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Original Article

In vitro Kinetic Release Study, *in vivo* Hydration and Moisturizing Effect of Peel-off Oil-in-Water (O/W) Nanoemulsion Containing Kojic Monooleate for Topical Application

Nur Farzana Jaslina[1,2], Nur Hana Faujan[1,2,3], Rosfarizan Mohamad[4,5], Siti Efliza Ashari[1,2,3,*]

[1]Integrated Chemical BioPhysics Research, Faculty of Science, Universiti Putra Malaysia, UPM, Serdang, Selangor, MALAYSIA.
[2]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, UPM, Serdang, Selangor, MALAYSIA.
[3]Centre of Foundation Studies for Agricultural Sciences, Universiti Putra Malaysia, UPM, Serdang, Selangor, MALAYSIA.
[4]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM, Serdang, Selangor, MALAYSIA.
[5]Bioprocessing and Biomanufacturing Research Complex, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM, Serdang, Selangor, MALAYSIA.

ABSTRACT

Background: Kojic monooleate (KMO) contains tyrosinase inhibitor and exhibits strong antioxidant activity makes it a good candidate to be incorporated into a formulation for topical application. A peel-off was chosen to ensure a better hydration effect and permeability of KMO into the skin. The objectives of this research were to analyze the kinetic release study of peel-off oil-in-water O/W nanoemulsion containing KMO and evaluate the moisturizing and hydration effect towards human volunteers. **Methods:** The peel-off formulation was developed by adding 27.61% w/w polyvinyl alcohol (PVA) and 1.05% w/w propylene glycol (PG). The study was performed by using Franz cell, tewameter and corneometer to analyze the release rate of KMO from peel-off O/W nanoemulsion, moisturizing, and hydration effect of formulation towards humans after 180 min of application, respectively. **Results:** The final formulation has a pH of 4.74, conductivity of 7.47 ± 4.05 x 10^{-3} μS/cm, viscosity 0.1058 Pa·s and spreadability of 61.86 ± 1.71 g·cm/s. It also disports 79.99 ± 2.53 % released of KMO after 180 min of study time. The results of the hydration effect of the formulation towards human volunteers' skin suggested that the peel-off O/W nanoemulsion containing KMO does increase the hydration of the skin by 12.33% due to the occlusive effect of KMO. **Conclusion:** In summary, this study presents new findings in the kinetic release study, hydration and moisturizing effect of peel-off O/W nanoemulsion containing KMO for topical application.

Key words: Cosmeceutical application, Thin film system, Franz cell, Transepidermal water loss, Hydration.

Correspondence

ChM. Dr. Siti Efliza Ashari

Research Laboratory, Integrated Chemical BioPhysics Research, Department of Chemistry, Faculty of Science and Centre of Foundation Studies for Agricultural Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, MALAYSIA.

Email id: ctefliza@upm.edu.my

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INTRODUCTION

Every year, the cosmetic industry is getting bigger and one of the most growing products is the brightening product.[1] At the end of 2017, the global brightening product market was valued at USD 5.2 billion and was expected to reach USD 8,479.08 million in 2022. The current trend in the cosmetic industry has reoccur to natural actives, with fewer side-effects towards the consumers.[2] Cosmetic products in the market are mostly used to reduce dryness, eczema, acne, aging, hyperpigmentation and to protect skin from free radicals of sunlight.[3]

Peel-off is a type of dosage form that is gently put into the surface of the facial skin and then peeled off after a few minutes. The fundamental purpose of a peel-off formulation is to promote metabolism due to its occlusive action.[4] It can entrap moisture beneath the film lead to the increment of skin hydration, which leads to the solvent swelling where the cross-sectional area for diffusion of polar molecules increased, thus, ease the permeability of a substance into the skin within a short period. Compounds utilized in this peel-off, on the other hand, must be able to permeate the skin effectively, can survive in the skin epidermis and possess no skin irritation.[5] The peel-off formulation has several advantages compared to other topical delivery systems which are increased hydration of the skin and increased penetration rate of actives into the skin within a short period. It also provides occlusive and moisturizer effect, deep pore cleansing, and blackheads removal.[6] Therapeutic benefits such as reducing dosing schedule, convenience, and consumer-friendliness can also be reached.[7]

Several factors need to be taken into consideration while developing a peel-off such as molecular size, polarity, pH of the substance, and state of the skin hydration. A chemical penetration enhancer can improve percutaneous penetration by changing the stratum corneum's barrier characteristics.[8,9] However, the therapeutic effects may vary for individuals as there are other factors such as the nature of the stratum corneum, the thickness of the skin, and density of appendages that affect the rate of penetration of substance into the skin.[10] Plasticizers, solvents, moisturizers, preservatives, surfactants, perfumes, and active compounds are commonly found in peel-off compositions.[11]

