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*Research Article*

# Formulation of Microemulsion Systems for Dermal Delivery of Silymarin

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***Abstract.*** Silymarin is a standardized extract from *Silybum marianum* seeds, known for its many skin benefits such as antioxidant, anti-inflammatory, and immunomodulatory properties. In this study, the potential of several microemulsion formulations for dermal delivery of silymarin was evaluated. The pseudo-ternary phase diagrams were constructed for the various microemulsion formulations which were prepared using glyceryl monooleate, oleic acid, ethyl oleate, or isopropyl myristate as the oily phase; a mixture of Tween 20®, Labrasol®, or Span 20® with HCO-40® (1:1 ratio) as surfactants; and Transcutol® as a cosurfactant. Oil-in-water microemulsions were selected to incorporate 2% *w/w* silymarin. After six heating–cooling cycles, physical appearances of all microemulsions were unchanged and no drug precipitation occurred. Chemical stability studies showed that microemulsion containing Labrasol® and isopropyl myristate stored at 40°C for 6 months showed the highest silybin remaining among others. The silybin remainings depended on the type of surfactant and were sequenced in the order of: Labrasol®>Tween 20®>Span 20®. *In vitro* release studies showed prolonged release for microemulsions when compared to silymarin solution. All release profiles showed the best fits with Higuchi kinetics. Non-occlusive *in vitro* skin permeation studies showed absence of transdermal delivery of silybin. The percentages of silybin in skin extracts were not significantly different among the different formulations ($p>0.05$). Nevertheless, some silybin was detected in the receiver fluid when performing occlusive experiments. Microemulsions containing Labrasol® also were found to enhance silymarin solubility. Other drug delivery systems with occlusive effect could be further developed for dermal delivery of silymarin.

**KEY WORDS:** dermal delivery; microemulsion; silybin; silymarin.

## INTRODUCTION

UV irradiation causes oxidative stress to the skin by inducing the generation of reactive oxygen species exceeding the antioxidant defense ability of cells. Thus, the use of naturally occurring herbal antioxidants has gained considerable interest to protect the skin from adverse biological effects of solar UV irradiation. Silymarin, flavonolignans isolated from milk thistle, is generally used for several liver disorder conditions such as cirrhosis, chronic hepatitis, and liver diseases associated with alcohol consumption [1–3]. Recently, topical application of silymarin has received attention because of its antioxidant, anti-inflammatory, and immunomodulatory properties which may prevent UV-induced skin disorders including erythema, photoaging, and skin cancer [4, 5]. To achieve skin benefits, effective amounts of silymarin require be solubilized and incorporated into the corresponding formulation. Due to poor water solubility (3.2 mg/100 mL) [6], the enhancement of silymarin solubility remains one of the most challenging aspects of drug development.

Microemulsions, as drug delivery systems, have several advantages such as enhanced drug solubility, high stability, and ease of manufacturing. Moreover, they improve percutaneous penetration of drugs [7]. Oil and/or surfactant phases contribute to the potential enhancing effect of microemulsions rather than the specific microemulsion structure [8]. Oil phase like oleic acid can interact with the lipids in the stratum corneum leading to an increase in their fluidity, such that drug mobility is also increased [9]. While, surfactants penetrate into the skin and enhance dermal and transdermal drug delivery either by disrupting the stratum corneum lipids or by increasing the partition coefficient of the drug between skin and formulation medium thus improving the drug solubility in the skin [8].

In this study, 2% *w/w* silymarin was incorporated into selected oil-in-water (O/W) microemulsions and their physical/chemical stabilities and release properties were investigated. *In vitro* drug permeation studies through excised pig skin were also evaluated to determine the suitability of the prepared silymarin microemulsions for skin delivery.

## MATERIALS AND METHODS

### Materials

Silymarin was procured from IVAX Pharmaceutical s.r.o. (Czech Republic) lot number 71315100408, Standard Silybin

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was purchased from Sigma-Aldrich (Germany), Glyceryl monooleate and ethyl oleate were obtained from Croda Co., Ltd. (Thailand), Labrasol® and Transcutol® were purchased from PC Intertrade Co., Ltd. (Thailand), Isopropyl myristate and HCO-40® were purchased from Namsiang Trading Co., Ltd. (Thailand), Oleic acid, Tween 20® and Span 20® was purchased from Srichand United Dispensary Co., Ltd. (Thailand). All chemicals were used as received without further purification.

## Methods

### *Construction of Pseudo-Ternary Phase Diagrams*

Oils and surfactants were selected according to their ability to solubilize and enhance the skin penetration of silymarin. The selection can be categorized into polar and nonpolar oils and high and low HLB surfactants. Four types of oils, three types of surfactants, and one cosurfactant were used to result in 12 different formulations.

Pseudo-ternary phase diagrams for all formulations were constructed in order to obtain the existing range of microemulsions. The composition of the studied pseudo-ternary phase diagrams are presented in Table I. For each pseudo-ternary phase diagram, the oil mixtures were prepared with the weight ratio of oil to surfactants/cosurfactant blend (Smix) at 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, respectively. To these mixtures, water was added drop-wise and mixed by vortex mixer (Scientific Industries, Inc., USA) at room temperature. Following the addition of each drop of water, the mixture was visually examined for transparency. The changes in the sample appearance from turbid to transparent and vice versa were observed. Transparent, single-phase, and low viscous mixtures were designated as isotropic region.

### *Characterization of Microemulsion*

Microemulsions were prepared and stored at room temperature for 3 days to reach equilibrium before checking them for transparency or phase separation by visual inspection. Microemulsions were also characterized in regard to viscosity, conductivity, and dilution test.

Viscosities of microemulsions were measured by Brookfield viscometer (Brookfield engineering laboratories, Inc, USA) and their electrical conductivities were measured using conductivity meter (Consort, Belgium). Both viscosity and conductivity measurements were conducted at 25±2°C in triplicate ($n$=3).

Dilution test was carried out by addition of water or oil used in the formulation to determine the type of emulsion. If water was easily dispersed in the continuous phase, the microemulsion was defined as oil-in-water and if oil was dispersible in the continuous phase, the microemulsion was defined as water-in-oil. If microemulsion becomes turbid upon dilution with oil or water, the microemulsion was defined as bicontinuous. Based on electrical conductivity, viscosity, and dilution test, the microemulsion was determined as O/W, water-in-oil (W/O), or unclassified microemulsion.

### *Stability Testing*

Physical and chemical stability testing of selected microemulsions containing 2% *w*/*w* of silymarin were performed under the accelerated conditions in triplicate ($n$=3). For physical stability testing, the heating–cooling cycle test was done by storing silymarin microemulsions in the refrigerator at 4±1°C for 48 h followed by hot air oven at 45±1°C for 48 h as one cycle. Six cycles were carried out. The clarity, phase separation, and precipitation of silymarin from microemulsions were investigated. For chemical stability testing, silymarin microemulsions were stored in hot air oven at 40±1°C for 6 months [10]. The concentrations of active component, silybin, in the tested microemulsions was determined at 0, 1, 2, 3, 4, 5, and 6 month (s) by high-performance liquid chromatography (HPLC) method [11].

