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Transdermal delivery of kojic acid from microemulgel

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ABSTRACT

The aim of this study was to develop microemulgel for skin delivery of kojic acid. Microemulsions (ME) containing either oleic acid (OA) or caprylic/capric triglycerides as the basic oily components were developed after construction of pseudoternary phase diagrams. Tween 80 was used as surfactant for oleic acid system both in presence and absence of ethanol or propylene glycol (PG) as cosurfactants. For the caprylic/capric systems, Tween 85 was the surfactant in presence or absence of ethanol as cosurfactant. Selected ME formulations were tested for transdermal delivery of kojic acid both in fluid state and after transformation into gel. Incorporation of cosurfactants expanded the microemulsion zone. The cosurfactant free ME were more viscous. Incorporation of kojic acid in ME systems increased the transdermal flux compared to saturated aqueous solution. caprylic/capric ME were more efficient than (OA) based ME. Transformation of the tested ME systems into gel produced significant enhancement in transdermal drug delivery compared with the saturated aqueous drug solution. However, the data revealed superior efficacy for the fluid ME systems over the corresponding microemulgel. In conclusion, both (OA) and caprylic/capric ME were promising for dermal and transdermal delivery of kojic acid even after gel formation.

INTRODUCTION

Kojic acid (5-hydroxy-2hydroxymethyl-4H-pyran-4one) is a depigmenting agent obtained from rice fermentation (Burdock *et al.,* 2001). It is a natural antibiotic produced by various bacterial or fungal strains such as *Aspergillus oryzae*, *Penicillium* or *Acetrobacter spp.* (Bentley, 2006; Brtko *et al.,* 2004; Burdock *et al.,* 2001). It exerts its effect by competitive inhibition of tyrosine enzyme due to the ability of chelating copper ion at the active site. It is thus considered as a slow binding inhibitor of the diphenolase activity of tyrosinase enzyme resulting in antimelanogenic action (Cabanes *et al.,* 1994). Unfortunately, the hydrophilic nature of kojic acid limited its ability to penetrate through the stratum corneum of the skin which is the first step for delivering the drug to deeper skin strata. This will hinder the delivery of the drug to the target sites, melanocytes which are localized at the dermal/epidermal border (Curto *et al.,* 1999; Yamaguchi *et al.,* 2007). Many strategies have been employed to enhance transdermal delivery of hydrophilic drugs. These include the use of permeation enhancers (Cornwell and Barry, 1994; Phillips and Michniak, 1995; Sinha and Kaur, 2000; Trommer and Neubert, 2006; Walker and Smith, 1996), employing vesicular drug delivery systems (El Maghraby *et al.,* 2001; Ntimenou *et al.,* 2012) delivering hydrophilic drugs using microneedles (Oh *et al.,* 2008) or augmenting transdermal flux using laser systems (Gómez *et al.,* 2008; Lee at al., 2001). Microemulsion provides another promising alternative for transdermal delivery for hydrophilic drugs (Cui *et al.,* 2011; Hosmer *et al.,* 2009; Kreilgaard *et al.,* 2000; Zhang and Michniak-Kohn, 2011).

Microemulsion is a transparent, thermodynamically stable single optically isotropic liquid system of water, oil and surfactants (Danielson and Lindmann, 1981). Microemulsions can be considered as ideal system for drug delivery of both hydrophilic and hydrophobic drugs. They have the advantages of being thermodynamically stable. They were shown to enhance skin penetration of drugs via different mechanisms (Heuschkel et al, 2008; Kogan and Grati, 2006). The benefit of microemulsion can be even greater if the selected oily phase has skin penetration enhancement ability. Accordingly, the objective of this study was to investigate the efficacy of penetration enhancer containing microemulsion as skin drug delivery system for kojic acid. Oleic acid (OA) or caprylic/capric triglycerides (CP) were selected as the oil phase in microemulsion preparation. The study was extended to incorporate optimum microemulsion formulation in gel based system.

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MATERIALS AND METHODS

Materials

Kojic acid, Tween85, caprylic/capric triglycerides and oleic acid were obtained from Santec Chemicals Crop., Fresh Meadows, U.S.A. Tween 80 was supplied from Klob Company, Hedingen, Switzerland. Ethanol, propylene glycol and glacial acetic acid were obtained from Sigma Aldrich GmbH, Switzerland. Aerosil 200 (colloidal silicone dioxide) was obtained from Evonik Industries, Essen, Germany.

