

## Development of SEDDS/SMEDDS of Sweet Basil Oil and Fingerroot Oil for Using in Drinking Water for Chickens

Prapaporn Boonme<sup>1,2</sup> Varaporn Buraphacheep Junyaprasert<sup>3,4\*</sup>

### *Abstract*

This study aimed to develop self-emulsifying drug delivery systems or self-microemulsifying drug delivery systems (SEDDS/SMEDDS) to thoroughly mix sweet basil oil and fingerroot oil in drinking water for chickens in order to utilize for coccidiosis prevention and treatment. Effects of surfactant and cosolvent type on the formulations were investigated. The selected formulations were then investigated for physical properties. In addition, the chemical stability of the formulations was studied by Gas Chromatography-Mass Spectrophotometry (GC-MS) after kept in three different conditions, i.e., 5±3 °C (in a refrigerator), 30±2 °C/75±5%RH, and 45±2 °C/75±5%RH, for four months. It was found that both sweet basil oil and fingerroot oil could be mixed with Tween80 to obtain clear yellowish liquids which further possibly formed into emulsions when diluted with water at appropriate concentrations. Both volatile oils provided small microemulsion regions when mixed with Tween80 and water. CremophorRH40 used as a surfactant had superior in mixing both volatile oils in SEDDS/SMEDDS formulations than Tween80 while polyethylene glycol 400 (PEG400) and propylene glycol (PG) showed similar efficacy as cosolvents. Finally, the SEDDS/SMEDDS formulations composed of CremophorRH40, PEG400 or PG, and sweet basil oil or fingerroot oil were successfully developed for homogeneously mixing in drinking water for chickens. All selected samples were clear liquid having spherical particles in nanometer-range (12.3-52.4 nm). The results also indicated that all samples were chemically stable when stored in well-closed containers at low temperature.

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**Keywords:** coccidiosis, drinking water for chickens, fingerroot oil, SEDDS/SMEDDS, sweet basil oil

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<sup>1</sup>Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand

<sup>2</sup>Drug Delivery System Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand

<sup>3</sup>Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Rajathavee, Bangkok 10400, Thailand

<sup>4</sup>Center of Excellence in Innovative Drug Delivery and Nanomedicine, Faculty of Pharmacy, Mahidol University, Rajathavee, Bangkok 10400, Thailand

\*Correspondence: varaporn.jun@mahidol.ac.th

## Introduction

Coccidiosis, a disease in chickens caused by protozoan parasites of the genus *Eimeria*, affects high economic loss in poultry farming. Chickens are risk to be infected by several species of *Eimeria*, e.g., *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*. Different species result in the damage to different parts of the gut; however, all species can cause death in the infected chickens. Therefore, coccidiosis prevention is necessary. Two basic means of coccidiosis prevention are chemoprophylaxis (using anticoccidial drugs) and vaccination (De Gussem, 2009). However, these drugs and vaccines may be unsuitable for human consumption and may lead to environmental residue.

At present, utilization of natural substances in agriculture has been promoted due to the safety for consumers and environment. Basil (*Ocimum basilicum* L.) administered fresh in water or dry in feed was reported to provide benefit in management of coccidial infection in broiler chicks since volatile oils in basil could make the negative effect on oocyst, a microscopic egg of coccidian (Onwurah et al., 2011). Recently, fifteen plant extracts were evaluated for anticoccidial sporulation and it was reported that only oil extracts with steam distillation from fingerroot (*Boesenbergia pandurata* (Roxb) Schitr.) and sweet basil (*Ocimum basilicum* L.) could efficiently provide this activity (Jitviriyanon et al., 2012). Therefore, sweet basil oil and fingerroot oil are potential to use as supplement for coccidiosis prevention and treatment.