Chromatography was performed using a Shimadzu LC-10 AD system (Shimadzu, Japan) with a BDS Hypersil® C18

**Table I.** Composition of the Studied Pseudo-Ternary Phase Diagrams

| | Surfactants | | |
|---|---|---|---|
| Oil | S1 | S2 | Cosurfactant |
| Glyceryl monooleate (GMO) | Tween 20®<br>Labrasol®<br>Span 20® | HCO-40® | Transcutol® |
| Oleic acid (OA) | Tween 20®<br>Labrasol®<br>Span 20® | HCO-40® | Transcutol® |
| Ethyl oleate (EO) | Tween 20®<br>Labrasol®<br>Span 20® | HCO-40® | Transcutol® |
| Isopropyl myristate (IPM) | Tween 20®<br>Labrasol®<br>Span 20® | HCO-40® | Transcutol® |

Smix is the blend of S1, S2, and cosurfactant at the weight ratio of 0.5:0.5:1, respectively

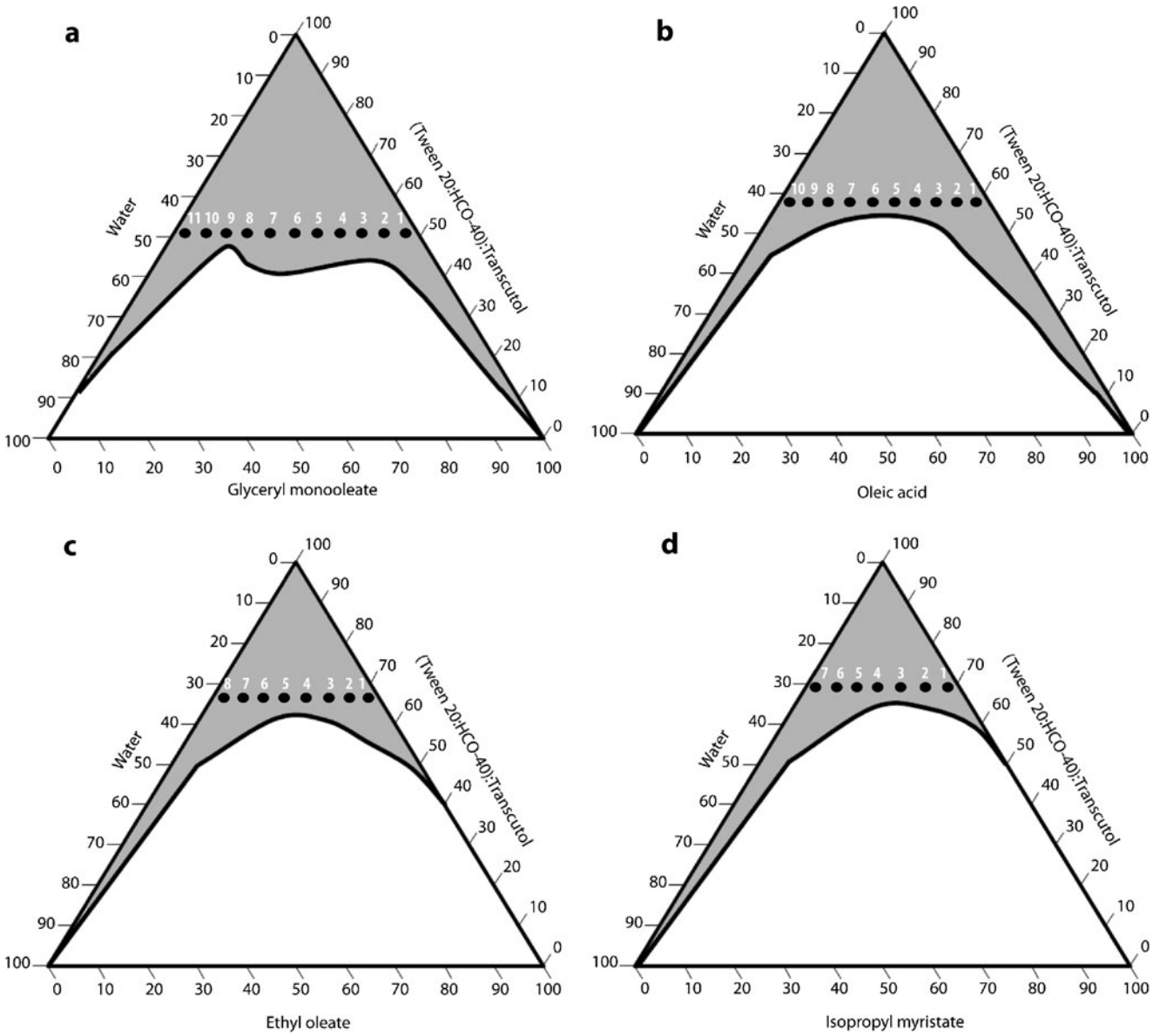


**Fig. 1.** The isotropic existence regions (*shaded area*) of formulations containing (Tween 20®/HCO-40®)/Transcutol® and four oils: **a** glyceryl monooleate, **b** oleic acid, **c** ethyl oleate, and **d** isopropyl myristate

250×4.6 mm, 5 μm column (Thermo Electron Corporation, England, UK). The mobile phase consisted of solution A (80:20:0.5 water/methanol/phosphoric acid) and solution B (80:20:0.5 methanol/water/phosphoric acid). In initial conditions (0–5 min), the mobile-phase composition was 85% A and 15% B; a linear gradient was applied to reach a composition of 55% A and 45% B after 15 min, maintained for 20 min and then set to return to initial conditions. The flow rate was 1 mL/min and the column temperature was set at 40°C. The total HPLC effluent was directed into a UV–VIS detector (SPD-10A, Shimadzu, Japan). Microemulsion was accurately weighed and suitably diluted with 30% ethanol in phosphate buffer saline (PBS) at pH 7.4 before loading it into the HPLC vials and a volume of 10 μL was injected into the HPLC. The HPLC analysis method was verified under the standard topics of specificity, linearity, accuracy, and precision.

