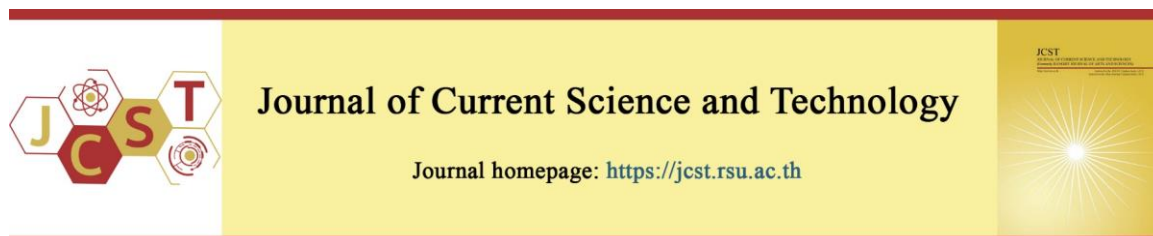


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Development and evaluation of *p*-chlorophenyl benzyl ether-loaded microemulsions for transdermal delivery

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Abstract

p-Chlorophenyl benzyl ether (CBE) has been reported to be a new skin brightening agent. Because of its strong inhibitory effect on tyrosinase and its high safety, CBE was selected as a model antityrosinase compound in this study. Unfortunately, the poor aqueous solubility of CBE limits its use. This study aimed to develop a CBE-loaded microemulsion (ME) with enhanced solubility and skin permeation capability for transdermal delivery. The physicochemical properties, loading efficiency, and skin permeation of CBE-loaded ME were investigated, and results revealed that maximum CBE solubility could be achieved in lemon oil (211.81 ± 15.19 mg/mL). Thus, lemon oil was selected as the oil phase for the ME formulation. Polysorbate 20 and ethanol at a ratio of 1:1 was employed as a surfactant and co-surfactant mixture (S_{mix}). A pseudo-ternary phase diagram was constructed to obtain the optimal concentration ranges of oil, S_{mix} , and water for ME formation. Here, lemon oil, S_{mix} , and water at a weight ratio of 20:70:10 was formulated with different amounts of CBE (3wt%, 5wt%, and 10wt%). The CBE-loaded ME had a particle size of 152–181 nm and negatively charged surfaces (-16.6 to -14.3 mV). The percentage loading efficiency of CBE was approximately 70%–100%. The ME preparation with 5wt% CBE was selected for skin permeation studies, and a 5wt% CBE aqueous suspension (free drug) was used as the control. The 5wt% CBE-loaded ME exhibited a significantly higher skin permeation flux (32.74 ± 1.36 $\mu\text{g}/\text{cm}^2/\text{h}$) compared with the 5% CBE suspension (2.35 ± 0.38 $\mu\text{g}/\text{cm}^2/\text{h}$); specifically, the flux of the CBE-loaded ME was approximately 14-fold greater than that of the free drug. Therefore, the prepared ME may potentially be used to improve the transdermal delivery of CBE. However, skin irritation must be further investigated due to the concern on skin irritation of lemon oil and ethanol.

Keywords: microemulsions; *p*-chlorophenyl benzyl ether; phase diagram; skin permeation; transdermal delivery.

1. Introduction

The popularity of skin-lightening products in Asia has grown dramatically over the last few decades (Li, Min, Belk, Kimura, & Bahl, 2008). Flawless skin without scars, freckles, and melasma is desired not only in Asia but also in North America, Latin America, Africa, and Europe. Interestingly, the demand for skin-lightening products among men is also rising (Masum,

Yamauchi, & Mitsunag, 2019). A survey revealed that approximately 80% of all Indian males use skin-lightening products (Pillaiyar, Manickam, & Namasivayam, 2017). The global sales of skin-lightening products has been estimated to amount to over US\$ 23 billion by the end of 2022 (Masum et al., 2019).

The skin color of humans is mainly influenced by a skin pigment called melanin

(Solano, Briganti, Picardo, & Ghanem, 2006). Overproduction of melanin can trigger abnormal skin conditions, such as freckles and melisma (Pandya & Guevara, 2000). Melanin is generated from melanocytes located at the bottom layer (i.e., the stratum basale) of the epidermis and then translocated to the upper layer of the skin (Solano et al., 2006; Tsatmali, Ancans, & Thody, 2002). Therefore, the primary mechanism of most skin-whitening agents involves the restraint of the synthesis and translocation of melanin. The synthesis of melanin, known as melanogenesis, is a complicated process related to various chemical and enzymatic reactions. Several enzymes, such as tyrosinase, tyrosinase-related protein 1 (TRP1), and TRP2, are involved in the regulation of melanogenesis (Kobayashi et al., 1994; Pawelek & Korner, 1982). Melanogenesis takes place in melanosomes, which are located in melanocytes, and tyrosinase is bound to the membrane of melanosomes. The first step of melanogenesis involves the change of tyrosine to L-DOPA via hydroxylation, followed by L-DOPA oxidation to generate *dopaquinone*. Both steps are catalyzed by tyrosinase enzyme, which is an essential enzyme in the melanogenesis pathway. *Dopaquinone* is then converted and modified via several reactions to form melanin (Cichorek, Wachulska, & Stasiewicz, 2013; Riam-Amatakun et al., 2019).

