25^{o}C).

Patch Preparation

NLC was poured into a 3.8 cm diameter backing layer mold and allowed to dry for 24 hours at room temperature. The rate-controlling membrane was casted on the drug reservoir using 10% (w/v) HPMC 606 in water added PEG 400 and menthol as plasticizer and permeation enhancer. The patch was then allowed to dry at room temperature for 12 hours.[13] Table 2 provides the patch formula.

Organoleptic Evaluation

The organoleptic evaluation of quercetin-loaded NLC consisted of a visual evaluation of each formula's consistency and color.

pH Determination

The pH of quercetin-loaded NLC in each formula was determined using a pH meter.

Viscosity Determination

Viscosity was determined using a Cone and Plate viscometer. A sample weighing approximately 2 mL was placed on the middle plate and then elevated to a position directly below the cone.[14] The sample was located in the shear area between the stationary plate and the rotating cone. The velocity was adjusted via a dial selector, which either raised or lowered the shear speed. The corresponding viscosity was then indicated on a scale.[15]

Particle Size and Polydispersity Index Evaluation

An amount of 50 mg of NLC was weighed, and 50 mL of distilled water was added. The mixture was then agitated for 30 minutes using a magnetic stirrer set at 500 rpm and subjected to sonication for 3 minutes. The determination was conducted using the DelsaTM nano submicron particle size analyzer.[14]

Zeta Potential

The zeta potential is the primary property required to estimate the surface charge of nanoparticles. It is an essential component of the physical stability of the NLC. It was SZ-100 HORIBA Scientific that conducted the zeta potential analyses. The samples were subsequently diluted with double-distilled water, and particulates were evaluated in triplicate at a temperature of 25°C via electrophoretic scattering of light at a 90° angle.[12]

Encapsulation Efficiency

Quercetin-loaded NLC was placed in a beaker glass and centrifuged it for 10 minutes at 15000 rpm. After collection, the supernatant was diluted 25 times and analyzed using a UV-Vis spectrophotometer set to the maximum wavelength at 371.4 nm. The equation is then used to calculate, as below:

$$EE\ (\%) = \frac{(Ct - Cf)}{Ct} x\ 100\%$$

Where Ct is the amount of quercetin used and Cf is the amount of drug in the water phase.[20]

Thickness Evaluation

The patch thickness test was conducted by employing a vernier caliper to precisely measure the thickness of three patches. The mean thickness and standard deviation of many measurements could be utilized to validate the appropriate thickness.[12]

Table 1: Quercetin-loaded NLC formula

Materials	Function	Concentration (%) (w/w)		
		F1	F2	F3
Quercetin	Active ingredients	1	1	1
Stearic acid	Solid lipid	6	7	8
Oleic acid	Liquid lipid	4	3	2
Tween 80	Surfactant	5	5	5
Phosphate buffer pH 6 ± 0.05	Aqueous phase	ad 100%		

Table 2: Membrane-type patch formula

Materials	Function	Formula Concentration	
		FI	FII
Quercetin-loaded NLC	Reservoir	410 mg/12.56 cm^2	-
Physical Mixture as cream	Reservoir	-	410 mg/12.56 cm^2
HPMC 606 (10%)	Rate controlling membrane	1 mL	1 mL
PEG 400	Plasticizer	117 mg	117 mg
Menthol (1%)	Enhancer	0.641 mL	0.641 mL

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Moisture Content Evaluation
The patch was placed in a desiccator with dry silica gel for 24 hours and weighed again. The following equation can be used to calculate the percentage of moisture in a patch:

$$Moisture\ Content\ (\%) = \frac{(Initial\ mass - final\ mass)}{initial\ mass} x\ 100\%$$

Flatness Evaluation
The patch was flattened by cutting it into three long strips, one on each of the right, left, and central side. The following equation was used to calculate the thickness of each strip:
$Contriction\ (\%) = (I1 - I2)\ x\ 100$
Where I1 is the thickness of the initial strip and I2 is the thickness of the final strip.[17]