*In Vitro Release Studies*

Release studies were carried out using modified Franz diffusion cells with cellulosic membrane (cutoff molecular weight 12,000–14,000). The cellulose membrane was first hydrated in water for 24 h and then soaked in the receptor solution for 1 h before the experiment. The membrane was clamped between the donor and the receptor chambers of vertical diffusion cells. The receptor chamber was filled with 40% ethanol in PBS at pH 7.4 to solubilize silymarin and to ensure sink conditions. The receptor chambers were thermostated at 37°C±1°C and their

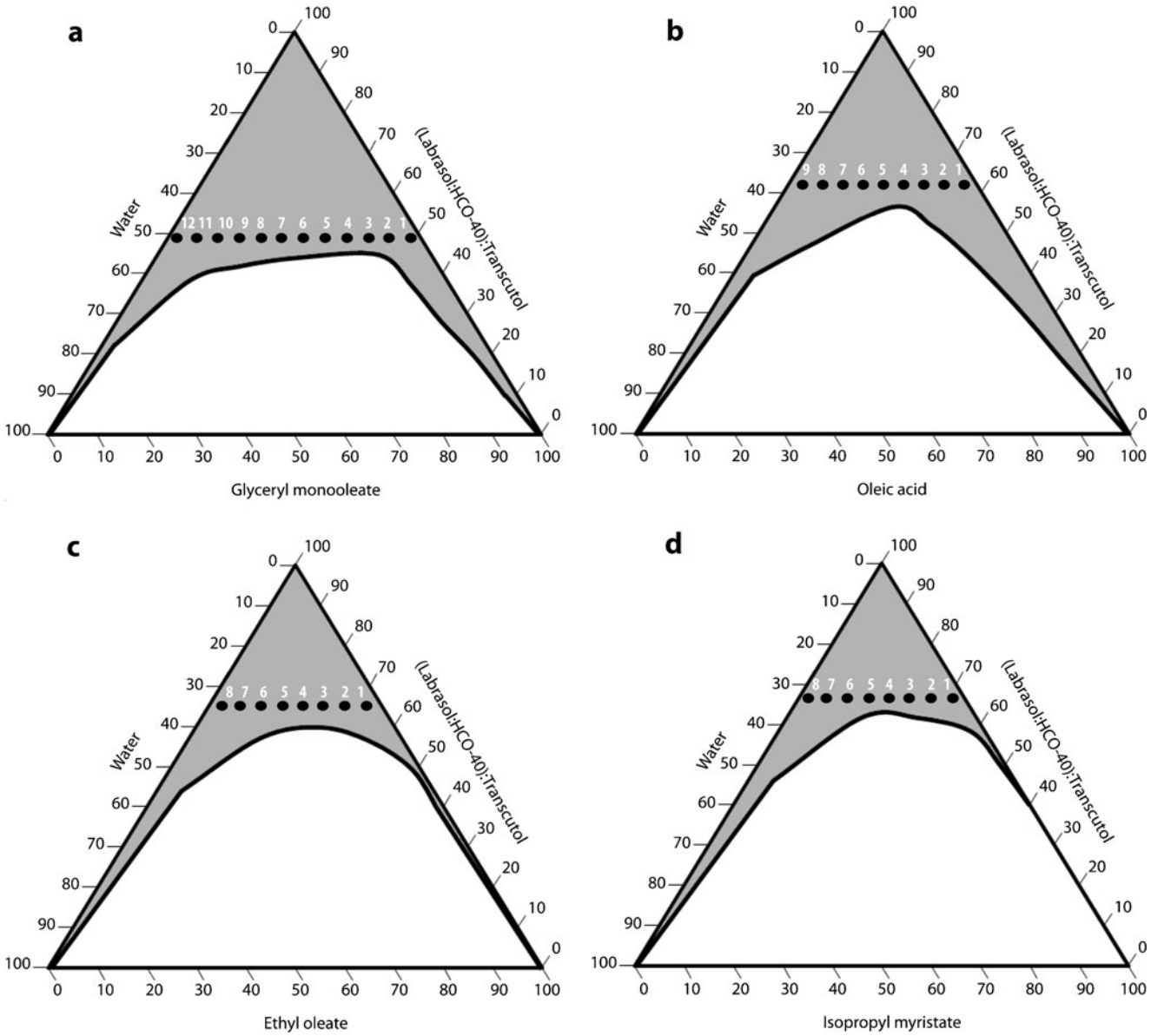


**Fig. 2.** The isotropic existence regions (*shaded area*) of formulations containing (Labrasol®/HCO-40®)/Transcutol® and four oils: **a** glyceryl monooleate, **b** oleic acid, **c** ethyl oleate, and **d** isopropyl myristate

solution was magnetically stirred at 600 rpm throughout the experiment. Silymarin microemulsions (250 μL) were gently placed in the donor chamber. At 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h, 5 mL of the solution in the receptor chamber were removed for UV determination (Shimadzu, Japan) at 329 nm and replaced immediately with an equal volume of fresh medium. The silymarin amount was analyzed by spectroscopic method since it had a good correlation with silybin content (data not shown). Each sample was performed in triplicate ($n$=3). Cumulative corrections were made to obtain the total amount of silymarin released at each time interval. The UV–VIS spectrophotometeric method was verified under the standard topics of specificity, linearity, accuracy, and precision.

The release profiles of silymarin were fitted to different release kinetics including release kinetic from spherical entities by Guy *et al.* [12], zero-order, first-order, and Higuchi kinetics as shown in Eqs. 1, 2, 3, 4, respectively. Pearson coefficients were calculated and used as one criteria for selecting the appropriate microemulsion formulations to perform the skin permeation studies.

$$\ln\left(1-\frac{M_t}{M_0}\right)=\frac{-3kt}{r^2} \quad (1)$$

where, $M_t/M_0$ is the fraction of released drug at time $t$ and $r$ is the droplet radius. Plotting the natural logarithm of the