Construction of pseudo-ternary phase diagrams

Oleic acid (OA) and caprylic/capric triglycerides (CP) were selected as the oily phase. Tween 80 was used as surfactant for (OA) and Tween 85 was selected as surfactant for (CP). These selections were based on the miscibility of the surfactant with the corresponding oil. Ethanol was used as a cosurfactant in both cases with propylene glycol being employed as cosurfactant in case of oleic acid only. Propylene glycol was not employed as a cosurfactant in case of (CP) due to poor miscibility with (CP). Cosurfactant was mixed with surfactants at a ratio of 1:1 (W/W). This ratio was selected after solubilizing the highest concentration of water on titration of a 1:1 mixture of the oil with surfactant/cosurfactant system (Alany *et al.,* 2001). Pseudo-ternary phase diagrams were constructed at ambient temperature using water titration method (Chen *et al.,* 2004; El Maghraby, 2008). For each phase diagram mixtures of oils and surfactant or surfactant/ cosurfactant mixtures were prepared at weight ratios of 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. These mixtures were titrated with water while magnetic stirring. The resultant mixtures were characterized visually after equilibration with transparent fluid systems being considered as microemulsion (El Maghraby, 2008; El Maghraby *et al.,* 2014). Highly viscous non-fluid systems were considered as gel (El Maghraby, 2008).

Preparation of microemulsions

The composition of the tested microemulsion formulation is presented in (Table 1). Formulations containing fixed concentrations of oil and water were employed in the current study. This allowed investigation of the effect of different variables which included the type of oil, presence or absence of cosurfactant and the type of cosurfactant used. The selected microemulsion formulations were prepared by simple mixing oil with the surfactant or surfactant/cosurfactant mixture with the aid of magnetic stirring. The required amount of water was then added while mixing. Excess drug was added to prepare saturated drug solutions with excess crystals being included to maintain saturation. These systems were equilibrated by continuous mixing in water bath maintained at 32 °C for 72 h before skin permeation studies (El Maghraby, 2008). Addition of excess drug did not lead to any phase change.

Characterization of the selected microemulsion formulations

The viscosity of the selected formulations was determined using RVDVE Brookfield Viscometer Version 1.1 using spindle 92 at 50 rpm (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA).

To determine the saturation solubility of drug in different formulations, excess drug was added and the mixtures were equilibrated in a thermostated shaking water bath which was maintained at 32 °C for 72 hours. Excess drug was separated by centrifugation and the supernatant was suitably diluted before HPLC analysis.

The physical stability was assessed by subjecting the selected microemulsion formulations to different stress conditions. These included centrifugation of the formulation at 4000 rpm for 15 min followed by visual inspection for any phase separation (Chen *et al.,* 2007). The tested formulations were also subjected to three freeze-thaw cycles. Each cycle included storage of the formulation at −20ºC for 24hours followed by 24 hours storage at 25 ºC (Brime *et al.,* 2002). The systems were then visually evaluated for any sign of phase change.

The pH values of those formulae were measured using a digital pH-Meter (Jenway 3310, U.K). The refractive index of microemulsion was measured by digital refractrometer (Atago, Tokyo, Japan).

All the tested microemulsion formulations were characterized using conductimetric measurements. Water was replaced with 0.8% w/v aqueous sodium chloride to allow conductivity measurement (El Maghraby *et al.,* 2014). The electrical conductivity of these formulations was recorded by electrical conductivity meter (HANNA-HI 8733, Michigan, USA).

Table 1: The composition of selected microemulsion formulations.

Material	OA ME	OA PG ME	OA ET ME	CP ME	CP ET ME
Oleic acid	15	15	15	--------	--------
Ethanol	--------	--------	35	--------	35
Propylene glycol	--------	35	--------	--------	--------
Tween 80	70	35	35	--------	--------
Tween 85	--------	--------	--------	70	35
Caprylic capric triglycerides	--------	--------	--------	15	15
Water	15	15	15	15	15
Aerosil required for gel formation (%)	3	9.5	6	8.5	8

Preparation of microemulgel formulations

Various gelling agents namely; xanthan gum, sodium alginate, hydroxylpropylmethyl cellulose (Methocel E5, E15 & K15), carrageenan and Aerosil200 (colloidal silicone dioxide) were evaluated for their ability to gel different microemulsion formulations. The gelling agent was dispersed slowly in the supersaturated microemulsion formulations with the aid of overhead stirring. Of these agents Aerosil 200 was the most suitable gelling agent for the tested systems. The concentration of Aerosil 200 used to gel each ME formula is represented in (Table 1).

Viscosity of microemulgel formulations

The viscosity of different microemulgel formulations was determined at 25 ± 1 ºC. Viscosity redeterminations employed a RVDVE Brookfield Viscometer Version 1.1 using spindle 96 at 1rpm (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA).