Novel skin-lightening products had been developed to fulfill consumer needs. Because tyrosinase is an essential enzyme for melanogenesis, many tyrosinase inhibitors have been

screened from various sources, including synthetic and natural products, to find prominent inhibitors of melanogenesis. Several tyrosinase inhibitors, including kojic acid and arbutin, are currently used as skin brightening agents (Masum et al., 2019).

In a previous study, 13 derivatives of phenyl benzyl ethers were synthesized, and their tyrosinase inhibition effects were evaluated. Findings revealed that *p*-chlorophenyl benzyl ether (CBE) shows significantly higher inhibitory activity compared with other compounds. For example, CBE showed higher inhibitory potency toward tyrosinase compared with kojic acid (Riam-Amatakun, Limpachayaporn, Pizon, Opanasopit, & Nuntharatanapon, 2019; Suttisintong, Palakhachane, Athipornchai, Pimtong, & Limpachayaporn, 2018). The molecular weight of CBE is 218.68 g/mol, log P value 4.42 ± 0.01 , its chemical formula is $C_{13}H_{11}ClO$, and its chemical structure is presented in Figure 1. *In vitro* inhibition of α -MSH-induced melanin synthesis in mouse melanoma B-16 cells and toxicity tests revealed that CBE and kojic acid similarly inhibit melanin production; however, CBE was superior to kojic acid in terms of safety (Riam-Amatakun et al., 2019; Suttisintong et al., 2018). Unfortunately, despite its many beneficial effects, the main drawback of CBE is its poor water solubility and high lipophilicity.

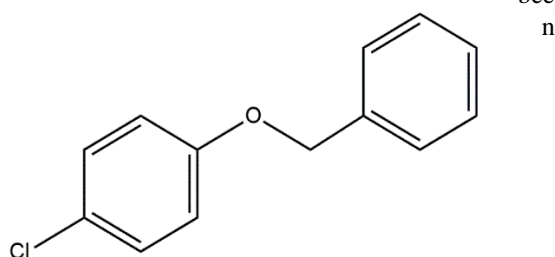


Figure 1 The chemical structure of *p*-chlorophenyl benzyl ether (CBE)

Emulsions are frequently used in pharmaceuticals and cosmetics. Emulsions are composed of liquids that do not typically blend, such as water and oil. These systems are

thermodynamically unstable and may undergo phase separation to reduce their free energy. Another emulsion system called microemulsion (ME) has been developed. MEs are mixtures of oil

and water stabilized by surfactants and co-surfactants. An ME is a translucent, homogeneous, and thermodynamically stable mixture with droplet sizes in the nanometer range. The advantages of MEs include stability, clarity, and ease of application and preparation (Jayne & Gareth, 2000; Agrawal, & Agrawal, 2012; Rao, Barot, Rajesh, & Jha, 2015). MEs may be an excellent solvent for drugs because they can solubilize hydrophobic and hydrophilic substances. Moreover, the penetration of drugs through the skin is enhanced by MEs (Kogan & Garti, 2006; Agrawal et al., 2012; Jayne & Gareth, 2000; Rao et al., 2015; Tsai, Lee, Huang, Huang, & Wu, 2010). MEs are the subject in this study. Because CBE is lipophilic, an ME could provide good incorporation efficiency because of its high oil content. Furthermore, the ME preparation process is simple and does not require special equipment, unlike other lipid-based delivery systems.

2. Objectives

This study aimed to formulate an ME to enhance the solubility and skin penetration of CBE.

3. Materials and methods

p-CBE was supplied by the Department of Chemistry, Faculty of Science, Silpakorn University, Thailand. Isopropyl myristate (IPM) and isopropyl palmitate (IPP) were obtained from Nikkol Chemical (Jurong Island, Singapore). Polysorbate 20 (Tween 20), soybean oil, lemon oil, and oleic acid were purchased from Sigma-Aldrich (MO, USA). Medium-chain triglyceride oil (MCT) was procured from Uniquema Asia Pacific (Kuala Lumpur, Malaysia). Vegetable and olive oils were purchased from a local market. All other chemicals were commercially obtained and used as received.