One of the convenient methods to use sweet basil oil and fingerroot oil as a feed additive in chickens is to add in drinking water; however, the problem is arise due to immiscibility of oils in water. Besides, substances with poorly water-soluble property generally give low oral bioavailability. Therefore, new formulations of self-emulsifying drug delivery systems (SEDDS) or self-microemulsifying drug delivery systems (SMEDDS) would overcome this drawback. These formulations are homogenous mixtures consisting oil phase and surfactant or a mixture of surfactant and cosolvent. After water dilution with mild agitation, SEDDS or SMEDDS become an oil-in-water (o/w) emulsion with a droplet size in a range of 100 to 300 nm or develop into an o/w microemulsion with a droplet size of smaller than 50 nm, respectively (Gursoy and Benita, 2004). Enhancement of oral bioavailability of poorly water-soluble actives loaded in SEDDS/SMEDDS has been intensively reported (Balakrishnan et al., 2009a; Balakrishnan et al., 2009b; Hong et al., 2006; Cui et al., 2009; Sermkaew et al., 2013; Singh et al., 2009). Furthermore, SEDDS/SMEDDS are easily produced by simply mixing.

The aim of this study was to develop SEDDS/SMEDDS of sweet basil oil and fingerroot oil for using in drinking water for chickens.

## Materials and Methods

**Materials:** Sweet basil oil and fingerroot oil were purchased from Thai-China Flowers and Fragrances Industry, Bangkok, Thailand. Polyoxyethylene (20) sorbitan monooleate (Tween80), polyoxyl

hydrogenated 40 castor oil (CremophorRH40), polyethylene glycol 400 (PEG400) and propylene glycol (PG) were purchased from local distributors in Thailand. All chemicals used were pharmaceutical or food grade and used without any modification. Distilled water was used throughout the experiment.

**Preparation of sweet basil oil and fingerroot oil in form of SEDDS/SMEDDS:** Initially, sweet basil oil or fingerroot oil, Tween80 and water at random ratios were mixed and observed for types of occurred association structures to determine the possibility of emulsion or microemulsion formation. In the second step, if emulsions/microemulsions could be observed in the first step, microemulsion regions in phase diagrams were constructed according to preparation of various samples containing different ratios of components. Briefly, oil, water and Tween80 at the determined accurate weight were added in clear-glass bottles and vigorously mixed by a vortex mixer for 1 min. The samples were kept at ambient temperature overnight to achieve their equilibrium. Afterwards, the samples were observed for their appearance and classified as emulsions or microemulsions when they were milky liquids or clear isotropic liquids, respectively. No attempt was done to classify other association structures. The data were finally plotted on the triangular graphs.

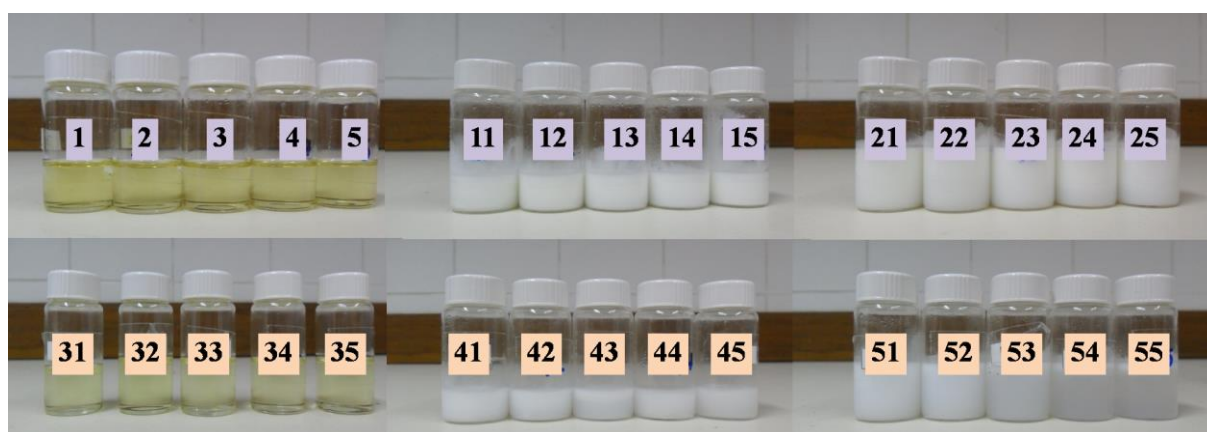
**Effect of surfactant and cosolvent type on preparation of SEDDS/SMEDDS of sweet basil oil and fingerroot oil:** Sweet basil oil or fingerroot oil, a surfactant and a cosolvent at serial ratios were mixed and observed for clarity. If the mixtures were clear, they were further diluted with water to evaluate the feasibility of emulsion or microemulsion formation. The studied surfactants were Tween80 and CremophorRH40 while the studied cosolvents were PEG400 and PG since they have been safe for oral administration. Moreover, these chemicals were reported to generally use in oral SEDDS/SMEDDS (Morozowich and Gao, 2009; Setthacheewakul et al., 2010; Sprunk et al., 2012; Yao and Li, 2011).