Nanoemulsion can influence the structure of the stratum corneum, increase hydration, and affect the solubility process by acting as a penetration enhancer. The ability of nanoemulsion systems to distribute active chemicals in a regulated manner and optimize penetration of active substances into the appropriate layers of skin has made their use in skin care products increasingly significant. Nanoemulsions are divided into two types: oil-in-water (O/W) and water-in-oil (W/O) emulsions, with droplet sizes ranging from 20 to 200 nanometers.[12-14] Advantages of nanoemulsions compared to other dosage forms are increased rate of

penetration of actives, helps solubilize lipophilic actives, and provides aqueous dosage form for water-insoluble actives. Apart from that, nanoemulsion increases bioavailability, protects actives from hydrolysis and oxidation as actives in the O/W nanoemulsion are not exposed to water.[15]

A potent tyrosinase inhibitor, the metabolite kojic acid (5-hydroxymethyl)-1, 4-pyrone is produced by fungus, such as *Aspergillus, Acetobacter*, and *Penicilium*.[16-18] Kojic monooleate (KMO) is a derivative of kojic acid that improves the storage instability, oil-solubility, and toxicity of kojic acid.[16-19] KMO was also found to be a better tyrosinase inhibitor compared to kojic acid and exhibits strong antioxidant activity.

Previous research reported a KMO nanoemulsion formulation, which released KMO by 4.94% at 1 hr and reach 16.96% at 3 hr, however, the formulation developed form a sticky and fragile thin film on the skin which makes it impossible to stay on the skin for a long period.[20] To solve the problem with the texture of the formulation and the penetration rate, peel-off was introduced and incorporated into the formulation. Peel-off is chosen to ensure better texture, better ability of the formulation to stay on the skin for an intended period, and better kinetic release of KMO. The objective of this study was to improve the kinetic release study of the KMO by using peel-off formulation.

MATERIALS AND METHODS

Materials

Sigma Aldrich, Saint Louis, USA, provided the polyvinyl alcohol (PVA), castor oil (CO), and phosphate-buffer saline (PBS). KMO (Figure 1) was obtained according to the previos method.[21] Xanthan gum, propylene glycol, and liquid germall plus (diazonlidinyl urea, iodopropynyl butylcarbamate and propylene glycol) were purchased of cosmetic grade from Shanghai Tianjia Biochemical Co., Ltd, China. Fisher, Hampton, USA, provided Tween 80 (hydrophile–lipophile balance [HLB] 15.0). Advantec, Japan, provided the synthetic membrane (cellulose acetate Whatman 10404112). Analytical, food, and cosmetic grade chemicals and reagents were also used.

Preparation of Polymeric Peel-off Formulation

Using the approach described in a previous study, a peel-off O/W nanoemulsion containing KMO (105.93 ± 0.21 nm) was created.[22] KMO, castor oil, tween 80, xanthan gum, deionized water, PVA as a plasticizer, and PG as solvent were used to make a peel-off nanoemulsion using a high and low energy emulsification approach.

PVA was first prepared by dispersing it in deionized water (1:1) and heating it to 80°C to dissolve it. Both the oil and aqueous phases were prepared separately. In the oil phase, a mixture of 10.0% (w/w) KMO and 3.37% (w/w) castor oil was used. Tween 80 (3.19% w/w), xanthan gum (0.70% w/w), deionized water (53.38% w/w), PVA (27.61% w/w), and PG (1.05% w/w) were blended together to make the aqueous phase. Both the aqueous and oil phases were continuously stirred at 30°C before being sonicated for 20 min at 70°C.

The aqueous phase was homogenized after sonication, and the oil phase was added drop by drop throughout the homogenization process, which took 15 min at 6000 rpm at room temperature using a high shear homogenizer (T25 digital; IKA-Werk, GmbH & Co. KG, Staufen im Breisgau, Germany). A low-shear homogenizer was then used to homogenize the mixture for 3 hr at 250 rpm (RW20 digital; IKA-Werk). Finally, the mixture was homogenized for 15 min at 250 rpm with 0.7% (w/w) liquid germall plus. The same sample formulation without KMO was prepared as a control formulation. KMO and Peel-off oil-in-water nanoemulsion containing KMO was shown in Appendix 1. Application and peeling of the Peel-off O/W nanoemulsion containing KMO on left hand dorsal surface was also shown in Appendix 2.