3.1 Solubility study

An excess quantity of CBE was added to tubes containing different oils, distilled water, or a surfactant/co-surfactant mixture (S_{mix}). The samples were shaken using an Intelli-mixer™ system at ambient temperature for 24 h and then centrifuged at 14000 rpm for 15 min. The supernatants were collected, filtered through a 0.45 μ m syringe filter to eliminate undissolved solids in the saturated solution, and then diluted with

isopropyl alcohol (IPA) prior to the determination of CBE content by HPLC. The solubility study was conducted in triplicate.

3.2 HPLC analysis

CBE contents were analyzed using an HPLC system (Agilent 1220, Agilent Technologies Inc., CA, USA) with a reversed-phase column (4.6 \times 150 mm, C18, 5 μ m). Elution was carried out using a mixture of acetonitrile and water (70:30) as the mobile phase flowing at a rate of 1.0 mL/min. The injection volume was 20 μ L, and the retention time of CBE was 7.6 min. Detection was performed using a UV detector at a wavelength of 254 nm. The quantity of CBE in the samples was estimated from the standard curve, which showed excellent linearity in the concentration range of 1–500 μ g/mL. The R^2 of the mean calibration plot was 0.9999.

3.3 Pseudo-ternary phase diagram construction

The water titration method was employed to determine the ME area from a pseudo-ternary phase diagram. The S_{mix} was composed of Tween 20 and ethanol at a weight ratio of 1:1. Lemon, which provided the highest solubility for CBE, was used as the oil phase and mixed with S_{mix} at various ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Water was added to each oily mixture until turbidity was observed. The amount of each component was recorded to construct a pseudo-ternary phase diagram. Clear or transparent regions were considered the ME (Rangsimawong et al., 2018).

3.4 Preparation of CBE-loaded ME

After the ME region was identified from the pseudo-ternary phase diagram, MEs consisting of three phases, namely, oil, water, and S_{mix} , were prepared at ambient temperature with magnetic stirring. Then, CBE (3wt%, 5wt%, and 10wt%) was added to these MEs. The ME formulation exhibiting a good appearance was selected, and its physicochemical properties, loading efficiency, and skin permeation were evaluated.

3.5 Determination of droplet size and surface charge

The droplet size, surface charge, and polydispersity index (PDI) of the ME were determined by dynamic light scattering experiments using a Zetasizer Nano series

(Malvern, UK) instrument. The measurements were conducted in triplicate.

3.6 pH measurement

The pH of the ME was measured at 25 °C by using a digital pH meter (LAQUAtwin pH-22, HORIBA, Japan). The pH meter was standardized prior to its use. Each sample was analyzed in triplicate.

3.7 Conductivity measurement

The conductivity of the ME was assessed at 25 °C by using a conductivity meter (ECTestr

11, Thermo Fisher Scientific Inc., USA). The conductivity obtained was used to indicate the type of ME produced.

3.8 Incorporation efficiency and CBE content

CBE was loaded into the ME at weight ratios of 3wt%, 5wt%, and 10wt%. The quantity of CBE incorporated in the ME was directly analyzed by HPLC after appropriate dilution with IPA. The analysis was performed in triplicate, and the incorporation efficiency of CBE in ME and CBE content were calculated using Eqs. 1 and 2, respectively.

$$\% \text{ Incorporation efficiency} = (W_m/W_i) \times 100 \quad (1)$$

Where W_m is the total amount of CBE in ME (mg) and W_i is the initial amount of CBE added to ME (mg)

$$\text{CBE content} = W_m(\text{mg})/M(\text{g}) \quad (2)$$

Where W_m is the total amount of CBE in ME and M is the weight of ME.

3.9 *In vitro* skin permeability

The skin from the abdominal region of a newborn, naturally dead pig was supplied by a farm in Nakornpathom Province, Thailand. The permeation test was conducted using Franz diffusion cells. The receptor chamber was filled with a mixture of phosphate buffer solution (pH 7.4) and IPA at a ratio of 1:1 and stirred with a magnetic stirrer. The temperature was maintained at 37 °C throughout the experiment. Porcine skin segments were affixed between the receptor and donor chamber. Exactly 500 μL of the CBE-loaded ME or a CBE suspension was

added to the donor chamber. Then, 700 μL of the receptor solution was withdrawn at different time points and analyzed for CBE content by HPLC. The receptor solution was immediately replenished with new medium to maintain the volume of the chamber. The cumulative amount (i.e., amount of drug permeated through the skin [$\mu\text{g}/\text{cm}^2$]) and transdermal flux (i.e., amount of drug permeated through the skin per unit time [$\mu\text{g}/\text{cm}^2/\text{h}$]) of CBE were calculated. The enhancement ratio (ER) was computed following Eq. 3 to investigate the effect of the permeation enhancer.

$$\text{ER} = \text{Flux of CBE-loaded ME} / \text{Flux of CBE in water} \quad (3)$$

3.10 Statistical analysis

The experiments were conducted in triplicate. The data of all experiments are reported as mean \pm standard deviation, and statistically significant differences were tested using one-way ANOVA at a significance level of $p < 0.05$.