Drug Content Evaluation
The patch was immersed in an Erlenmeyer flask containing 100 mL of 70% ethanol, agitated for a duration of 24 hours, and afterwards separated by filtration. The supernatant was subjected to UV/Vis spectrophotometry analysis at its maximum wavelength at 371.4 nm to quantify the drug concentration.[17]

Homogeneity Evaluation
The patch was placed in an Erlenmeyer with 100 mL of 70% ethanol, stirred for 36 hours, and filtered. Immediately after that, the filtrate's absorbance was assessed using UV/Vis spectrophotometry at the wavelength at 371.4 nm.[24]

Release and Penetration Evaluation
An amount of 500 ml of a solution consisting of phosphate buffer with a pH of 6.0, 1% Tween 80, and 20% ethanol 70% was utilized for a release and penetration study. The study lasted for 8 hours at a temperature of 37ºC. Subsequently, 5 mL aliquots were collected at hourly intervals, and the absorbance of the samples was measured using a UV/Vis spectrophotometer at the maximum wavelength of quercetin.[16] The release study employed cellophane as the membrane, while the penetration investigation utilized Wistar rat skin as the membrane. The cumulative amount of quercetin that has penetrated expanded the area of diffusion (µg/cm^2), which was computed using the following equation:[18]

$$Q = \frac{\mathrm{Cn.V} + \sum_{i=1}^{n-1} C.S}{\mathrm{A}}$$

Where Q is cumulative amount of a quercetin through the membrane, Cn is quercetin concentration (µg/mL) at the n-th minute, V is medium volume, $\sum_{i=1}^{n-1}$ is penetration concentration at previous minutes (µg/mL), S is sampling volume and A is membrane surface area (cm^2). The drug penetration rate per unit time was determined as the flux based on first Fick's, which is the following equation:[19]

$$J = \frac{\mathrm{M}}{\mathrm{A.t}}$$

Where J is flux (µg/cm^2.hour), M is the amount of active substance penetration (µg) A is membrane surface area (cm^2), t is time (minute).

Statistical Analysis
The drug release parameter and initial data on physicochemical characteristics were tested using statistical analysis using a one-way analysis of variance (ANOVA), provided that the data were normally distributed and exhibited homogeneity. Non-parametric statistical tests, such as the Kruskal-Wallis test, were employed when the data did not exhibit homogeneity or follow a normal distribution.[16]

Results and Discussion

Quercetin-loaded NLC Physicochemical Characteristics
Quercetin-loaded NLC physicochemical properties among formulae are showed in Table 3. All formulae of quercetin-loaded NLC had a similar color, a greenish yellow with a distinct odor, as shown in Figure 1. The concentration ratio of lipid liquid significantly impacts the particle size of NLC. Bhatt *et al*.[20] mentioned that the highest concentration ratio of liquid lipid yielded the smallest particle size. This might be explained by the possibility that more solid lipid may affect the melting and lead to the formation of agglomerates during the manufacture of NLCs. Additionally, greater concentration of solid lipids may have a tendency to fuse or form aggregates during the solidification phase of NLC manufacture. These aggregates may be unable to break apart, resulting in the formation of large particles with a wide size distribution.[21] This is further confirmed by the results, which show that the PDI value increases when the solid lipid to lipid ratio increases. Since all pH formulae provide results between pH 4-6, they are in compliance with the skin's pH and can be used topically.[22] Topical formulations' viscosity is an important factor because it affects how the formulation spreads on the skin's surface, which can have an impact on the concentration and penetration of the active ingredient.[22] Formula 3 has the highest viscosity because it has the highest solid lipid concentration. Formula 1 is ideal for topical use because of its viscosity, which is neither too thick nor too liquid. All formulas showed zeta potential value less than -20 mV and have no significant differences. NLC with zeta potential value less than -20 mV was classified as stable nanodispersion.[23] In the results of entrapment efficiency, all formulae gave high values, i.e above 98% and if statistical tests were performed, no significant difference was found. The use of stearic acid and oleic acid as a lipid matrix result in the high quercetin entrapment efficiency because both of these lipids can make quercetin more soluble, which increases the likelihood that it will be trapped in NLC particles.[24,25]