***In vitro* drug release**

The in vitro drug release was using vertical glass Franz diffusion cells. These cells has a diffusional surface area of 2.27 cm^2 with the volume of the receptor compartment being 14 ml. Semipermeable membrane (Cellulose tubing, molecular weight cut off is 12000 Da, Sigma diagnostics, St. Louis, MO, USA) was mounted between donor and receptor compartments of these cells. The release studies were conducted at 32± 1 °C to mimic the skin permeation studies.

This was achieved by incubation of the diffusion cells into thermostatically controlled water bath. The receptor compartments were filled with distilled water which was used as the receptor fluid. The cells were left to equilibrate to the required temperature. The tested formulations (2ml) were loaded into the donor compartments which were occluded with parafilm. Receptor samples were taken at predetermined time intervals and replaced with fresh receptor. The drug content in each sample was determined by HPLC analysis (see below). The cumulative amount of kojic acid released was calculated and plotted as a function of time to produce the release profiles. These profiles were used to determine the release rate.

Preparation of skin samples

The study utilized freshly excised full thickness skin obtained from the Rabbit ear. This model was successfully adopted to investigate the skin delivery of a variety of drugs including hydrophilic and lipophilic drugs (El Maghraby, 2010, El Maghraby *et al.*, 2014; Nicoli et al, 2008).

Freshly excised ears of male rabbits, weighing 2-3 kg were used. The full thickness skin was simply peeled from the underlying cartilage after cutting along the tips of the ears. The skin was cut into sections and was used immediately.

Skin permeation studies

The study employed the same setup as the release experiments. The skin samples were mounted between the donor and receptor compartments with the stratum corneal side uppermost Water was employed as a receptor. The receptor compartments were filled with distilled water and incubated in a thermostated water bath which was adjusted to ensure that the skin surface was maintained at 32± 1 °C to mimic *in vivo* conditions. The whole assembly was left to equilibrate for overnight. The tested formulations (2ml) were loaded into the donor compartments which were then occluded as before.

Receptor samples were taken periodically and replaced with fresh receptor. The amount of drug permeated was determined after HPLC analysis of each sample. Saturated aqueous solution of the drug containing excess drug crystals to maintain saturation was used as the control. All the tested formulations contained the drug at saturation with excess crystals being included as well.

This allowed investigation of skin permeation at equal thermodynamic activity in all systems and provide better chance for detecting any effect for the formulation variables on skin permeation (El Maghraby, 2008, 2010; El Maghraby *et al.*, 2014).

Chromatography

The concentration of kojic acid in each sample was determined using high pressure liquid chromatograph (Shimadzu LC20-20A, Japan) equipped with a double wavelength UV/Visible detector and an auto-sampler unit for injection. Shimadzu LC solution software V 1.24 SP1 was employed for chromatographic data collection and handling. Separation was conducted on a reversed phase column Thermo HYPERSIL C_{18} (150 × 4.6 mm, 5µm).

The mobile phase was a filtered degassed phosphoric acid solution which was prepared by diluting 0.7 ml of phosphoric acid to 1000 ml with distilled water. This was pumped at a flow rate of 0.8 ml/ min with the effluent being monitored at 270 nm. Samples were suitably diluted with distilled water before injecting 30 µl.

Data analysis

The skin permeation profiles were obtained by plotting the cumulative amounts of the drug recorded in the receptor as a function of time. The obtained profiles were typical steady state plots which are expected after occlusive application of saturated systems (Figure 1).

The transdermal drug flux was obtained from the slope of the regression line fitted to the linear portion of the permeation profile. Extrapolation of this line will intercept with the x axis at a time equal to the lag time (El Maghraby, 2008, 2010, 2012a; El Maghraby *et al.*, 2014). The Student's t-test was used for statistical analysis.