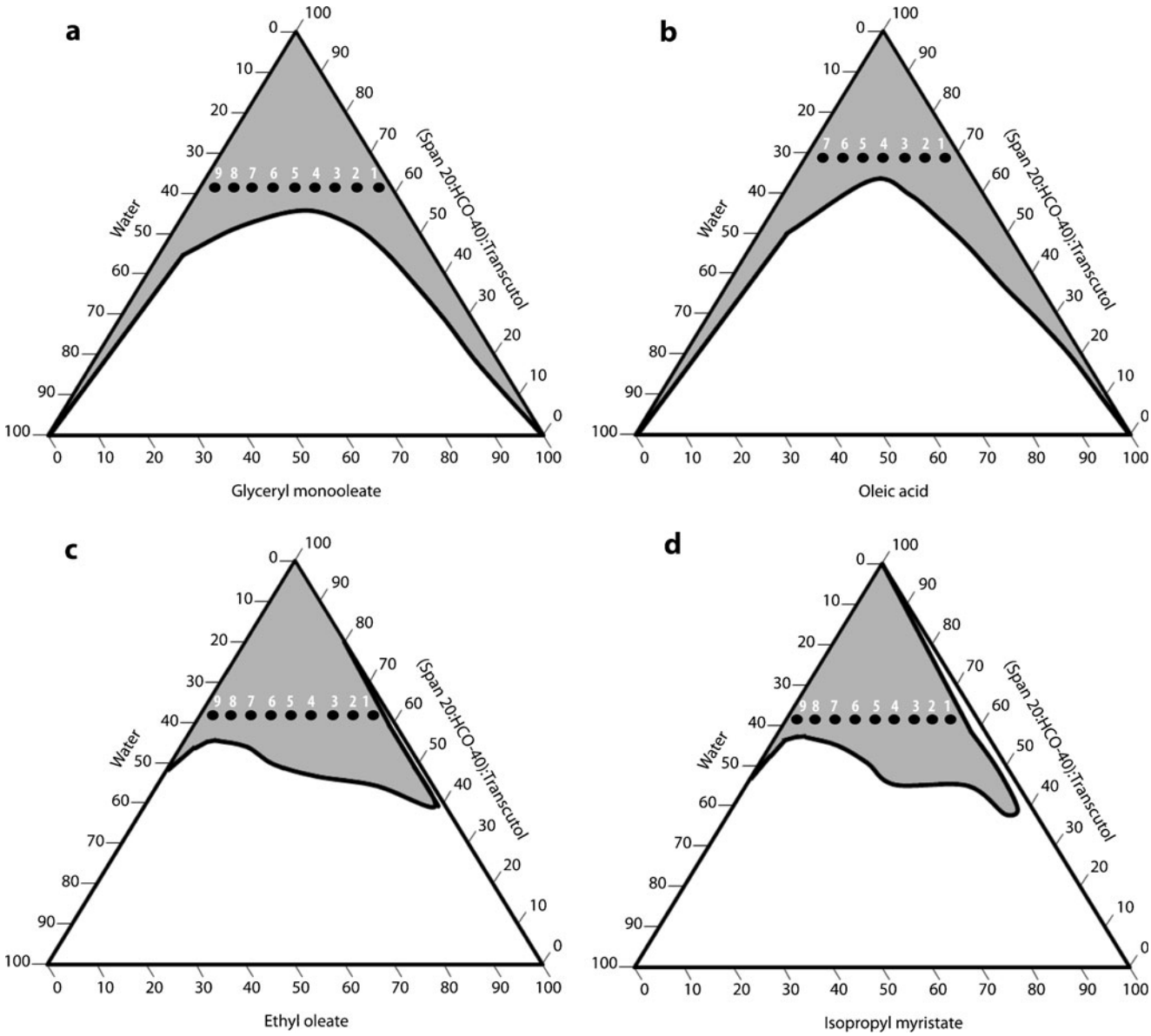


**Fig. 3.** The isotropic existence regions (*shaded area*) of formulations containing (Span 20®/HCO-40®)/Transcutol® and four oils: **a** glyceryl monooleate, **b** oleic acid, **c** ethyl oleate, and **d** isopropyl myristate

fraction of remaining drug against time, release curve whose slope was $-3\ k/r^2$ was obtained.

$$M_t = M_0 + k_0 t \tag{2}$$

$$\ln\ M_t = \ln\ M_0 + k_1 t \tag{3}$$

$$M_t = k_H t^{\frac{1}{2}} \tag{4}$$

where, $M_t$ is the cumulative amount of drug released at time $t$, $M_0$ is the initial amount of drug in the formulation, $k_0$, $k_1$, $k_H$ are the zero-order, first-order, and Higuchi release rate constant.

*In Vitro Skin Permeation Studies*

Newborn pig abdominal skin was obtained from a local slaughterhouse immediately after the animal's death (Ratchaburi, Thailand). Subcutaneous fat was removed, and skin was washed and examined for integrity. Skin was soaked in the receptor solution for 1 h before the experiment and was then clamped between the donor and the receptor chamber of vertical diffusion cells. The receptor chamber was filled with PBS at pH 7.4 and thermostated at 37°C±1°C. The solutions in the receptor chambers were magnetically stirred at 600 rpm throughout the experiment. Silymarin microemulsions (400 μL) were gently placed in the donor chamber. Non-occlusive experiments were performed in non-occluded donor compartments and the applied formulation was allowed to dry,

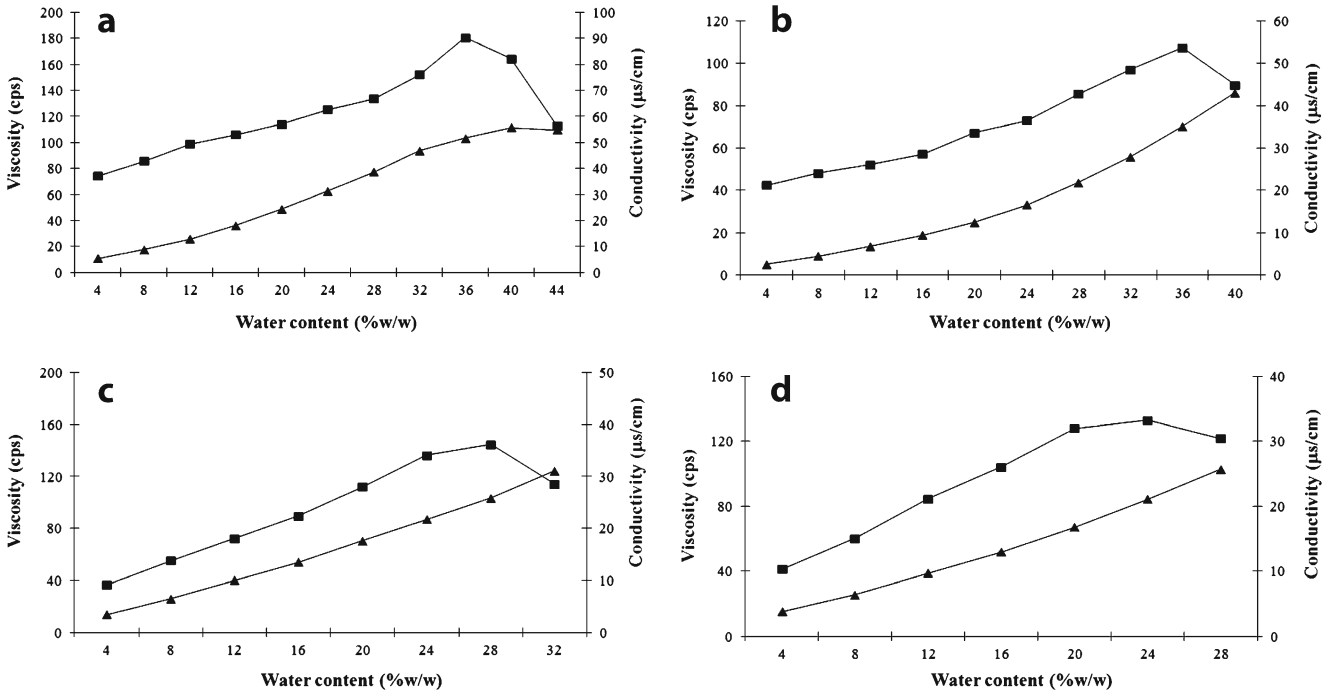


**Fig. 4.** Conductivity (*filled triangle*) and viscosity (*filled square*) of microemulsions containing: **a** glyceryl monooleate, **b** oleic acid, **c** ethyl oleate, and **d** isopropyl myristate as oil phase, Tween 20® and HCO-40® (1:1) as surfactant, and Transcutol® as cosurfactant

whereas occlusive experiments were carried out in occluded donor compartments covered with parafilm to avoid any evaporation process.