4. Results and discussion

CBE shows promise as a skin-whitening agent. Unfortunately, CBE is poorly soluble in water. The solubility study was conducted to determine the appropriate oil phase for ME. The S_{mix} used in the ME formulations was co composed of Tween 20 and ethanol (1:1). The solubility of CBE in various oils, Tween 20, ethanol, and water are listed in Table 1. CBE is a hydrophobic

substance; thus, an ME system was applied to overcome its solubility limitation. Whereas the solubility of CBE in water was extremely low at only 0.0017 mg/mL, its solubility in lemon oil was 211.81 mg/mL. The highest solubility of CBE was achieved in lemon oil followed by vegetable oil, IPM, IPP, soybean oil, MCT, olive oil, and oleic acid. Thus, lemon oil was selected as the oil phase for the ME. Our results are consistent with those of Hashem, Shaker, Ghorab, Nasr, & Ismail (2011) who reported that lemon oil is the most applicable solubilizer for the hydrophobic drug clotrimazole (Hashem et al., 2011). Lemon oil is an essential oil composed of terpenoid components, among which *d*-limonene is the most abundant. Lemon oil has been reported to be a

safe and effective penetration enhancer for topical delivery (Valgimigli et al., 2012).

Table 1 Solubility of CBE in various solvents * $p < 0.05$

<i>Solvents</i>		<i>Solubility (mg/mL)</i>
<i>Oils</i>	Lemon oil	*211.812 ± 15.191
	Vegetable oil	114.931 ± 2.102
	Isopropyl myristate (IPM)	103.504 ± 2.241
	Isopropyl palmitate (IPP)	100.237 ± 2.540
	Soybean oil	90.076 ± 0.047
	Medium chain triglyceride oil (MCT)	68.472 ± 1.182
	Olive oil	60.211 ± 2.406
	Oleic acid	56.493 ± 2.443
	<i>Tween 20</i>	36.015 ± 0.003
	<i>Ethanol</i>	41.850 ± 0.082
<i>Tween 20 : Ethanol (1:1)</i>		87.510 ± 2.440
<i>Water</i>		0.002 ± 0.000

A pseudo-ternary phase diagram was constructed to determine the optimal concentration of each component in the ME. The translucent ME region is shown in the pseudo-ternary phase diagram in Figure 2. The colored area represents the transparent ME zone, whereas the clear areas indicate turbid zones. The findings are in accordance with previous studies

investigating the effects of surfactant type on the formation of MEs using lemon oil as the oil phase (Hashem et al., 2011; Rao & McClements, 2012; Valoppi, Frisina, & Calligaris, 2017).

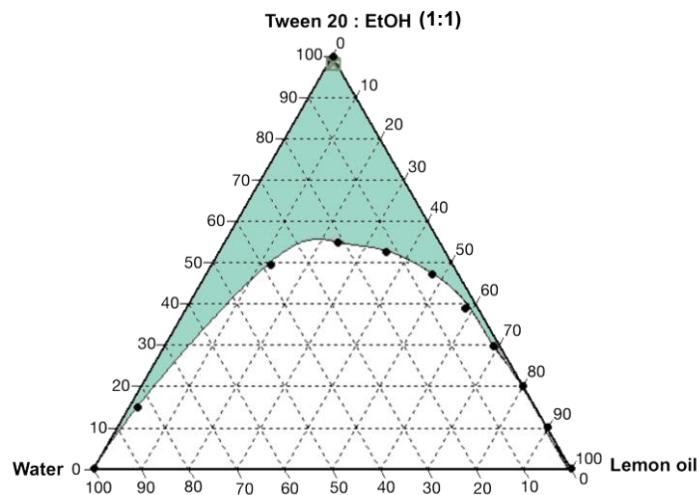
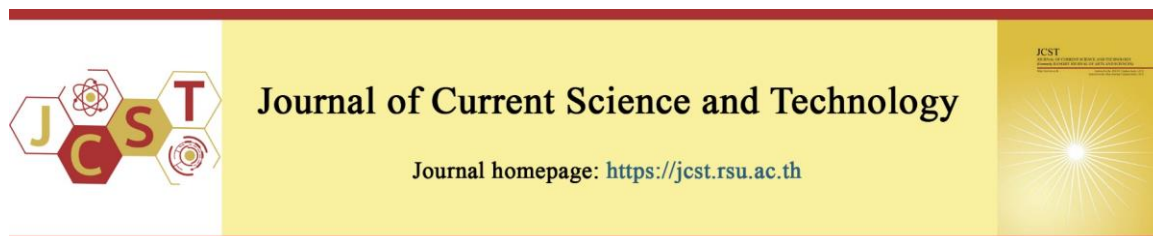


Figure 2 Pseudo-ternary phase diagram of lemon oil, Tween 20:ethanol (1:1, S_{mix}), and water

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Translucent areas in the phase diagram were randomly selected to obtain four formulations of ME (Table 2). The results showed that F2, which is composed of lemon oil, S_{mix} , and water at

a ratio of 20:70:10, has a good physical appearance and is neither greasy nor sticky. Therefore, this formulation was selected as the ME formulation for CBE loading.