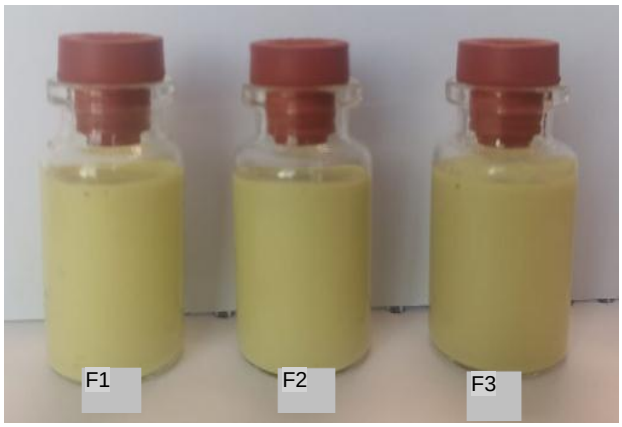

Figure 1 : The result of organoleptic of quercetin-loaded NLC in various lipid ratio. F1 (6:4); F2 (7:3) and F3 (8:2)

Table 3: Physicochemical properties of quercetin-loaded NLC (Mean ± SD, N=3)

Formula code	Particle Size (nm)	PDI	pH	Viscosity	Zeta Potential (mV)	Entrapment Efficiency (%)
F1	533 ± 50	0.343 ± 0.01	5.84 ± 0.07	322 ± 12 cps	-32.9 ± 2.7	98.4 ± 0.5
F2	661 ± 40	0.426 ± 0.07	5.81 ± 0.07	335 ± 10 cps	-29.9 ± 4.6	98.4 ± 0.5
F3	923 ± 67	0.436 ± 0.02	5.89 ± 0.04	472 ± 29 cps	-32.1 ± 3.9	98.5 ± 0.9

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Patch Membrane-Type Physicochemical Characteristics

Patch membrane type physicochemical properties with quercetin-loaded NLC as reservoir can be seen in Table 3. The SEM image captures the surface of the membrane-type patch (Figure 2). A patch containing NLC F1 and a physical mixture of the same formula were used as patch samples. NLC F1 was selected since the release test results revealed that it had the greatest flux value of any formula. The pores on the patch let the drug escape from the matrix and larger pores allow the medication to exit more easily. Penetration enhancement is an enhancing system that leads to an increase in the number of drug molecules passing through the skin due to many characteristics such as natural origin, good penetration enhancement, and partitioning action in the skin by oils.[26]

All patch compositions have a thickness of less than 1 mm. The drug release is influenced by the thickness of a membrane patch. As the thickness of the membrane patch increases, the amount of drug release decreases.[27] The moisture content values of all patch formulae show no statistically significant differences. The moisture content of all formulae is within the range of 2 to 10%. It is advisable to maintain a low moisture level in order to optimize stability, minimize fragility, promote good density, and reduce susceptibility to bacterial decomposition.[28] Since none of the patch formulae exhibit any constriction, the patch can be said to be completely flat. Drug content of patch of F1 to F3 formulation ranges from 76.8 ± 0.16 to 86.5 ± 1.21 %. Furthermore, F2 shows the maximum drug content. However, as the homogeneity value in the F2 patch formula increases, the release from the F2 patch decreases. Patch formula F1 has the lowest homogeneity due to its lower viscosity, making it easier to spread NLC during the reservoir pouring process.

In Vitro Release Study of Patch

In vitro release study from membrane-type patch with quercetin-loaded NLC as reservoir was investigated using paddle over disc with phosphate buffer pH 6, Tween 80 (1%) and ethanol 70% (20%) mixture as a release medium. Although that medium is unable to mimic the physiological conditions of skin, it has been utilized in a number of previous studies to make hydrophobic drugs more soluble in water and preserve sink conditions.[22,29,30] According to result as seen in Figure 3, F1 has the highest release compared to the other formula. On the other hand, F1 has the highest flux value. Patch formula flux values for F1, F2, and F3 are 0.6517, 0.6121, and 0.6108 µg/cm^2/min, respectively.