At the end of each permeation experiment (24 h), silybin in the donor and receptor chambers as well as in the skin were quantified using the validated HPLC. This was done by collecting the formulation remaining on the skin and rinsing the donor compartment with ethanol and the collected sample was analyzed for silybin. The drug deposited within the skin was extracted by cutting the skin into small pieces and then shaking the cut skin in methanol at 100 rpm for 3 h followed by three cycles of 30 min sonication. The methanolic skin extract was then evaporated under $N_2$ purge until dryness. Receptor solutions were collected and evaporated at 40°C using a thermostatic water bath until dryness. The residues (skin extract and receptor compartment) were reconstituted

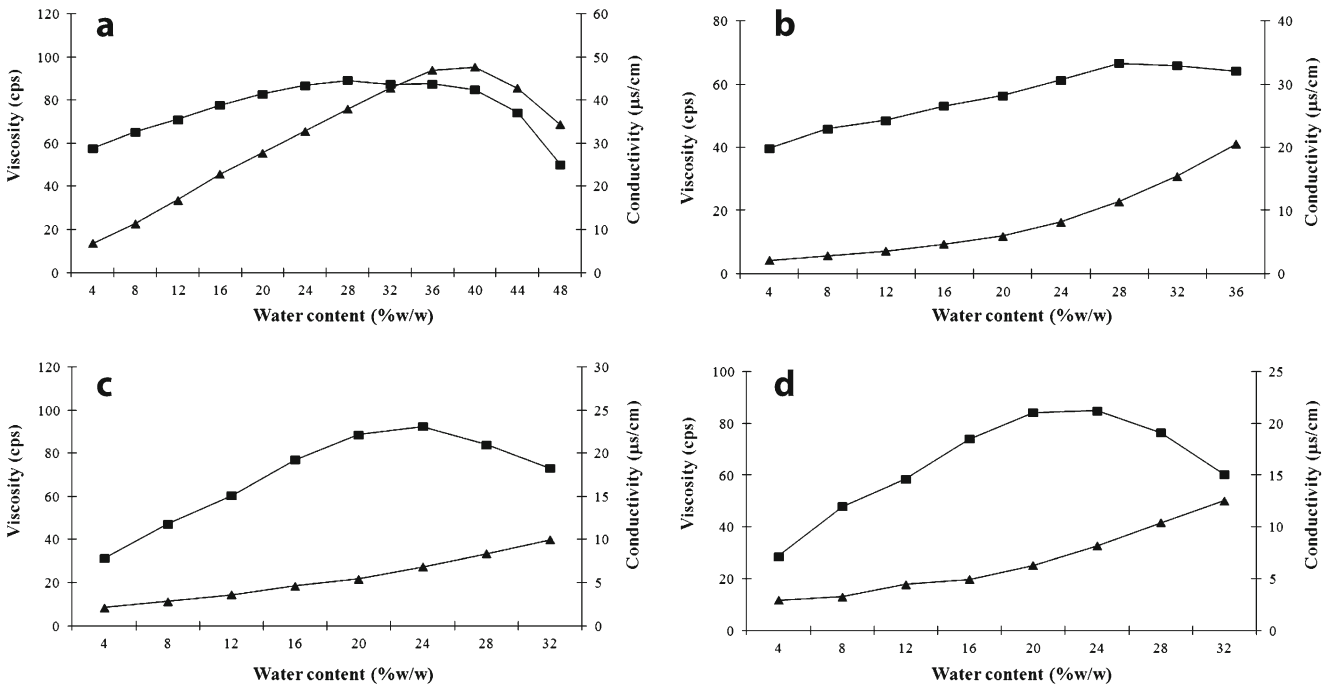


**Fig. 5.** Conductivity (*filled triangle*) and viscosity (*filled square*) of microemulsions containing: **a** glyceryl monooleate, **b** oleic acid, **c** ethyl oleate, and **d** isopropyl myristate as oil phase, Labrasol® and HCO-40® (1:1) as surfactant, and Transcutol® as cosurfactant

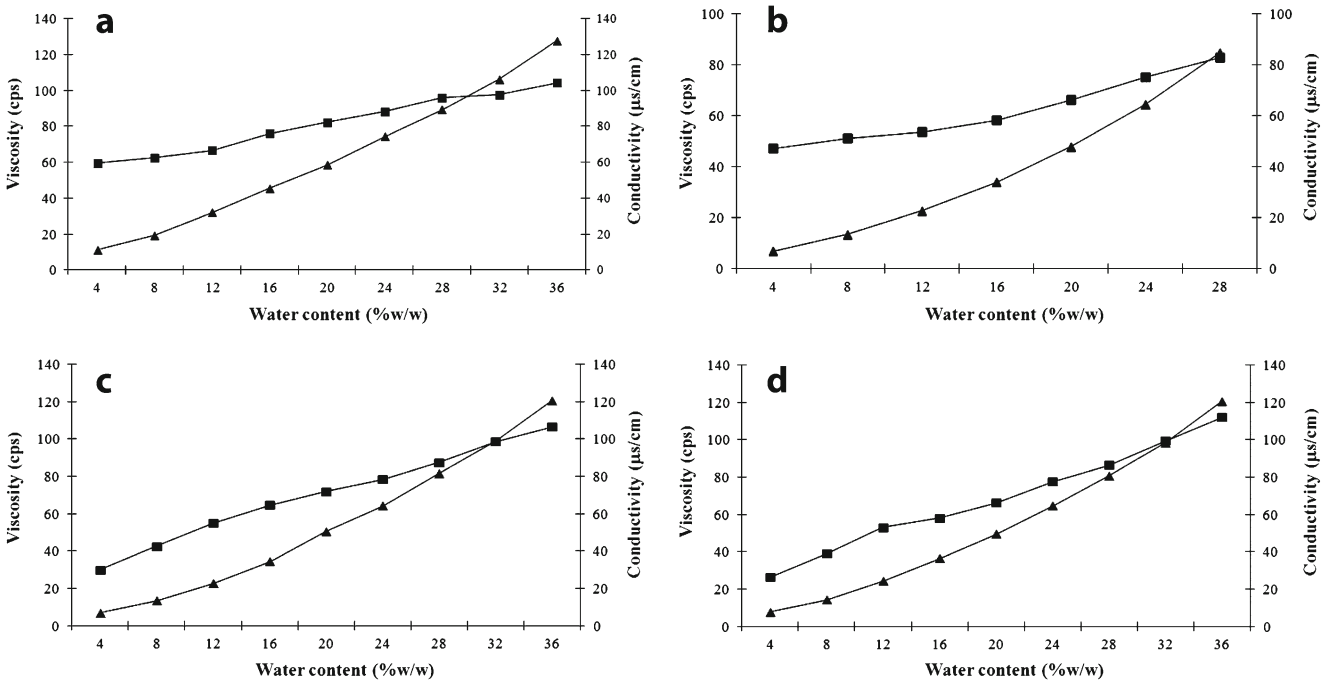


**Fig. 6.** Conductivity (*filled triangle*) and viscosity (*filled square*) of microemulsions, which containing: **a** glyceryl monooleate, **b** oleic acid, **c** ethyl oleate, and **d** isopropyl myristate as oil phase, Span 20® and HCO-40® (1:1) as surfactant, and Transcutol® as cosurfactant

with 40% ethanol in phosphate buffer saline pH 7.4 and the reconstituted solutions were centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed for silybin content using HPLC for each compartment. The experiment was performed in six replicates ($n=6$).