Table 2 Compositions (% w/w) and physical appearance of the four ME formulations

Formulation	Oil phase (%)	S_{mix} (%)	Water phase (%)	Appearance
F1	10	80	10	sticky
F2	20	70	10	good
F3	30	60	10	greasy and sticky
F4	40	55	5	greasy and sticky

CBE (3wt%, 5wt%, and 10wt%) was loaded into the ME by dissolution in the oil phase followed by mixing with S_{mix} . Then, water was added to the formulation. After CBE was loaded into the ME, the physical appearance of the latter became clear/translucent. However, at higher amounts of CBE (10wt%), precipitation was observed after overnight storage at ambient conditions. The physicochemical parameters of the blank and 3wt%, 5wt%, and 10wt% CBE-loaded MEs are shown in Table 3. Mean particle size and surface charge are essential parameters affecting the physical stability of colloidal dispersion systems (Kogan & Garti, 2006). The particle sizes of the CBE-loaded ME formulations were in the range of 152–181 nm. Larger particles were obtained as the CBE content increased. The observed increase in droplet size could be due to increases in the amount of drug loading. Because CBE is lipophilic, the addition of large amounts of the compound may alter the oil phase ratio in the ME region and result in larger droplet sizes. This result agrees with the findings of a previous study (Subongkot & Ngawhirunpat, 2017). The PDI of all MEs was less than 0.3, which suggests a narrow size distribution of the droplets. The surface charge of the droplets was negative, and their zeta potentials were between -16.60 and -14.33 mV. All formulations had a pH in the range of 5–6. Electrical conductivity measurements are a helpful method to differentiate

the type of ME formed. In w/o MEs, the water forms separate droplets and is not present in the continuous phase. ME formulations with a conductivity of less than 10 $\mu\text{S}/\text{cm}$ are considered w/o MEs. By contrast, o/w MEs contain water in the continuous phase and cause measurable conductivity. Thus, o/w MEs provide a relatively higher conductivity compared with w/o MEs (approximately 10–100 $\mu\text{S}/\text{cm}$) (Mehta & Kaur, 2011; Ngawhirunpat, Worachun, Opanasopit, Rojanarata, & Panomsuk, 2013; Yotsawimonwat et al., 2006). The electrical conductivity of all MEs was greater than 10, thus suggesting that oil-in-water MEs had been formed. The properties of MEs are considered important factors in the development of cosmetic products. Because the stratum corneum is the major barrier against the absorption of molecules, the characteristics of an ME influence its skin permeation and the success of delivery to the target site. The droplet size in nano-range and large surface area/volume ratio help to increase the penetration. The type of ME is an important consideration for cosmetic development. Formulations providing a good skin feeling are highly desired. The pH of the product must also be suitable for the skin. The pH of skin products must be in the range of 5–7 because formulations that are excessively acidic or basic may lead to skin irritation.

Table 3 Characterization of CBE-loaded MEs containing various concentrations of CBE

Formulations	Droplet size (nm)	PDI	Zeta potential (mV)	pH	Conductivity ($\mu\text{S}/\text{cm}$)
Blank-MEs	127.90 \pm 0.96	0.23 \pm 0.01	-10.80 \pm 0.17	5.57 \pm 0.13	11.87 \pm 0.25

Initial CBE (%w/w)	% Incorporation efficiency	CBE content (mg/g)
3	31.12 \pm 0.33	31.12 \pm 0.33
5	57.49 \pm 0.10	57.49 \pm 0.10
10	74.85 \pm 0.40	83.17 \pm 0.44

3 %CBE-loaded MEs	152.40 \pm 3.32	0.25 \pm 0.02	-14.33 \pm 0.25	5.54 \pm 0.04	12.63 \pm 0.23
5%CBE-loaded MEs	168.60 \pm 1.36	0.25 \pm 0.01	-14.40 \pm 0.66	5.61 \pm 0.02	11.90 \pm 0.17
10%CBE-loaded MEs	181.00 \pm 0.70	0.22 \pm 0.01	-16.60 \pm 0.10	5.28 \pm 0.19	12.63 \pm 0.45