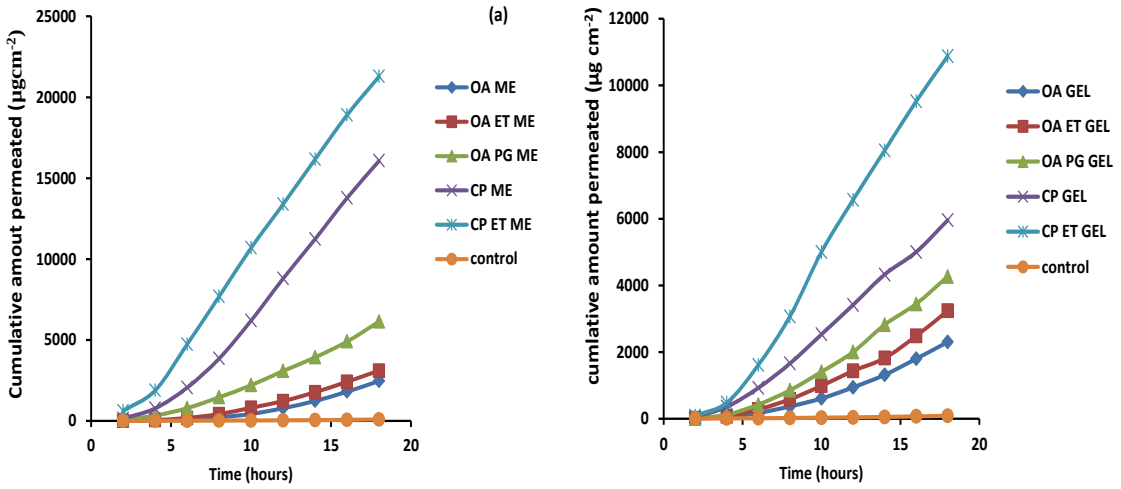

Fig. 1: The in *vitro* skin permeation profiles of kojic acid from microemulsion (a) and microemulgel formulations (b) after application to rabbit ear skin. The control is saturated aqueous solution of the drug. Formulation details are in Table 1.

RESULTS AND DISUSSION

Pseudo-ternary phase diagrams

Oleic acid (OA) and medium chain glycerides were separately utilized as the oily phase as they were successfully used to enhance the transdermal delivery of hydrophilic drugs like kojic acid (Hosmer *et al.*, 2009; Okumura *et al.*, 1991; Ongpipattanakul *et al.*, 1991; Sinha and Kaur, 2000; Tanojo *et al.*, 1997). For (OA) based systems, Tween 80 was used as the surfactant in the presence or absence of propylene glycol or ethanol which were used as cosurfactants. For (CP) ME; Tween 85 was used as the surfactant in the presence or absence of ethanol as a cosurfactant. The selection of surfactant and cosurfactant was based on the ability of these materials to solubilize large amounts of water when mixed with the corresponding oil (Alany *et al.*, 2001; El Maghraby, 2008). Figures 2 and 3 shows the pseudo-ternary phase diagrams of the two systems in the presence and absence of different cosurfactants.

For oleic acid based systems, Tween 80 was able to form microemulsion in absence of cosurfactants (Figure 2a). The microemulsion zone occupied about 16% of the total area of the phase diagram. The gel phase occupied about 15% of the total area with the rest of the phase diagram being identified as coarse emulsion (Figure 2a).

The maximum amount of water that can be solubilized in microemulsion was 26%. This was achieved only at very low oil concentration and was reduced progressively with increasing oil concentrations. With respect to the fluidity of the microemulsion, there was a reduction in the fluidity with increasing the concentration of water in the system. Further increase in water concentration resulted in phase change with the system changing to gel structure especially at high surfactant concentration. Further dilution resulted in coarse emulsion formation.

Similar phase behavior was recorded for the same oil (El Maghraby, 2012 a). Incorporation of propylene glycol as cosurfactant increased the maximum amount of water to be incorporated in microemulsion to about 70% compared to 26% in the cosurfactant free system at the lowest concentration of oil. The area occupied by the microemulsion zone was increased to 17% with the gel phase occupying only 7% of the total area of the phase diagram (Figure 2b). Replacing propylene glycol with ethanol resulted in further increase in the area occupied by the ME zone to reach 34%. This effect was associated with complete absence of the gel phase in the total area of the phase diagram (Figure 2c). Incorporation of short chain alcohols as cosurfactant was previously shown to increase the ability of the system to accommodate water and was able to disrupt the gel structure (Alany *et al.*, 2000; El Magrhaby, 2008). For (CP) based systems, Tween 85 was able to form microemulsion in absence of cosurfactants (Figure 3a). The microemulsion zone occupied about 20% and the gel phase occupied about 48% of the total area of the pseudo- ternary phase diagram with the rest of the phase diagram being characterized as coarse emulsion. This finding is expected based on the bases that gel structure is more likely to dominate in medium chain glycerides microemulsions at high surfactant concentration (Prajapati *et al.*, 2012). At very low oil concentration the maximum amount of water that can be incorporated in a microemulsion system was 16%. Incorporation of ethanol as cosurfactant in this system resulted in further increase in the area occupied by ME zone to reach 25% compared to 20% in the cosurfactant free system. In addition, the cosurfactant containing system did not form any gel structure (Figure 3b). Breaking of the liquid crystalline and gel structure of the ternary system was previously recorded after addition of short chain alcohols including ethanol as cosurfactant (Alany *et al.*, 2001; El Magrhaby, 2008).