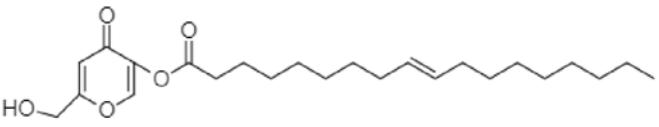
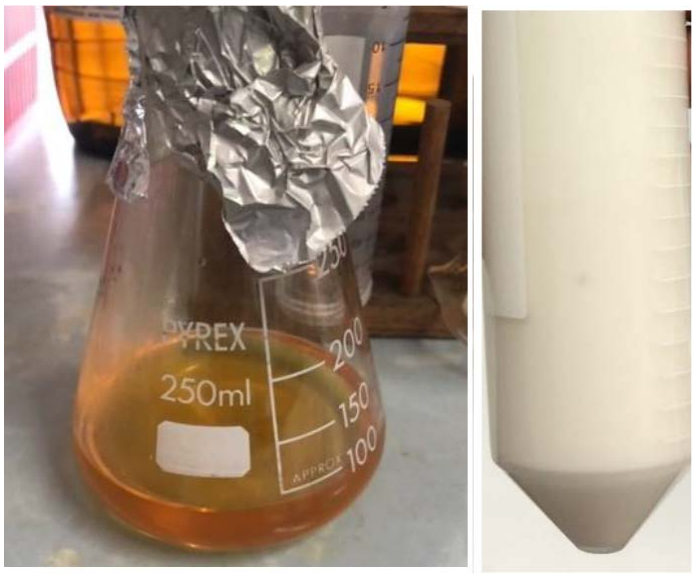


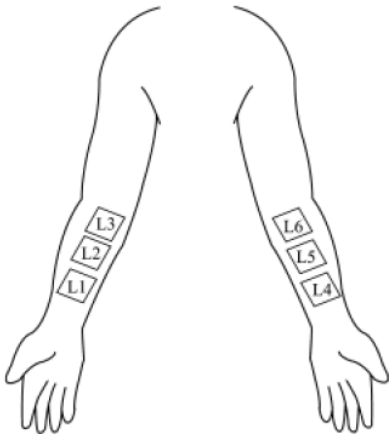
Figure 1: Structure of KMO.

pH and Conductivity

A Delta 320 pH meter was used to determine the pH of the peel-off O/W nanoemulsion containing KMO in triplicate (Mettler Toledo, Columbus, OH, USA). Three pH standard buffer solutions which are phthalate (pH 4.01), phosphate (pH 6.98), carbonate (pH 10.01) were measured and calibrated before pH measurements of peel-off O/W nanoemulsion containing KMO were taken.



KMO and Peel-off oil-in-water nanoemulsion containing KMO



Three sites (3.0 cm x 3.0 cm) of an application designated on the volar forearm of each arm (L1 to L6)

Viscosity

The viscosity of polymeric peel-off was measured using a rheometer (Haake Rheowin, Thermo Scientific, USA) which measure the torque and the deflection angle of the measuring bob. The experiment was conducted out at a temperature of 25°C. To reach equilibrium, one drop of the sample was placed on top of the lower plate and allowed to stand for 10 sec. From 0.1 to 100 s^{-1}, the steady rheological behavior of the samples was investigated. To investigate the influence of KMO on viscosity, a formulation without KMO (control formulation) was tested. Equation 1 was used to fit the experimental data to the power-law model.[20]

$$\eta = ky^{n-1} \quad \text{(Eq. 1)}$$

Where n is the flow behaviour index, k is the consistency index, y is the shear rate (s^{-1}), and n is the viscosity (Pa·s).

Spreadability

The setup consists of two glass slides placed on a level surface on which 1 g of peel-off O/W nanoemulsion containing KMO was applied in between two glasses. The glass plate was placed on a level surface. The other glass plate was put onto the sample, after which a 100 g brass weight was added on top. The time taken was recorded and the longest sample diameter was measured using a ruler. The measurement was repeated three times and the values were reported as average values. The spreadability can be calculated by using Equation 2.[23]

$$S = \frac{m \times l}{t} \quad \text{(Eq. 2)}$$

Where, S = Spreadability, m = Weight placed on upper slide, l = Longest sample diameter and t = Time taken. The results were considered as the mean of three measurements.