The initial amount of CBE was varied (3wt%, 5wt%, and 10wt%) and the %incorporation efficiency of the resulting ME formulations was determined. High drug loading and excellent physical characteristics of the ME are the primary objectives of this study. MEs loaded with 3wt% and 5wt% CBE were translucent and revealed %incorporation efficiencies of 100.61% \pm 1.09% and 109.24% \pm 0.19%, respectively. The results showed that the %incorporation efficiency of CBE decreased as the initial amount of CBE increased from 5wt% to 10wt%. Precipitates were noted when CBE was loaded to a greater extent (10wt%), and the %incorporation efficiency of this formulation decreased to 74.85% \pm 0.40%. The

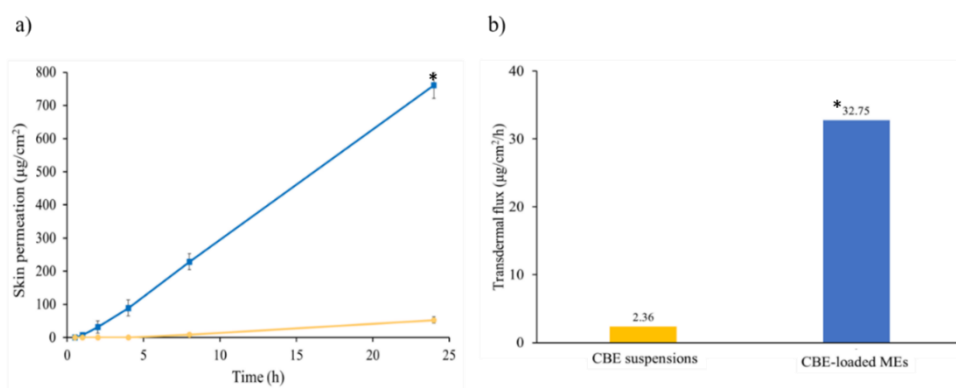
CBE contents in the 3wt%, 5wt%, and 10wt% CBE-loaded ME formulations were 31.12 \pm 0.33, 57.49 \pm 0.10, and 83.17 \pm 0.44 mg/g, respectively (Figure 3). These results show that the ME formulation of lemon oil, Tween 20 and ethanol (1:1), and water (20:70:10) can be loaded with CBE at initial concentrations of as high as 10wt%. However, given the formation of precipitates at this concentration, 5wt% CBE-loaded ME was selected and used for the skin permeation study. These results imply that large amounts of CBE could be solubilized in the ME formulation and that the loading capacity of CBE in ME could be improved.

Figure 3 Effect of initial CBE amount on the (open squares) CBE content and (filled circles) % incorporation efficiency of CBE incorporated ME formulations * $p < 0.05$ vs 3wt% CBE-loaded ME

An experiment on the permeation abilities of the 5wt% CBE-loaded ME and a 5wt% CBE aqueous suspension (free drug) through porcine abdominal skin was conducted using Franz diffusion cells. The results of the *in vitro* skin permeability assay are presented in Figure 4a. After 24 h, the skin permeation of the ME preparation was greater than that of the CBE

aqueous suspension. Transdermal flux was determined from the slope of the graph in Figure 4a. Although the result of the free drug suspension showed that the CBE molecule itself may be directly transported into the stratum corneum, the transdermal flux of the CBE-loaded ME was nearly 14-fold higher than that of the CBE suspension, as presented in Figure 4b.

Figure 4 (a) Skin permeation and (b) transdermal flux of the (■) 5wt% CBE-loaded ME and (■) 5wt% CBE aqueous suspension * $p < 0.05$ vs 5wt% CBE aqueous suspension



This result demonstrates the potential use of ME as an effective penetration enhancer for CBE delivery through the skin. The findings are consistent with those of previous studies showing that ME could improve the skin permeation of hydrophobic drugs, such as clotrimazole (Hashem et al., 2011), ketoprofen (Ngawhirunpat et al., 2013), capsaicin (Huang et al., 2008), and curcumin (Liu, Chang, & Hung, 2011). Many factors may affect the flux of the microemulsions. A penetration enhancer is one of the factors that

may have a considerable effect on the flux of the formulation. In this study, lemon oil, Tween 20, and ethanol functioned as skin penetration enhancers.

Taken together, the results of this work comprehensively reveal that a suitable ME formulation could enhance the skin permeation of a loaded compound. Besides the use of penetration enhancers, the small droplet size of the ME may also play an essential role in improving its skin permeability. The formulation contains lemon oil and a large amount of ethanol. However, although lemon oil and ethanol have been reported to be safe for topical delivery, some studies reveal that they can cause skin irritation and dermatitis

(Chandrashekar & Hiremath, 2008; Lachenmeier, 2008; Valgimigli et al., 2012). Further investigations on the effects of MEs on skin irritation must be conducted.

5. Conclusion

The new skin-whitening agent *p*-CBE has poor solubility in water and low skin permeation. In this work, a 5% CBE-loaded ME composed of lemon oil, Tween 20/ethanol, S_{mix} and water at the ratio of 20:70:10 was successfully formulated. The ME could increase the solubility and skin permeability of CBE. Thus, the ME formulation had the potential to be used as a transdermal delivery system of CBE.