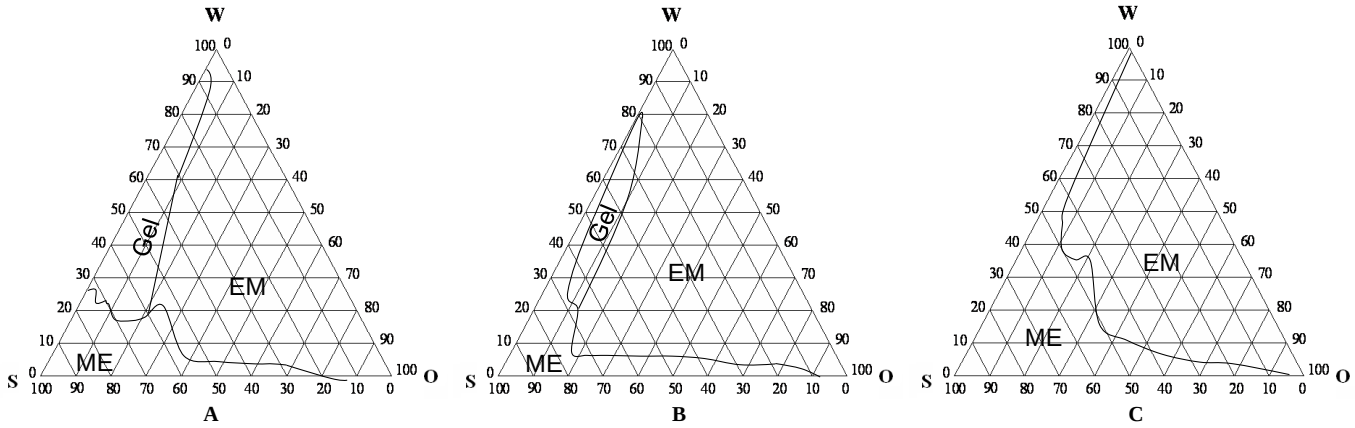

Fig. 2: The pseudo-ternary phase diagrams of oleic acid/ Tween 80 and water systems both in absence (a) and presence of either propylene glycol (b) or ethanol (c) as cosurfactant. W means water, O means oil and S means surfactant/cosurfactant mixture.

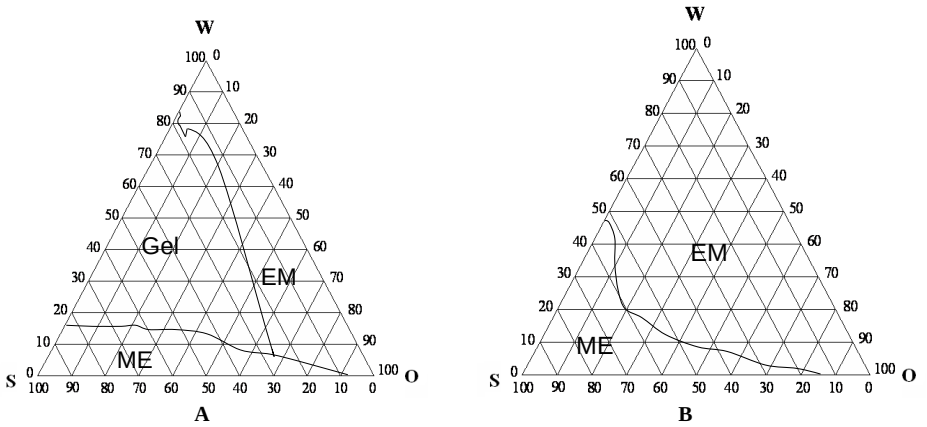

Fig. 3: The pseudo-ternary phase diagrams of caprylic/capric triglycerides with Tween 85 and water both in absence (a) and presence of ethanol (b). W means water, O means oil and S means surfactant/cosurfactant mixture.

The area occupied by microemulsion zone in the phase diagram depends on the physicochemical properties of the oil phase and the type of surfactant with some essential conditions required for microemulsion formulation. These conditions include the presence of very low surface tension at the oil-water interface and the existence of non-viscous surfactant film at the water oil interface. Penetration and association of oil molecules with the interfacial surfactant film is also required (Schulman *et al.,* 1959). Based on this, the miscibility of surfactant with the oil phase can be taken as initial indication for the suitability of the surfactant in formulation of microemulsion using this oil. The presence of fluidizing group such as double bond in the lipophilic chain of surfactant can provide a chance for microemulsion formation using this surfactant as a single surfactant (Lawrence, 1994). This explains the ability of Tween 80 to form microemulsion with oleic acid due to its miscibility with oil and presence of unsaturated acyl chain. The ability of cosurfactant to increase the area occupied by the microemulsion zone can be explained on its ability to reduce the surface tension with high capacity to increase the fluidity of the interfacial film. This explanation agrees with the previously published reports on the use of short chain alcohols as cosurfactants (Aboofazeli and Lawrence, 1994; Stilbs *et al.,* 1983).