In vitro and *in vivo* Studies

In vitro Release Study

Following the previously reported method, franz diffusion cells were used to evaluate the diffusion of a peel-off O/W nanoemulsion containing KMO through artificial membranes.[24] A cellulose acetate membrane with a pore size of 0.2 µm was used in the experiment as it allows better discrimination of substance released. After fixing the membrane between the donor and receptor compartments, 0.1 mL of the peel-off O/W nanoemulsion containing KMO was equally applied to the membrane. Diffusion had a total effective area of 0.636 cm^2 (d=9 mm). The receptor compartments were filled with a pH 7.4 phosphate buffer, which was agitated with a magnetic bar and kept at 37°C. At 0, 15, 30, 45, 60, 90, 135 and 180 min after applying the peel-off O/W nanoemulsion containing KMO, 2 mL of the receptor medium was withdrawn and replaced with an equivalent volume of new phosphate buffer to keep the volume in the lower chamber at 5 mL. The KMO content of these samples was determined using UV-VIS at 304 nm. All of the studies were done in triplicate, with the results provided as mean values.

Hydration Profile in Skin Layer using Human Volunteering

Ten healthy Malaysian females and males (aged 21 to 36 years) with healthy skin took part in the study and completed the consent form. On the day of the test, participants were not allowed to use any skin care products on their volar forearm. The volunteers were kept in a controlled room at 20.0 ± 2.0°C for 30 min before the measurements. All participants were given a peel-off O/W nanoemulsion containing KMO (P), a base formulation without KMO as a control formulation (C), and a marketed formulation (M), with each formulation being applied to 20 different sites. On the volar forearm of each arm, three locations (3.0 cm × 3.0 cm) were selected (refer to Appendix 3). Firstly, 0.1 mL of each formulation was put over each site, and the plastic film generated by the formulations (P, C, and M) was removed 20 min later. The formulations were measured before application, 45 min, 90 min, and 135 min after application. The formulations (P, C, and M) created a plastic film that was removed 20 min after application. The short-term treatment study's experimental design (application sites, topical formulations, and volunteer numbers) are shown in the Table 1. The IIUM Research Ethics Committee (IREC) accepted the study with ID NO (IREC2019-107). All measurements were taken three times, with the average result recorded.

Tewameter Measurement

The effect of the formulations towards the Transepidermal water loss (TEWL) was evaluated using Tewameter TW210 (Courage + Khazaka, Cologne, Germany) following the previously reported method.[25] The temperature and relative humidity were set to standardize at 20-22°C and 40–60%. The samples (0.1 mL) were measured using a syringe and applied to the designated volar arm of the volunteers and left to dry for 20 min. The measurement was taken using the probe before application, 45 min, 90 min and 135 min after application of samples. All measurements were repeated three times and the values were reported as mean values.

Corneometer Measurement

A Corneometer CM 825 PC (Courage + Khazaka, Cologne, Germany) was installed on a Multi Probe Adapter MPA 5 (Courage + Khazaka, Cologne, Germany) to evaluate skin hydration. The moisture content of the stratum corneum was measured using the capacitance value. The temperature and relative humidity were set to standardize at 20-22°C and 40–60% respectively.[25] A 0.1 mL sample were measured using a syringe and applied to the designated volar arm of the volunteers and left to dry for 20 min. The measurement was taken using the probe before

Properties of Peel-off O/W nanoemulsion containing KMO (Jaslina *et al.*, 2020)

Properties	Peel-off O/W nanoemulsion containing KMO	% RSE
Droplet size (nm)	105.93 ± 0.21	0.11
PDI	0.13 ± 4.50 x 10^{-3}	-
Zeta potential (mV)	-37.37 ± 0.86	-
Conductivity (µS cm^{-1})	7.47 ± 4.05 x 10^{-3}	
pH	± 0.02	

Table 1: Experimental design of the short-term treatment study.

	Volunteers									
	1	2	3	4	5	6	7	8	9	10
L1	P	C	M	P	M	C	P	C	M	P
L2	C	P	C	M	P	M	C	P	C	M
L3	M	C	P	C	M	P	M	C	P	C
L4	P	M	C	P	C	M	P	M	C	P
L5	M	P	M	C	P	C	M	P	M	C
L6	C	M	P	M	C	P	C	M	P	M

P, nanoemulsion formulation containing KMO; C, control formulation (without KMO); M, marketed peel-off mask.; L, sites of application.

application, 45 min, 90 min and 135 min after application of samples. The measurement was taken in triplicate.

Statistical Analysis

GraphPad Prism 8.0.2 was used for statistical analysis. Each experiment was repeated three times, and the results were presented as a mean ±standard deviation (SD).