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7. References

- Agrawal, O. P., & Agrawal, S. (2012). An overview of new drug delivery system: microemulsion. *Asian Journal of Pharmaceutical Science & Technology*, 2(1), 5-12.
- Baspinar, Y., & Borchert, H. H. (2012). Penetration and release studies of positively and negatively charged nanoemulsions--is there a benefit of the positive charge?. *International Journal of Pharmaceutics*, 430(1-2), 247-252. DOI: <http://www.doi.org/10.1016/j.ijpharm.2012.03.040>
- Chandrashekar, N. S., & Hiremath, S. R. (2008). In vivo immunomodulatory, cumulative skin irritation, sensitization and effect of d-limonene on permeation of 6-mercaptopurine through transdermal drug delivery. *Biological and Pharmaceutical Bulletin*, 31(4), 656-661. DOI: <http://www.doi.org/10.1248/bpb.31.656>
- Cichorek, M., Wachulska, M., & Stasiewicz, A. (2013). Heterogeneity of neural crest-derived melanocytes. *Open Life Sciences*, 8(4). DOI: <http://www.doi.org/10.2478/s11535-013-0141-1>
- Gillet, A., Compere, P., Lecomte, F., Hubert, P., Ducat, E., Evrard, B., & Piel, G. (2011). Liposome surface charge influence on skin penetration behaviour. *International Journal of Pharmaceutics*, 411(1-2), 223-231. DOI: <http://www.doi.org/10.1016/j.ijpharm.2011.03.049>
- Hashem, F. M., Shaker, D. S., Ghorab, M. K., Nasr, M., & Ismail, A. (2011). Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. *AAPS PharmSciTech*, 12(3), 879-886. DOI: <http://www.doi.org/10.1208/s12249-011-9653-7>
- Huang, Y. B., Lin, Y. H., Lu, T. M., Wang, R. J., Tsai, Y. H., & Wu, P. C. (2008). Transdermal delivery of capsaicin derivative-sodium nonivamide acetate using microemulsions as vehicles. *International Journal of Pharmaceutics*, 349(1-2), 206-211. DOI: <http://www.doi.org/10.1016/j.ijpharm.2007.07.022>
- Jayne, L., & Gareth, R. (2000). Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews*, 45, 89-121. DOI: [http://www.doi.org/10.1016/s0169-409x\(00\)00103-4](http://www.doi.org/10.1016/s0169-409x(00)00103-4)
- Kobayashi, T., Urabe, K., Winder, A., Jimenez-Cervantes, C., Imokawa, G., Brewington, T., . . . Hearing, V. J. (1994). Tyrosinase related protein 1 (TRP1) functions as a DHICA oxidase in melanin biosynthesis. *EMBO Journal*, 13(24), 5818-5825.
- Kogan, A., & Garti, N. (2006). Microemulsions as transdermal drug delivery vehicles. *Advances in Colloid and Interface Science*, 123-126, 369-385. DOI: <http://www.doi.org/10.1016/j.cis.2006.05.014>
- Lachenmeier, D. W. (2008). Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *Journal of Occupational Medicine and Toxicology*, 3, 26.