**Characterization of the samples:** The samples were optically observed for their appearance. The particle size (z-ave), polydispersity index (PI) and zeta potential of the selected samples were measured at 25°C by Zeta potential analyzer (model ZetaPALS, Brookhaven Instruments Corporation, USA) after diluted with appropriate amount of water before measurement to avoid multiple scattering. For measurement of z-ave and PI values, the real and the imaginary refractive indices were set at 1.590 and 0.000, respectively. The particle size analysis was determined by the Mie theory while the zeta potential analysis was calculated by the Helmholtz-Smoluchowsky equation. For observation of morphological shapes, each sample was diluted with an appropriate amount of distilled water by observing the turbidity of the diluted mixture. Afterwards, it was dropped on Formvar carbon film on 200 mesh copper grid and left at ambient temperature until dried. Finally, the dried sample on the grid was observed under transmission electron microscope or TEM (JEM-2010, JEOL, Japan).

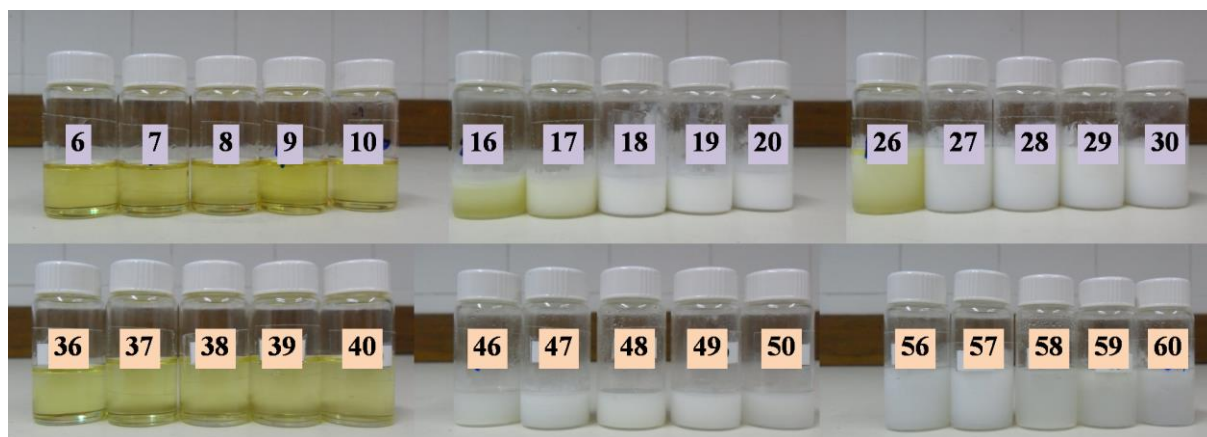
**Stability of the samples:** The selected samples were kept in three different conditions, i.e.,  $5\pm 3^{\circ}\text{C}$  (in a refrigerator),  $30\pm 2^{\circ}\text{C}/75\pm 5\%$  RH, and  $45\pm 2^{\circ}\text{C}/75\pm 5\%$  RH, for four months. They were studied for chemical stability every month by Gas Chromatography-Mass Spectrophotometer or GC-MS (GC-2010-Shimadzu, Japan) which equipped with Column capillary GC DB-5 MS 30 m x 0.25 mm x 0.25  $\mu\text{m}$  (Agilent, USA). The analysis method was previously validated before using. The samples were extracted with hexane. Briefly, 0.05 mg of each sample was mixed with 3 ml of hexane using vortex mixer for 5 min. The obtained mixture was centrifuged at 4000 rpm for 20 min and the supernatant was collected. Afterwards, amounts of sweet basil oil extracted in the supernatants (remained in the formulations) were analyzed using methyl chavicol as the marker. Those of fingerroot oil were analyzed using methyl cinnamate as the marker. The quantitative amounts of sweet basil oil and of fingerroot oil were calculated from the standard curve of peak areas of methyl chavicol and sweet basil oil concentrations and that of peak areas of methyl cinnamate and fingerroot oil concentrations, respectively.