RESULTS

pH and Conductivity

The pH reading is critical since it must be between 4.0 and 7.0 to be compatible with human skin. Range of pH 4 to 6 was reported as safe and not irritate human facial skin.[26,27] On the other hand, other researchers stated that the skin pH was 4.5 to 6.[28] The peel-off O/W nanoemulsion containing KMO had a pH of 4.74 ± 0.02, indicating that it could be employed as a topical formulation.

The number of free ions and water in the system determines the conductivity value. The kind of nanoemulsion can be determined by the conductivity value as either oil-in-water (O/W) or water-in-oil (W/O). The conductivity of the emulsion is determined by the exterior phase. The formulation's high conductivity implies that the aqueous phase is the system's exterior phase, whereas the oil phase is non-conductive. According to one study, O/W emulsion is conductive when conductivity is greater than 0.00 S/cm, whereas W/O emulsion is non-conductive when conductivity is less than 0.00 S/cm.[29] The conductivity of the peel-off O/W nanoemulsion containing KMO was 7.47 ± 4.05 x 10^{-3} µS/cm, showing that it was an O/W emulsion (refer to Appendix 3).

Viscosity

The flow behavior indices (n), consistency coefficients (k), and regression coefficients (R^2) of the KMO-containing peel-off O/W nanoemulsion are shown in Table 2. Using the power-law model, both the peel-off O/W nanoemulsions containing KMO and the control formulation exhibit shear-thinning behaviour as the flow index of n < 1 in the table. Under high shear circumstances, the shear thinning formulation had a low viscosity characteristic. Because topical formulation products are easy to apply, the beauty and pharmaceutical industries frequently need shear thinning characteristics.[20] Previous studies reported a nanoemulsion containing 1.5%, 2% and 2.5% HPMC with a viscosity of 0.2584, 0.4585 and 0.5856 Pa·s, respectively.[30] To compare, the viscosity of the peel-off O/W nanoemulsion containing KMO was lower indicating the formulation would be easier to be applied on a surface area or skin.

Spreadability

The peel-off O/W nanoemulsion containing KMO should have a good spreadability to satisfy the ideal quality in topical applications. The large diameter signifies better spreadability.[31] From the observation, the longest diameter was obtained at 7 s with a value of 4.33 ± 0.12 cm. From the calculation, the spreadability of peel-off O/W nanoemulsion containing KMO was 61.86 ± 1.71 g·cm/s. Previous research also reported a nanoemulsion gel with a spreadability range between 42.85 to 85.70 g·cm/s.[32]

To correlate between viscosity and spreadability, as the viscosity increase, the spreadability is reduced. Hence, a formulation with higher viscosity would be difficult to spread and apply on the surface area. As a comparison, peel-off O/W nanoemulsion containing KMO had a viscosity of 0.1055 Pa·s and spreadability of 61.86 g·cm/s while a nanoemulsion reported by previous study had a viscosity range of 11.712 – 24.603 Pa·s and a spreadability range of 3.17 ± 0.53 to 3.85 ± 0.32 g·cm/s, respectively.[31] This finding suggests that Peel-off O/W nanoemulsion containing KMO has a better spreadability and is easy to be applied.

Table 2: Flow behaviour indices (n), consistency coefficients (k), and regression coefficients (R^2) of the peel-off O/W nanoemulsion containing KMO.

Formulation	k	n	R^2	Viscosity (Pa·s)
Peel-off O/W nanoemulsion containing KMO	1.1252	0.6803	0.8962	0.1058
Control formulation	0.6874	0.9124	0.9772	0.0567

In vitro and *In vivo* Studies

In vitro Kinetic Release Study

The *in vitro* kinetic release studies of the peel-off O/W nanoemulsion containing KMO was investigated over 180 min using a cellulose acetate membrane. This membrane can be used to show how quickly samples diffuse across the skin.[33] Figure 2 displays the percentage release of KMO from the peel-off O/W nanoemulsion containing KMO after 180 min of study time.