## RESULTS AND DISCUSSIONS

### Pseudo-Ternary Phase Diagrams

The constructed pseudo-ternary phase diagrams are shown in Figs. 1, 2, and 3a–d. Isotropic regions could be observed in all systems. Larger isotropic regions were obtained either from formulations with polar oils (i.e., glyceryl monooleate and oleic acid) and high HLB surfactant mixtures (i.e., Tween 20®/HCO-40® and Labrasol®/HCO-40®) or from formulations with less polar or nonpolar oils (i.e., ethyl oleate and isopropyl myristate) and low HLB surfactant mixture (i.e., Span 20®:HCO-40®). The surfactant ability to migrate to the oil–water interface contributes to the interfacial tension depression which is also related to the solubilizing power and microemulsion formation. Surfactants and oil polarities may indicate the extent of preference for surfactant migrating into the interface between water and oil phases [13]. Since the HLB values relatively represent the polarity of the surfactants, Tween 20® and Labrasol® with high HLBs may prefer to localize at the interface of high polar oils such as glyceryl monooleate and oleic acid owing to their hydroxyl and carboxyl groups, respectively. While Span 20® with low HLB may prefer to migrate into the interface of less polar or nonpolar oils containing ester groups such as ethyl oleate and isopropyl myristate.

**Table II.** Microemulsion Type as Determined using the Dilution Test (Presented as % Water Content)

| Systems | | | | Water content (%*w*/*w*) | | |
|---|---|---|---|---|---|---|
| Oil | Smix | | Water | W/O | unclassified | O/W |
| GMO | Tween20®:HCO-40® (Fig. 1a) | Transcutol® | Water | <16 (point 1–3) | 16–36 (point 4–9) | >36 (point 10–11) |
| | Labrasol®:HCO-40® (Fig. 2a) | | | <20 (point 1–4) | 20–40 (point 5–10) | >40 (point 11–12) |
| | Span 20®:HCO-40® (Fig. 3a) | | | <24 (point 1–5) | 24 (point 6) | >24 (point 7–9) |
| OA | Tween20®:HCO-40® (Fig. 1b) | Transcutol® | | <20 (point 1–4) | 20 (point 5) | >20 (point 6–10) |
| | Labrasol®:HCO-40® (Fig. 2b) | | | <20 (point 1–4) | 20 (point 5) | >20 (point 6–9) |
| | Span 20®:HCO-40® (Fig. 3b) | | | ≤20 (point 1–5) | – | ≥ 24 (point 6–7) |
| EO | Tween20®:HCO-40® (Fig. 1c) | Transcutol® | | <20 (point 1–4) | 20 (point 5) | >20 (point 6–8) |
| | Labrasol®:HCO-40® (Fig. 2c) | | | <12 (point 1–2) | 12–16 (point 3–4) | >16 (point 5–8) |
| | Span 20®:HCO-40® (Fig. 3c) | | | All (point 1–9) | | |
| IPM | Tween20®:HCO-40® (Fig. 1d) | Transcutol® | | <16 (point 1–3) | 16–20 (point 4–5) | >20 (point 6–7) |
| | Labrasol®:HCO-40® (Fig. 2d) | | | <20 (point 1–4) | 20 (point 5) | >20 (point 6–8) |
| | Span 20®:HCO-40® (Fig. 3d) | | | All (point 1–9) | | |

Points refer to the selected microemulsions and are depicted as dot in the pseudo-ternary phase diagrams (Figs. 1, 2, and 3a–d)

**Table III.** Composition of the Selected Oil-in-Water Microemulsions (%*w*/*w*)

| Formulation | GT10 | GL11 | GS8 | OT9 | OL8 | OS6 | ET7 | EL7 | IT6 | IL7 |
|---|---|---|---|---|---|---|---|---|---|---|
| GMO | 8 | 6 | 7 | | | | | | | |
| Oleic acid | | | | 5 | 6 | 8 | | | | |
| Ethyl oleate | | | | | | | 6 | 7 | | |
| Isopropyl myristate | | | | | | | | | 7 | 6 |
| Tween20®:HCO-40® | 26 | | | 29.5 | | | 33 | | 34.5 | |
| Labrasol®:HCO-40® | | 25 | | | 31 | | | 32.5 | | 34 |
| Span 20®:HCO-40® | | | 30.5 | | | 34 | | | | |
| Transcutol® | 26 | 25 | 30.5 | 29.5 | 31 | 34 | 33 | 32.5 | 34.5 | 34 |
| Water | 40 | 44 | 32 | 36 | 32 | 24 | 28 | 28 | 24 | 28 |

Microemulsion formulations were chosen from each pseudo-ternary phase diagram by keeping the surfactant concentration constant at the minimal percentages (5% above the highest boundary point) to ensure the achievement of microemulsions and varying the water and oil contents. The selected microemulsions from each pseudo-ternary phase diagram are depicted as dots presented in Figs. 1, 2, and 3a–d with a total of 107 microemulsion formulations being tested.

### Characterization of the Selected Microemulsions

Some structural changes occur during the transition of W/O to O/W microemulsion during which the inversion may gradually happens while the system remains isotropic [14]. Macroscopic changes of microemulsions such as viscosity and electrical conductivity can be used as indication of phase inversion. Conductivity and viscosity measurements were preformed along the line where the surfactant concentration was kept constant and the water to oil ratios was varied to evaluate the structural changes of microemulsions along this line (Figs. 4, 5, and 6).

An increase in the dispersed phase (water) of microemulsion is known to increase the viscosity and droplet size. The peak point in the viscosity–water content profile is denoted as the transition point of W/O to O/W microemulsion [15, 16]. In this study, the peak points were only found in microemulsion systems using high HLB surfactants (i.e., Tween 20® and Labrasol®) but not in the ones using low HLB surfactants (i.e., Span 20®). Absence of peak point may imply either no transformation has occurred or other transformation has occurred but could not be detected by viscosity changes.