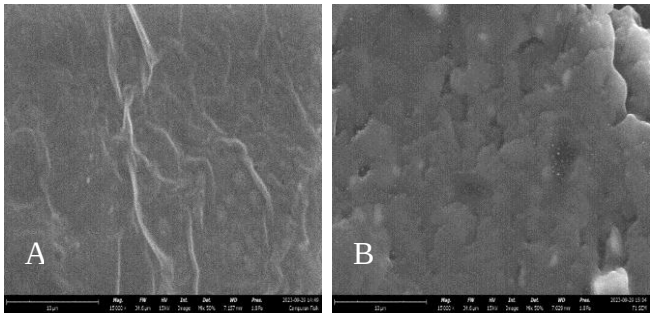

Figure 2 : Membran-type patch surface captured by Scanning Electrone Microscope. A: Membrane-type surface with physical mixture (cream quercetin) as reservoir. B: Membrane-type surface with NLC quercetin F1 as reservoir. (All patches were seen at a magnification of 15,000x)

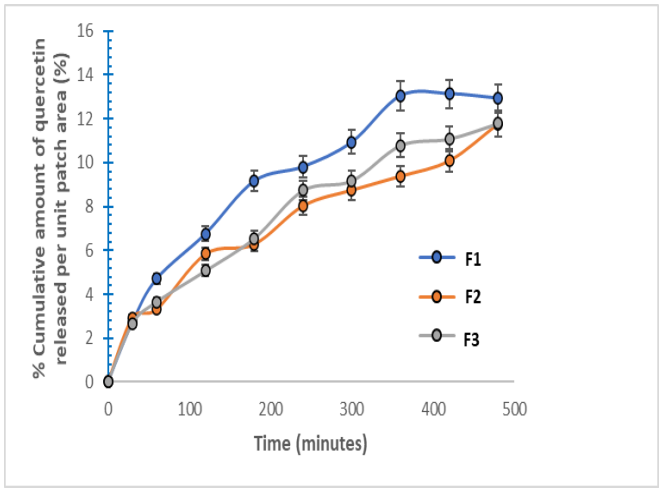

Figure 3: The relationship between cumulative amount of quercetin released versus time at 37^{o}C in the dissolution medium. Data is the mean of three replications.

Table 4: Physicochemical properties of membrane type patch with quercetin-loaded NLC as reservoir (Mean ± SD, N=3)

Formula code	Thickness (mm)	Moisture content (%)	Flatness (%)	Drug Content (%)	Homogenity (Coefficient of variations)(%)
F1	0.32 ± 0.02	3.14 ± 0.6	100	76.8 ± 0.16	18.3
F2	0.37 ± 0.01	3.00 ± 0.7	100	86.5 ± 1.21	25.5
F3	0.46 ± 0.02	3.13 ± 0.1	100	79.7 ± 1.44	22.8

Table 5: Drug release kinetics of membrane-type patch with quercetin-loaded NLC as reservoir

Release kinetics model		F1	F2	F3
Zero order	Regression equation	0.1056x + 10.642	0.0857x + 8.1891	0.0938x + 7.6689
	r	0.8998	0.9351	0.9448
First order	Regression equation	0.0018x + 0.4267	0.0017x + 0.3716	0.0018x + 0.3609
	r	0.7142	0.7612	0.785
Higuchi	Regression equation	2.6244x – 0.8087	2.0933x – 0.6283	2.2806x – 1.835
	r	0.9846	0.9891	0.9894
Korsmeyer-Peppas	Regression equation	0.6526x + 0.0501	0.6083x + 0.0569	0.6239x + 0.0368
	r	0.9922	0.9891	0.9954
Hixson-Crowel	Regression equation	-0.0004x + 4.5313	-0.0003x + 4.5406	-0.0004x + 4.5427
	r	0.9061	0.9402	0.9494