The maximum percentage release of KMO from the peel-off O/W nanoemulsion containing KMO was 79.77% at 180 min, as shown in the Figure. The KMO was delivered in a nearly linear pattern across the cellulose acetate membrane. To compare with the KMO nanoemulsion formulation developed by the previous researcher, the KMO released was only 4.94% at 60 min and reach 16.96% at 180 min.[34] This finding shows that the development of the peel-off O/W nanoemulsion containing KMO improved the rate of kinetic release across the cellulose acetate membrane. This finding is in line with other findings which stated that their peel-off formulation was able to enhance kinetic release.[35]

In addition, Figure 3 shows the cumulative KMO penetration per unit area as a function of time. After 180 min of research time, the total KMO permeated over the membrane per unit area is 21.84 mg/cm^2, with a flux (J) of 7 mg/cm^2·h and a permeation coefficient (Kp) of 3.35 cm/h (Table 3). Meanwhile, the flux of KMO-enriched O/W nanoemulsion was 2.39 mg/cm^2·h with a permeation coefficient of 1.24 cm/h.

Kinetic Release Analysis

To provide a relationship between the results obtained and a possible release mechanism, the data were fitted to various kinetic models such as zeroth order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas. Table 4 shows the coefficient of determination (R^2) for all kinetic

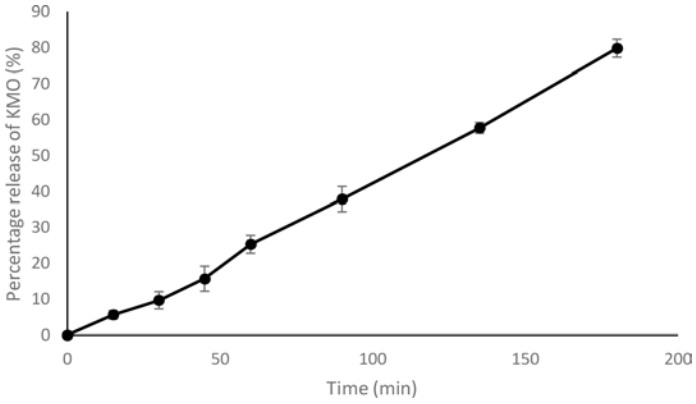


Figure 2: Percentage release of KMO from the peel-off O/W nanoemulsion containing KMO over time (min). Data was analyzed by using triplicate results and values are given in (Mean ± SD, *n*=3).

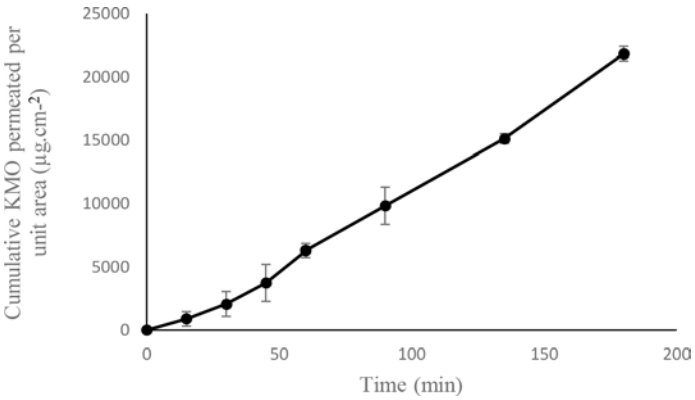


Figure 3: Cumulative KMO permeated per unit area (µg.cm^{-2}) as a function of time (min). Data was analyzed by using triplicate results and values are given in (Mean ± SD, *n*=3).

Table 3: The permeation parameter of the peel-off O/W nanoemulsion containing KMO.

Flux at 180 min, J (mg/cm^2.h^1)	Permeated amount of KMO at 180 min (%)	Permeation coefficient, K_p (cm/h)
7	79.77 ± 0.04	3.35

Table 4: The coefficient of determination (R^2) of all the kinetic models for KMO release from the peel-off O/W nanoemulsion containing KMO (Appendix 4).

Kinetic Model	Coefficient of Determination (R^2)
Zeroth order	0.9934
First-order	0.9403
Hixson-Crowell	0.8791
Higuchi	0.9585
Korsmeyer-peppas	0.9699

models for KMO release from a peel-off O/W nanoemulsion containing KMO.

Based on Table 4, the R^2 for the KMO release from the peel-off O/W nanoemulsion containing KMO was best fitted with the zeroth-order model (0.9934), followed by Korsmeyer-Peppas (0.9669), Higuchi (0.9585), first-order (0.9403) and Hixson-Crowell (0.8791).

Following the zeroth-order model, the release of the compounds was only time-dependent and independent of its concentration.[36,37] The release behaviors described were found to be in good agreement with the graph of percentage release of KMO from the peel-off O/W nanoemulsion containing KMO where the release of KMO seems to be in a linear pattern throughout the study time.