The electrical conductivity is dramatically different among O/W, W/O, and bicontinous microemulsions. The conductivity is usually similar to normal aqueous medium in O/W, very low in W/O, and significantly high in bicontinous. So a drastic increase in conductivity can be used to detect phase conversion where the aqueous droplets are interlinking and clustering [14, 17]. Unfortunately, a drastic increase in conductivity could be observed only in the system containing Labrasol® and GMO (Fig. 5a) but not in other systems. For this reason, additional information on the type of microemulsions formed was obtained by performing dilution tests.

Microemulsion type as determined by the dilution test is reported as percent water content of the microemulsion systems in Table II. Results indicate that all microemulsions using ethyl oleate or isopropyl myristate with Span 20® were W/O type which may be due to the hydrophobicity of the surfactant mixtures with low HLB. In the contrary, O/W microemulsions were found to be formed with Span 20® when using polar oils (i.e., glyceryl monooleate and oleic acid) which could be due to the penetration of glyceryl monooleate and oleic acid into the palisade layer of the surfactant mixture, thus rendering the surfactant layer to be more hydrophilic.

O/W microemulsions were chosen to incorporate silymarin due to several preferences. For instance, hydration effect from the external aqueous phase aids the drug permeation through

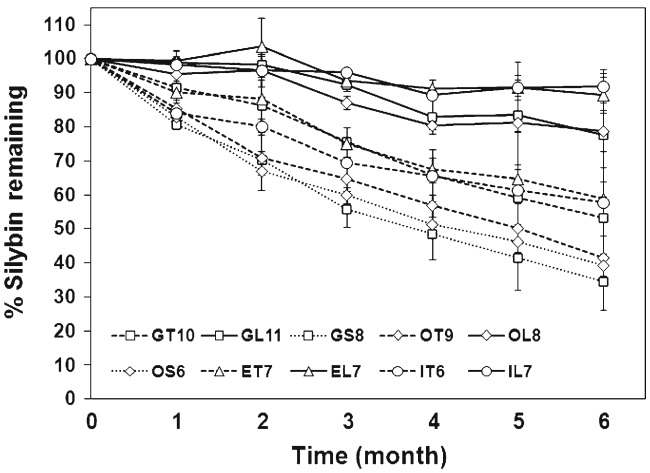


**Fig. 7.** Chemical stability of silymarin in selected O/W microemulsions ($n$=3)

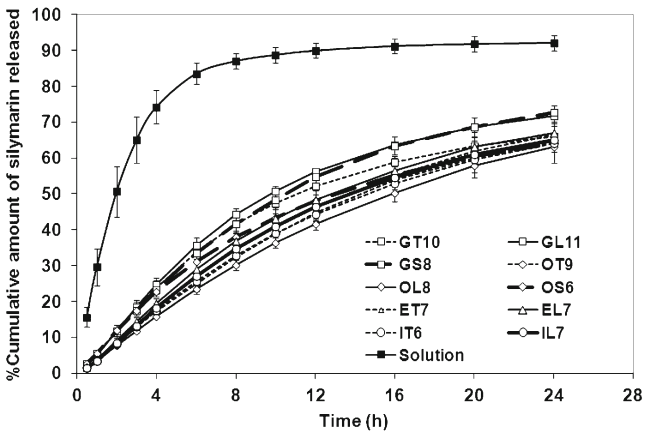


**Fig. 8.** Release profiles of silymarin from selected microemulsions ($n$=3) using modified Franz diffusion cell, cellulose acetate membrane, 40% ethanol in pH 7.4 PBS at 37±1°C, 600 rpm and under occlusive conditions

**Table IV.** Pearson Coefficients as Calculated According to Guy's Model, Zero-Order, First-Order, and Higuchi Kinetics

| Formulation | Guy's model[a] | Zero-order[b] | First-order[c] | Higuchi[d] |
|---|---|---|---|---|
| GT10 | 0.961 | 0.897 | 0.636 | 0.982 |
| GL11 | 0.973 | 0.902 | 0.631 | 0.983 |
| GS8 | 0.986 | 0.926 | 0.668 | 0.990 |
| OT9 | 0.994 | 0.959 | 0.699 | 0.993 |
| OL8 | 0.998 | 0.970 | 0.709 | 0.994 |
| OS6 | 0.975 | 0.920 | 0.653 | 0.992 |
| ET7 | 0.994 | 0.953 | 0.676 | 0.994 |
| EL7 | 0.988 | 0.938 | 0.657 | 0.993 |
| IT6 | 0.993 | 0.955 | 0.676 | 0.995 |
| IL7 | 0.989 | 0.945 | 0.665 | 0.993 |

[a] The extent of a linear relationship between $\ln\left(1-\frac{M_t}{M_0}\right)$ and $t$
[b] The extent of a linear relationship between $M_t$ and $t$
[c] The extent of a linear relationship between $\ln M_t$ and $t$
[d] Tthe extent of a linear relationship between $M_t$ and $\sqrt{t}$ ; where, $M_0$ is a percentage of released silymarin at $t=0$ and $M_t$ is a percentage of released silymarin at $t=t$

the skin [18]. In addition, the oil droplets might penetrate into the epidermis easier than the water droplets of the W/O type at the same surfactant concentration owing to the lipophilic nature of the stratum corneum [19]. In term of chemical stability, silymarin should be more stable in O/W microemulsion because the drug is protected in the internal phase (oil) from oxidative degradation since the external phase (water) can act as a barrier for oxygen diffusion thus preventing the oxidation of potent antioxidant silymarin. To minimize skin irritation, oil and surfactant contents were preferably used at low level. Surfactant mixtures were kept at 5% above the highest boundary point in the constructed pseudo-ternary phase diagrams and oils were chosen in the range of 5–8%. The chosen O/W microemulsions are shown in Table III and all the formulations remained clear after drug loading.

Silymarin is insoluble in water and has poor wettability. Therefore, maximum silymarin loading was desired and only two percentage of silymarin (2% *w*/*w*) was successfully incorporated into the selected O/W microemulsions while keeping considerable physical stability during storage. Two percent *w*/*w* (20 mg/g) silymarin is believed to be sufficient to show various skin benefits. In one study, Bonne and Sincholle [20] proposed that topical compositions containing from 0.01% to 1% and especially 0.1–0.5% by weight of the extract of fruits of *Silybum marianum* can oppose the degrading effects of free radicals which are partly responsible for skin aging. Moreover, Han *et al.* [21] reported that topical application of silymarin (50 μg in acetone/olive oil 4:1) reduces chemical-induced irritant contact dermatitis which was comparable to that of 0.1% hydrocortisone in BALB/c mice.