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The flux of the patch was explored using several mathematical models to determine the best representation of quercetin release kinetics from the reservoir. Zero-order, first-order, Higuchi, Korsmeyer Peppas dan Hixson Crowel kinetic models are among the mathematical models employed. As shown in Table 5, the Korsmeyer Peppas kinetic model displays the strongest correlation coefficient (r), which causes the patch's release of quercetin to match this model's kinetics. F1 to F3 displayed exponent n values ranging from 0.6083 to 0.6517, indicating the occurrence of non-Fickian diffusion or anomalous transport. At n = 0.45, the release mechanism adheres to Fickian diffusion. However, for values of n between 0.45 and 0.89, the release mechanism exhibits either anomalous diffusion or non-Fickian diffusion. F1 has thinner viscosity and smaller particle size due to the presence of more liquid lipids, which may make it easier for NLC particle to be separated from the base.[22]

Penetration Study of Patch

The penetration study was carried out using the abdominal skin of male Wistar rats as a membrane model. This research had been certified by the committee of animal care and use of Faculty of Veterinary, Universitas Airlangga for animal usage in the experiment. This study was carried out on the F1 and the physical mixture of the same formula in the form of cream as the reservoir. The study used the same tools and medium that were utilized for the release study. According to Figure 4, the amount of quercetin that penetrated the rat skin membrane increased when a patch containing NLC as a reservoir was used. The flux values of the quercetin-loaded NLC as a reservoir and the quercetin cream are 0.0013 µg/cm^2/min and 0.007 µg/cm^2/min, respectively. Several studies have revealed that the nanosize of NLCs plays a crucial role in allowing the nanocarriers to closely engage with the skin's outer membrane, improving membrane fluidity and moving the drug deeper into the skin layers.[31,32] Similarly with the results of penetration and release studies, the flux value increases as the size of the NLC particles decreases. The skin penetration of NLC occurs through the occlusive effect created by the formation of a lipid film. Smaller particles have enhanced adhesion and occlusion relative to bigger particles, hence augmenting the skin penetration of drugs.[33] As therefore, NLC quercetin has the ability of adhering to the surface of the skin and releasing quercetin that can permeate the layers of skin, thereby increasing the flux value. This is in contrast to quercetin cream, which does not consist of a nanoparticle system with sizes in the micrometer range.[34] However, if the patch is to be supplied transdermally, the value of the penetrated quercetin flux remains quite small.[35]

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Conclusion

It is feasible to develop quercetin into an NLC system, which is subsequently utilised as a reservoir in a membrane-type patch. When compared to physical combinations in cream form, membrane-type patches containing quercetin-loaded NLC as a reservoir can improve the release and penetration of quercetin. Particle size, polydispersity index, viscosity, and entrapment efficiency are a few variables that can affect this. Increasing the amount of liquid lipid in the solid lipid to liquid lipid ratio can result in NLC and patches with the best physical properties such as small particle size, low viscosity, thin patch thickness, and good homogeneity. Furthermore, NLC with a larger concentration of liquid lipids can induce higher release and flux penetration.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.\

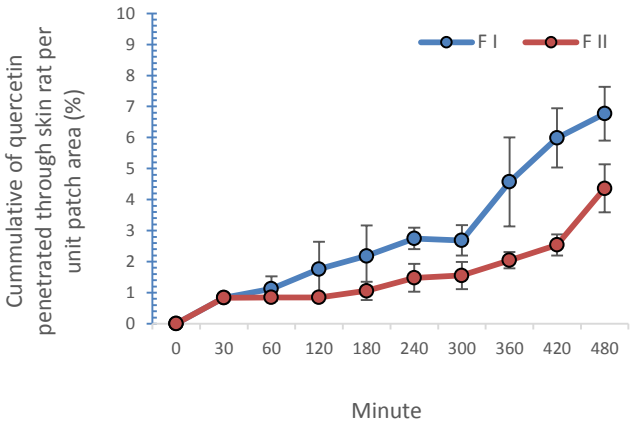

Figure 4: The relationship between cumulative amount of quercetin penetrated versus time at 37^{o}C in the dissolution medium. Data is the mean of three replication.

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