- Li, E. P. H, Min, H. J., Belk, R. W., Kimura, J., & Bahl, S. (2008). Skin lightening and beauty in four Asian cultures. *Advances in Consumer Research*, 35, 444-449.
- Liu, C. H., Chang, F. Y., & Hung, D. K. (2011). Terpene microemulsions for transdermal curcumin delivery: effects of terpenes and cosurfactants. *Colloids and Surfaces B :Biointerfaces*, 82(1), 63-70. DOI: <http://www.doi.org/10.1016/j.colsurfb.2010.08.018>
- Masum, M., Yamauchi, K., & Mitsunaga, T. (2019). Tyrosinase Inhibitors from Natural and Synthetic Sources as Skin-lightening Agents. *Reviews in Agricultural Science*, 7, 41-58. DOI: <https://doi.org/10.7831/ras.7.41>
- Mehta, S., & Kaur, G. (2011). Microemulsions: thermodynamic and dynamic properties. In M. Tadashi (Ed.), *Thermodynamics* (pp. 382-409): IntechOpen. DOI: <http://www.doi.org/10.5772/12954>
- Ngawhirunpat, T., Worachun, N., Opanasopit, P., Rojanarata, T., & Panomsuk, S. (2013). Cremophor RH40-PEG 400 microemulsions as transdermal drug delivery carrier for ketoprofen. *Pharmaceutical Development and Technology*, 18(4), 798-803. DOI: <http://www.doi.org/10.3109/10837450.2011.627871>
- Pandya, A. G., & Guevara, I. L. (2000). Disorders of hyperpigmentation. *Dermatologic Clinics*, 18(1), 91-98. DOI: [http://www.doi.org/10.1016/s0733-8635\(05\)70150-9](http://www.doi.org/10.1016/s0733-8635(05)70150-9)
- Pawelek, J. M., & Korner, A. M. (1982). The biosynthesis of mammalian melanin. *American Scientist*, 70(2), 136-145.
- Pillaiyar, T., Manickam, M., & Namasivayam, V. (2017). Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 32(1), 403-425. DOI: <http://www.doi.org/10.1080/14756366.2016.1256882>
- Rangsimawong, W., Wattanasri, P., Tonglairoum, P., Akkaramongkolporn, P., Rojanarata, T., Ngawhirunpat, T., & Opanasopit, P. (2018). Development of Microemulsions and Microemulgels for Enhancing Transdermal Delivery of Kaempferia parviflora Extract. *AAPS PharmSciTech*, 19(5), 2058-2067. DOI: <http://www.doi.org/10.1208/s12249-018-1003-6>
- Rao, J., & McClements, D. J. (2012). Lemon oil solubilization in mixed surfactant solutions: Rationalizing microemulsion & nanoemulsion formation. *Food Hydrocolloids*, 26(1), 268-276. DOI: <http://www.doi.org/10.1016/j.foodhyd.2011.06.002>
- Rao, S., Barot, T., Rajesh, K. S., & Jha, L. L. (2015). Formulation, optimization and evaluation of microemulsion based gel of Butenafine Hydrochloride for topical delivery by using simplex lattice mixture design. *Journal of Pharmaceutical Investigation*, 46(1), 1-12. DOI: <http://www.doi.org/10.1007/s40005-015-0207-y>
- Riam-Amatakun, W., Limpachayaporn, P., Pizon, J. R. L., Opanasopit, P., & Nuntharatanapon, N. (2019). Anti-Melanogenic Activity of p-Chlorophenyl Benzyl Ether in α -MSH-Induced Mouse Melanoma B16F10 Cells. *Key Engineering Materials*, 819, 118-123. DOI: <http://www.doi.org/10.4028/www.scientific.net/KEM.819.118>
- Solano, F., Briganti, S., Picardo, M., & Ghanem, G. (2006). Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. *Pigment Cell Research*, 19(6), 550-571. DOI: <http://www.doi.org/10.1111/j.1600-0749.2006.00334.x>
- Subongkot, T., & Ngawhirunpat, T. (2017). Development of a novel microemulsion for oral absorption enhancement of all-trans retinoic acid. *International Journal of Nanomedicine*, 12, 5585-5599. DOI: <http://www.doi.org/10.2147/IJN.S142503>
- Suttisintong, K., Palakhachane, S., Athipornchai, A., Pimtong, W., & Limpachayaporn, P. (2018). Synthesis and evaluation of anti-tyrosinase activity of phenyl benzyl ether derivatives: Effects of functional groups and their positions. *Science, Engineering and Health Studies*, 12,

- 111-123. DOI:
<http://www.doi.org/10.14456/sehs.2018.10>
- Tsai, Y. H., Lee, K. F., Huang, Y. B., Huang, C. T., & Wu, P. C. (2010). In vitro permeation and in vivo whitening effect of topical hesperetin microemulsion delivery system. *International Journal of Pharmaceutics*, 388(1-2), 257-262. DOI: <http://www.doi.org/10.1016/j.ijpharm.2009.12.051>
- Tsatmali, M., Ancans, J., & Thody, A. J. (2002). Melanocyte function and its control by melanocortin peptides. *Journal of Histochemistry and Cytochemistry*, 50(2), 125-133. DOI: <http://www.doi.org/10.1177/002215540205000201>
- Valgimigli, L., Gabbanini, S., Berlini, E., Lucchi, E., Beltramini, C., & Bertarelli, Y. L. (2012). Lemon (Citrus limon, Burm.f.) essential oil enhances the trans-epidermal release of lipid-(A, E) and water-(B6, C) soluble vitamins from topical emulsions in reconstructed human epidermis. *International Journal of Cosmetic Science*, 34(4), 347-356. DOI: <http://www.doi.org/10.1111/j.1468-2494.2012.00725.x>
- Valoppi, F., Frisina, R., & Calligaris, S. (2017). Fabrication of Transparent Lemon Oil Loaded Microemulsions by Phase Inversion Temperature (PIT) Method: Effect of Oil Phase Composition and Stability after Dilution. *Food Biophysics*, 12(2), 244-249. DOI: <http://www.doi.org/10.1007/s11483-017-9480-9>
- Yotsawimonwat, S., Okonoki, S., Krauel, K., Sirithunyalug, J., Sirithunyalug, B., & Rades, T. (2006). Characterisation of microemulsions containing orange oil with water and propylene glycol as hydrophilic components. *Pharmazie*, 61(11), 920-926.