**Table V.** Release Rate Constants of Silymarin from Microemulsions Following Higuchi Kinetics ($n=3$)

| Formulation | Higuchi release rate constant (μg $cm^{-2}$ $h^{-1/2}$) |
|---|---|
| GT10 | 347.75±20.65 |
| GL11 | 370.85±10.22 |
| GS8 | 371.05±25.22 |
| OT9 | 330.99±9.76 |
| OL8 | 321.51±8.65 |
| OS6 | 309.79±31.59*,** |
| ET7 | 339.11±15.48 |
| EL7 | 350.64±16.09 |
| IT6 | 325.85±5.64 |
| IL7 | 338.71±10.23 |

Statistical analysis was performed by one-way ANOVA followed by Tukey HSD's test
*$p<0.05$, statistically significant different when compared to GL11
**$p<0.05$, statistically significant different when compared to and GS 8, respectively

## Stability of Silymarin Microemulsions

### *Physical Stability*

After six heating–cooling cycles, physical appearances of silymarin microemulsions were unchanged in term of transparency and phase separation. Moreover, drug precipitation was not detected. Therefore, the studied silymarin microemulsions were considered physically stable.

### *Chemical Stability*

Chemical stability testing under accelerated conditions (40°C) for 6 months showed that silybin content decreased to different extents in the different formulations. Microemulsion containing Labrasol® and isopropyl myristate (IL7) showed

**Table VI.** Silybin Percentages in Donor Compartment and Skin Extracts after 24 h Non-Occlusive Permeation Studies ($n=6$)

| % Silybin of loaded dose | Donor | Skin extract |
|---|---|---|
| GT10 | 94.73±3.92 | 0.039±0.032 |
| GL11 | 96.50±1.06 | 0.051±0.032 |
| GS8 | 96.36±1.45 | 0.027±0.011 |
| OL8 | 96.86±1.17 | 0.028±0.012 |
| EL7 | 93.44±4.68 | 0.023±0.017 |
| IL7 | 97.41±1.97 | 0.020±0.008 |
| 40%Ethanol in PBS pH 7.4 | 98.59±0.52 | 0.255±0.119* |

Statistical analysis was performed by one-way ANOVA followed by Tukey HSD's test
*$p<0.05$, statistically significant different when compared to silymarin microemulsions

excellent chemical stability with the highest silybin content amounting to 91.98%.

Since oxidative degradation is the most expected chemical instability of silymarin, the location of antioxidant substances in the microemulsion is very important and will significantly influence its stability toward oxidation. Due to its higher solubility in oils and surfactants, silymarin may primarily localize in the oil and/or surfactant layer [22, 23]. Therefore, the surfactant structure may also become an important factor in silymarin stability since silymarin may be protected from oxidation by solubilization in the surfactant film. As seen in Fig. 7, the percentages of silybin remaining were sequenced in the order of Labrasol®>Tween 20®>Span 20®. Since the oxyethylene group of surfactants has the ability to form hydrogen bond with phenol group of silybin, this may play a role in drug stability. While Span 20® has no oxyethylene group in its structure, Tween 20® and Labrasol® have twenty and eight oxyethylene groups, respectively. By number of mole normalization, the numbers of oxyethylene group of Tween 20® and Labrasol® were comparable in each formulation. The decreased chemical stability of microemulsions containing Tween 20® compared to Labrasol® may be due to the sorbitan ring of Tween 20® which may cause steric hindrance against the formation of hydrogen bonding. On the other hand, silybin being greatly localized in Labrasol® has resulted in superior stability from being oxidized compared to other formulations.

### *In Vitro* Release Studies

*In vitro* release studies were conducted to ensure drug release prior to *in vitro* skin permeation studies. The release profiles of silymarin from the different microemulsion formulations compared to silymarin solution are illustrated in Fig. 8. Because silymarin is insoluble in water, 2% *w/w* aqueous solution of silymarin was prepared in 40% ethanol in PBS at pH 7.4. Silymarin microemulsions showed prolonged release when compared to silymarin solution. The release was between 60% and 73% in 24 h with no burst effect and no drug precipitation. Silymarin release from microemulsions may be strongly influenced by the interactions present between the drug and surfactants used and/or partitioning of the drug between the oil and water phases.

To describe the kinetics of drug release from the test microemulsions, Guy's model, zero-order, first-order, and Higuchi's models were used. The data were transformed for linear regression analysis for each case. The Pearson values are listed in Table IV. In all cases, best fits were found with Higuchi kinetics which is in accordance with the findings of Špiclin *et al*. [24]. The Higuchi equation suggests that the drug release is by diffusion; therefore, we may conclude that the rate-determining step for silymarin release from microemulsions is the diffusion of silymarin from the oil droplet.

As seen in Table V, only the release rate constant of silymarin from the OS6 formulation was significantly lower than GL11 and GS8 ($p<0.05$). Release rate constant and chemical stability were used as the criteria for selecting candidates for permeation study. Silymarin release rates were slightly higher from formulations containing glyceryl monooleate (i.e., GT10, GL11 and GS8) while formulations containing Labrasol® showed superior chemical stability (i.e., OL8, EL7 and IL7). Based on these findings, six formulations were chosen for permeation studies (Table VI) and results were compared to silymarin solution.

### *In Vitro* Skin Permeation Studies

Same silymarin solution as in the *in vitro* release studies was used as the control in permeation studies. No silybin was detected in the concentrated receiver fluid in non-occlusive experiments for all studied microemulsions and silymarin solution (Table VI). Absence of transdermal delivery for the solution may be due to evaporation of ethanol which also caused drug precipitation. Some silybin was found in skin extracts; however, the detected quantities from all formulations were insignificant but all formulations were significantly different when compared to the ethanolic solution. As shown by Vicentini *et al*. [25], the topical quercetin W/O microemulsion successfully prevented the UVB-induced skin damages using hairless mice with no transdermal delivery. Additionally, several previous studies showed the benefits of silybin to the keratinocytes [26] and melanocytes [27] in which both cells locate in the skin's epidermis. Therefore, it is reasonable to believe that the developed microemulsions in this work should be effective; however, additional *in vivo* efficacy studies are recommended to show the potential of these microemulsion systems as a dermal delivery system of silymarin.

Since GL11 formulation showed the second highest release rate constant (Table V) and showed considerably good chemical stability (Fig. 7), it was selected for preliminary investigation under the occlusive conditions. About 0.1% silybin was found in the skin extracts and 0.01% in the concentrated receiver fluid. With these preliminary results, other drug delivery systems with occlusive effect like lipid nanoparticles could be further developed for dermal deliver of silymarin.

## CONCLUSION

The studied microemulsions enhanced silymarin solubility while maintaining adequate physical and chemical stability especially microemulsions containing Labrasol®. The kinetics of drug release from all tested microemulsions were perfectly described by Higuchi model and showed prolonged release when compared to silymarin solution. *In vitro* occlusive skin permeation studies showed that some silybin was detected in the concentrated receiver fluid. Lipid nanoparticles with occlusive effect may be suggested for further development as a potential dermal delivery system of silymarin.

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