Hydration Profile in Skin Layer using Human Volunteering

Transepidermal Water Loss

Figure 4 illustrates the effects of peel-off O/W nanoemulsion containing KMO (P), control formulation (C) and marketed formulation (M) on transepidermal water loss (TEWL). The result indicates an immediate increase in TEWL for all tested formulations at 45 min upon application

Results of the physical appearance evaluation of the Peel-off o/w nanoemulsion containing KMO for each storage condition.

Parameter	Time (day)				
	0	7	14	21	28
	4 ± 2°C – low temperature				
Color	t	t	t	t	t
Odour	Normal	Normal	Normal	Normal	Normal
Applicability	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	6.67 ± 0.48	6.67 ± 0.48
TFS	√	√	√	√	√
Stability	√	√	√	√	√
	25 ± 2°C – exposed to sunlight				
Color	t	t	t	t	t
Odour	Normal	Normal	Normal	Normal	Normal
Applicability	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47
TFS	√	√	√	√	√
Stability	√	√	√	√	√
	25 ± 2°C – protected from sunlight				
Color	t	t	t	t	t
Odour	Normal	Normal	Normal	Normal	Normal
Applicability	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47
TFS	√	√	√	√	√
Stability	√	√	√	√	√
	45 ± 2°C - oven				
Color	t	t	t	t	py
Odour	Normal	Normal	Normal	Normal	Normal
Applicability	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47	7.67 ± 0.47
TFS	√	√	√	√	√
Stability	√	√	√	√	

Abbreviations: t, translucent; py, pale yellow

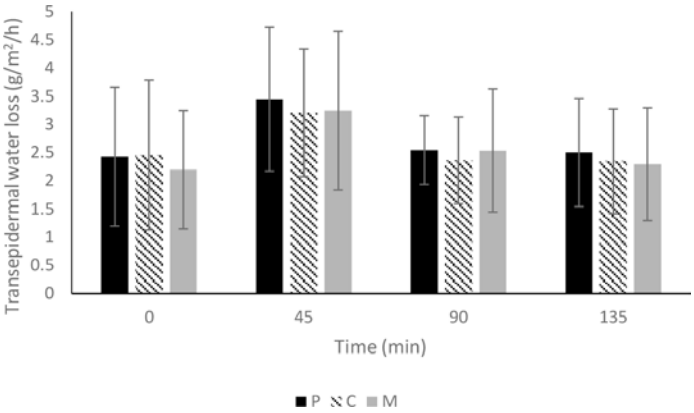


Figure 4: Effects of P, C and M on transepidermal water loss (TEWL) (g/m^2/h) over time (min). Data was analyzed by using triplicate and repeated measurements and values are given in (Mean ± SD; *n* =3).

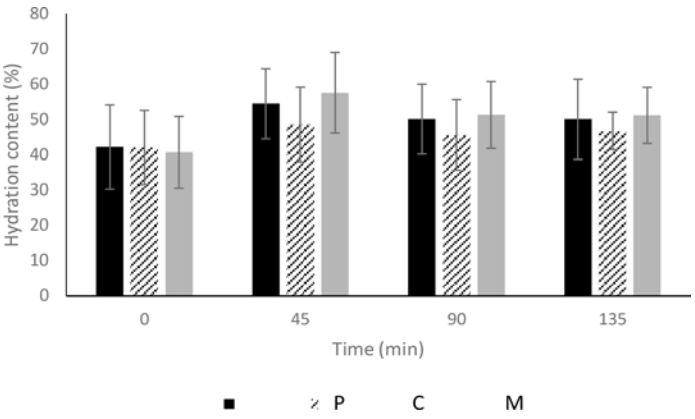


Figure 5: Effects of P, C and M on hydration content (%) over time (min). Data was analyzed by using triplicate and repeated measurements and values are given in (Mean ± SD; *n* =3).

with P (3.45 ± 1.28 g/m^2/h), C (3.21 ±1.13 g/m^2/h) and M (3.25 ± 1.41 g/m^2/h). At 90 min, the TEWL values decreased and shows almost similar results as 0 min (before application). An immediate increase in TEWL at 45 min indicates that the water loss from the skin increased. The TEWL return to a normal level at 90 min and maintain at that level at 135 min, indicating there were no significant effects of all formulations towards TEWL. The increment of water loss from the skin suggested that there was no moisturizing effect for all formulations. Furthermore, no significant differences existed between P, C, and M. Similarly, there were no significant differences concerning the presence or absence of KMO towards the moisturizing effect.