Characterization of the selected microemulsion formulations

The tested microemulsions formulations were selected so that all formulations contained the same concentration of oil and water (15% of each) with the rest of the formulation being the surfactant/cosurfactant system. This selection will thus allow the investigation of the effect of oleic acid and caprylic/capric triglycerides as oils on the skin delivery of kojic acid from microemulsion.

The selected formulations were characterized with respect to the viscosity, drug solubility, electrical conductivity, pH and refractive index.

These parameters are presented in (Table 2). The viscosity of the tested formulations depended on their composition with cosurfactant free systems being more viscous compared with the corresponding cosurfactant containing preparations.

This trend was recorded both in case of (OA) based systems and (CP) based systems (Table 2). Similar behavior was recorded for the viscosity of microemulsion systems after incorporation of short chain alcohols of 3-4 carbon atoms (Alany *et al.,* 2000; El Maghraby, 2008). Preparation of microemulgel resulted in significant increase in the viscosity which is expected after addition of the gelling agent (Table2).

Table 2: The characteristics of the selected microemulsion formulations.

Parameter	OA ME	OA PG ME	OA ET ME	CP ME	CP ET ME
Viscosity of microemulsion (CP)	1156.67 (5.8)	324.3 (12.5)	107.3 (6.4)	818.67 (10.3)	127.67 (7.5)
drug solubility* (mg/ml)	10.8 (0.19)	27.91 (1.1)	37.96 (1.6)	19.2 (0.99)	41.8 (2.2)
PH	5.8 (0.005)	5.00 (0.005)	5.44 (0.01)	5.9 (0.01)	5.22 (0.005)
Refractive Index	1.4606 (0.0003)	1.4428 (0.0002)	1.4301 (0.0004)	1.4219 (0.007)	1.4490 (0.015)
Conductivity (mS/cm)	22.8 (0.1)	56.3 (0.05)	140.2 (0.1)	9.18 (0.005)	129 (0.05)
Viscosity of microemulgel (CP)	OA Gel	OA PG Gel	OA ET Gel	CP Gel	CP ET Gel
	75,640 (40)	880,100 (100)	384,433 (152)	483,716 (104)	910,366 (550)

*The saturation solubility of kojic acid in water was 55.8 (0.87) mg/ml at 32 ºC. Values between brackets are SD (n=3).

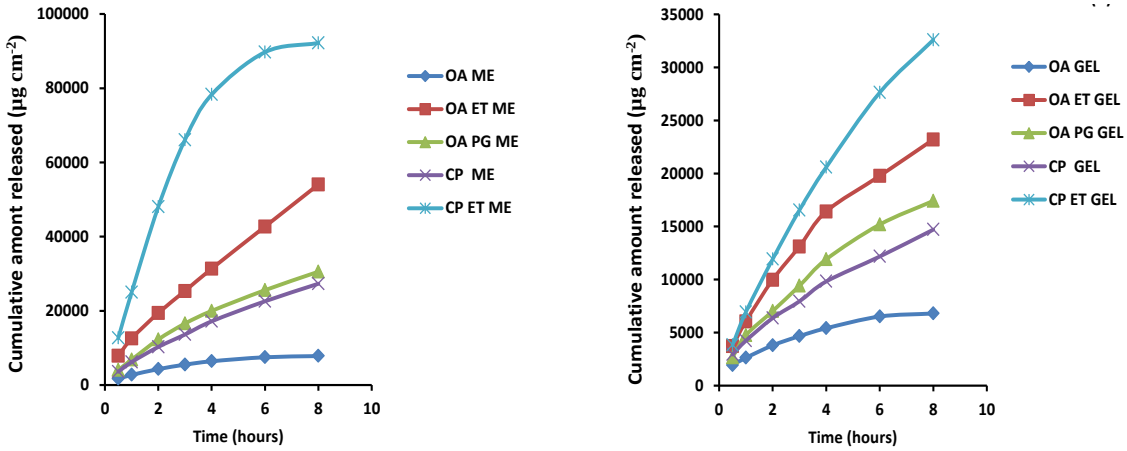

Fig. 4: The in vitro release profiles of kojic acid from microemulsions (a) and the corresponding microemulgel formulations (b). Formulation details are in Table 1.

With respect to the solubility of the drug in microemulsion systems, there was a dependence on the composition of the formulation with those containing ethanol or propylene glycol dissolving greater amounts of the drug compared with the corresponding microemulsions. However, the overall solubility was lower than the drug solubility in water. This is expected for such hydrophilic drug (Table 2).

(Table 2) presents the results of pH of different ME formulations. All the formulations had pH values in the range of 5-5.9. Also refractive index results are presented in (Table 2) and all the formulations have refractive index results between 1.4219-1.4606.

The electrical conductivity of all formulations was measured with the goal of determination of the type of microemulsion. The tested cosurfactant free formulations exhibited relatively low electrical conductivity values indicating that the tested systems are of W/O microemulsion type (Table 2). This is expected taking into consideration the fact that the water content of the formulations was 15% w/w. Despite of fixed water content, the cosurfactant containing systems revealed higher conductivity values compared with the corresponding cosurfactant free formulations but the difference does not imply phase inversion but suggests possible formation of bicontinuous systems.

The selected microemulsion formulations were tested for physical stability by subjecting them to different stress conditions. Upon centrifugation of microemulsion formulations at 4000 rpm for 15 min, no phase separation was noticed by visual inspection in all formulations. Also no sign of phase change was noticed visually after subjecting all the formulations to three successive freeze and thaw cycles. These findings reflect the physical stability of the formulations.

In Vitro drug release

The in vitro release of kojic acid was monitored using semipermeable membrane with the experimental conditions being adjusted to the same skin permeation conditions. This allowed correlation between release data and skin permeation data (El Maghraby, 2008, 2010). The in vitro release profiles of kojic acid obtained from different ME formulations in fluid and gel state are shown in (Figure 4). The apparent release kinetic model was determined for each of microemulsion and gel formulations. This involved fitting the release data to zero order, first order and Higuchi equations before comparing the correlation coefficient obtained from the linear regression of each model. The drug release data were best fitted to Higuchi kinetics (Figure 4). This finding was expected to the gelled microemulsion but was unexpected for the fluid system. The release kinetics of the drug from the fluid ME can be explained on the bases that the ME was of w/o type which entraps kojic acid in its internal aqueous phase. This means that the drug will diffuse through the oily phase before being released from the whole system. This may explain the recorded kinetic model for drug release from the fluid formulations. This means that drug diffusion through the oily phase is the rate limiting step in its release from microemulsions. Similar release kinetics was recorded for other drugs from microemulsion systems (Chauhan *et al.*, 2013; Panapisal et al,

2012; Üstündağ-Okur *et al.,* 2014). With respect to the drug release rate there was a dependence on the composition of the tested formulation and the solubility of the drug in such formulation which can affect the concentration gradient in the release study through artificial membranes (Table 3). Accordingly, caprylic ethanol ME system liberated the drug at the fastest rate followed by the oleic acid ethanol microemulsion followed by oleic acid PG ME then caprylic acid ME and oleic acid ME. This release rate correlates with the solubilizing power of the ME to kojic acid (Table 2). Formulation of the ME in the form of gel resulted in a significant reduction in the release rate compared with the corresponding fluid formulation (Table 3). This is expected taking consideration the increase in the viscosity of the formulation after gel formation. The kinetics of drug release followed matrix diffusion release kinetics which is expected with the gel matrix. Similar release pattern was recorded for other drugs from a gel matrix after incorporation of microemulsion in the gel structure (Chudasama *et al.,* 2011; Cojocaru *et al.,* 2015)

Skin permeation of kojic acid from tested formulations

Full thickness skin obtained from the inner side of freshly excised rabbit ears was used in this study. This skin has been successfully utilized to monitor skin permeation of a variety of drugs from different delivery systems including microemulsion (Corbo *et al.,* 1990; El Maghraby, 2010; Touitou *et al.,* 2000). This skin model was also shown to be a successful barrier for skin permeation studies for both lipophilic and hydrophilic materials (Nicoli *et al.,* 2008). The permeation profiles are shown in (Figure1) and the calculated transdermal permeation parameters are presented in (Table 3). Application of drug as aqueous solution (control) resulted in a very low permeation rate of the drug. This is clear from the recorded transdermal flux value (Table 3). This is expected taking into consideration the hydrophilic nature of kojic acid which has a very low partition coefficient (log P = -2.45). Other investigators recorded poor skin permeation for the same drug after application of aqueous solution (Oliveira *et al.,* 2010). Incorporation of kojic acid in different microemulsion formulations significantly increased the transdermal drug flux compared with the saturated aqueous control (Table 3). The efficacy of microemulsion formulations depended on their composition. For (OA) based systems the basic formulation comprised the oil with Tween 80 and water. This formulation was considered as the prototype and was modified by incorporation of either propylene glycol or ethanol as cosurfactants. Incorporation propylene glycol as cosurfactant in microemulsion resulted in a significant increase in the transdermal flux of the drug ($p < 0.05$) compared with the prototype formulation (Table 3). Replacing propylene glycol with ethanol in this formulation resulted in significant alteration in the transdermal flux compared with the prototype formulation ($P < 0.05$). The recorded result with respect to the effect of ethanol is similar to that recorded for the same cosurfactant with eucalyptus oil microemulsion (El Maghraby, 2008). With respect to the potentiating effect obtained after incorporation of propylene glycol can be explained on the base of the synergism between oleic acid and propylene glycol. Synergistic transdermal penetration enhancing effect was recorded after mixing oleic acid with propylene glycol. The pattern was also recorded for the same combination in microemulsion formulation (Barry, 1987; El Maghraby, 2012a; Tanojo *et al.,* 1997).