All formulations used in this study contained propylene glycol (PG) and polyvinyl alcohol (PVA) as their ingredients. PG is a component that can change the structure of the stratum corneum. It is highly hygroscopic, which makes it able to form hydrogen bonds with water, hence PG can enhance the concentration of water on the skin's surface and increase the softness and tenderness of the skin. The presence of PVA in all formulations suggests a reduction of TEWL due to its occlusive effects.[38] As a result of the presence of polyols and PVA in all formulations, it was expected that TEWL would decrease due to the increasing amount of water in the skin. However, it was determined in this investigation that occlusion of the peel-off formulation would not result in a decrease in TEWL.

Hydration of the Upper Skin Layers

Effects of P, C and M on hydration content were displayed in Figure 5. From the Figure, it can be observed that an increment in hydration of the skin was shown for M, higher than that obtained for the P. The presence of KMO in a formulation affected the hydration content on human volunteers' skin as C had a lower hydration value of 48.53 ± 10.55 % compared to P with 54.47 ± 9.93 %. The result obtained suggest that the increment of hydration of the skin was higher immediately at 45 min after application of samples. It can be assumed that a peel-off increase skin hydration probably due to the Occlusion effect.

DISCUSSION

Present study based on *in vitro* kinetic release study, *in vivo* hydration and moisturizing effect of peel-off O/W nanoemulsion containing KMO. The viscosity and spreadability of the formulation was measured and the analysed data clearly showed the formulation has low viscosity and good spreadability, making it easy to be applied on a surface area or skin. In the kinetic release study, it was found that the peel-off O/W nanoemulsion containing KMO improved the rate of kinetic release across the cellulose acetate membrane. The permeability efficacy may be attributed to the presence of penetration enhancers used in peel-off O/W nanoemulsion containing KMO as well such as tween 80, water, and PG.[39,40] According to Azhar *et al*., (2018), the droplet size of KMO nanoemulsion decreased as the concentration of Tween 80 increased, which was caused by the ability of Tween 80 to act as an emulsifier to lower the interfacial tension thus reducing droplet deformation.[20] Due to the optimization method performed while developing peel-off formulation containing KMO, the droplet size obtained was lower compared to the study by Azhar (2018) by approximately 5 nm (Jaslina *et al*., 2020).[20,22] The smaller droplet size obtained (105.93 ± 0.21 nm) may be contributed to the improved kinetic release of the KMO across the membrane layer as well.[22] In case of moisturizing effect study, it was found that there was no moisturizing effect for all formulations due to the increment of water loss from the skin over time. Meanwhile, in hydration study it was found that the formulation increased hydration state of the skin. The presence of KMO in the formulations causes a significant increase in skin hydration, indicating the efficacy of the KMO to boost hydration of the skin in a short-term study. A previous study has stated that skin hydration plays a vital role in the permeability of substances into the skin.[41] The presence of KMO in the formulations causes a significant increase in skin hydration, indicating the efficacy of the KMO to boost hydration of the skin in a short-term study. Other literature also mentioned that any substance that is able to alter the hydration state of the skin can be considered as a permeability enhancer.[42-44] It is expected for the peel-off O/W nanoemulsion containing KMO to have a good skin permeability, as the skin hydration increase leads to increment of the penetration rate of substance into the skin. However, further investigation using *in vitro* drug permeation study using animal or human skin is required to further understand the relationship between hydration and skin permeability of KMO.

CONCLUSION

The peel-off O/W nanoemulsion containing KMO exhibit pH of 4.74, conductivity 7.47 ± 4.05 x 10^{-3} μS/cm, viscosity 0.1058 Pa·s and spreadability of 61.86 ± 1.71 g·cm/s. Peel-off O/W nanoemulsion containing KMO also disports 79.99 ± 2.53 % released of KMO across the cellulose acetate membrane after 180 min of study time. The results of the hydration effect of peel-off O/W nanoemulsion containing KMO towards human volunteer's skin suggest that the formulation does increase skin hydration in a process related to the occlusive effect.

ABBREVIATIONS

O/W: Oil-in-Water; **KMO:** Kojic Monooleate; **W/O:** water-in-oil; **PG:** propylene glycol; **PVA:** polyvinyl alcohol; **C:** control formulation; **M:** marketed formulation; **TEWL:** transepidermal water loss.

Author Contribution Statement

Nur Farzana Jaslina carried out the experiments, examined the data, and authored the paper. The experiments were planned and designed by Siti Efliza Ashari. Data analysis was done by Rosfarizan Mohamad and Nur Hana Faujan. The authors state that their authorship and publication of this contribution did not include any conflicts of interest.

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CONFLICTS OF INTEREST

The author declare no conflicts of interest.

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