With respect to the (CP) based system, the basic formulation comprised the oil with Tween 85 and water. This formulation was considered as the prototype and was modified by incorporation ethanol as cosurfactants. This formulation was even more efficient than that containing oleic acid with respect to enhancing kojic acid transdermal delivery. Incorporation ethanol as cosurfactant in microemulsion resulted in a trend of increased transdermal flux of the drug compared with the prototype formulation (Table 3).

Incorporation of the microemulsion formulations into the gel matrix resulted in a reduction in the transdermal flux of the drug compared with the corresponding fluid microemulsion but the microemulgel systems delivered the drug through the skin at significantly higher rate compared with the aqueous control. The superiority of the fluid systems over the corresponding gel phase systems is expected as the former can provide intimate contact between the colloidal structure and the microarchitecture of the skin surface. The efficiency of the tested microemulgel systems correlated with the efficacy of the corresponding fluid microemulsion with respect to the rank order.

The mechanisms of enhanced transdermal drug delivery from microemulsions have been reviewed with different possible mechanisms being suggested (El Maghraby, 2012b).

Table 3: The in vitro drug release rate and the transdermal permeation parameters of kojic acid obtained from different microemulsion and microemulgel formulations.

Formulation	Release rate (µg cm^{-2} $h^{-0.5}$)	Flux (µg cm^{-2} h^{-1})	Lag time (h)
Control	N.D	4.85 (2.7)	3.17 (1.1)
OA ME	3433 (70)	347.2 (22.9)	9.22 (1.28)
OA PG ME	12591.4 (3690)	633.7 (117)	7.8 (0.63)
OA ET ME	19711.5 (1536)	394.9 (122)	10.3 (2.6)
CP ME	10914 (2182)	1038.5 (75)	2.9 (0.9)
CP ET ME	46676 (6216)	1335 (237)	1.94 (0.5)
OA Gel	2430 (131)	222 (53)	7.9 (0.53)
OA PG Gel	7103 (374)	323.4 (83)	5.4 (1.07)
OA ET Gel	9358 (61)	256.41 (56)	6.6 (2.4)
CP Gel	5542 (320)	366 (38)	2.9 (1.2)
CP ET Gel	13864 (2212)	671.9 (200)	2.88 (1.1)

The control was saturated aqueous solution of drug. Values between brackets are SD (n=3).

The high drug loading capacity of microemulsions was considered as the first possible mechanism (Kreilgaard *et al.*, 2000). However, this mechanism can be applied to lipophilic drugs suffering from poor solubility but does not apply for the current study which deals with freely water soluble drug. Second hypothesis is the possibility of supersaturation which results from phase transition of the microemulsion after application on the skin. This can modulate the thermodynamic activity and the driving force for the transdermal drug transfer (Kemken *et al.*, 1992). The third possible mechanism depends on the ability of the microemulsion droplet to come into intimate contact with the microenvironment of the skin surface due to very small droplet size and very low surface tension. This mechanism explains the superiority of fluid formulation over microemulgel (El Maghraby, 2008, 2012 a).

The forth possible explanation for enhanced transdermal delivery from microemulsions may be the penetration enhancing effect of the microemulsion components. The later possibility has high probability taking into consideration the nature of the main components of the tested microemulsion systems. For example oleic acid was shown to exert skin penetration enhancing effect for both hydrophilic and hydrophobic dugs from microemulsions (El Maghraby, 2012a; Malakar *et al.*, 2011).

It was also able to enhance skin penetration from microemulgel (Sabale and Vora, 2012). As for oleic acid, medium chain glycerides have been used as a penetration enhancer for drugs from microemulsions (Hosmer *et al.*, 2009; Lopes *et al.*, 2010; Zhang *et al.*, 2010).

CONCLUSIONS

Microemulsions containing oleic acid or caprylic/capric triglycerides are promising for dermal and transdermal delivery of kojic acid. Incorporation of cosurfactants in the microemulsions augmented the transdermal drug delivery potential of kojic acid from the colloidal system. The prepared systems were successfully formulated in gel form which retained good fraction of the penetration enhancing ability of the corresponding fluid microemulsion.

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