## Results

**Preparation of SEDDS/SMEDDS of sweet basil oil and fingerroot oil:** Figure 1 shows feasibility in SEDDS/SMEDDS formation of sweet basil oil. After mixing sweet basil oil with Tween80 at the ratios of 5:5, 5.5:4.5, 6:4, 6.5:3.5 and 7:3 by weight (in Bottle Numbers 1-5, respectively), it was found that all samples were yellowish clear liquids. After water dilution at the ratios of 1:5 (in Bottle Numbers 11-15, respectively) and 1:10 (in Bottle Numbers 21-25, respectively), all mixtures of sweet basil oil and Tween80 provided milky emulsions. In an attempt to decrease amount of used surfactant and increase amount of diluted water, higher ratios of sweet basil oil and Tween80 at 7:3, 7.5:2.5, 8:2, 8.5:1.5 and 9:1 were formulated (in Bottle Numbers 31-35, respectively) and diluted with water at the ratios of 1:50 (in Bottle Numbers 41-45, respectively) and 1:100 (in Bottle Numbers 51-55, respectively). It could be seen that all 7:3 to 9:1 mixtures of sweet basil oil and Tween80 were yellowish clear liquids. After water dilution at the ratios of 1:50, all mixtures of sweet basil oil and Tween80 provided milky emulsions. Nevertheless, when dilution ratio was increased to 1:100, only 7:3 ratio of sweet basil oil and Tween80 mixture could result in milky emulsions while other ratios provided unstable dispersions (two-layer liquids).



**Figure 1** Appearance of mixtures of sweet basil oil and Tween80 at various ratios before and after water dilution at different ratios



**Figure 2** Appearance of mixtures of fingerroot oil and Tween80 at various ratios before and after water dilution at different ratios

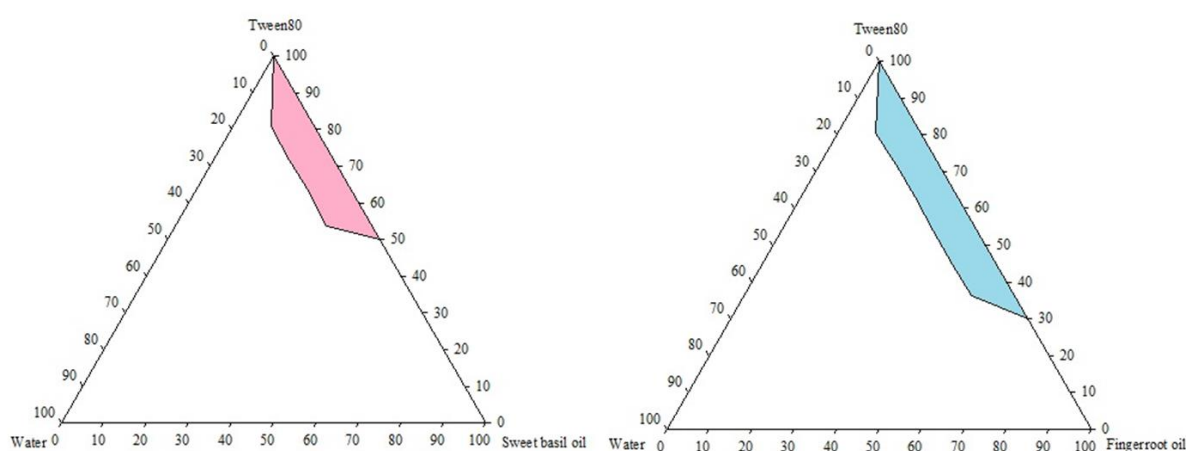
Figure 2 shows feasibility in SEDDS/SMEDDS formation of fingerroot oil. After mixing fingerroot oil with Tween80 at the ratios of 5:5, 5.5:4.5, 6:4, 6.5:3.5 and 7:3 by weight (in Bottle Numbers 6-10, respectively), it was found that all samples were yellowish clear liquids. After water dilution at the ratios of 1:5 (in Bottle Numbers 16-20, respectively) and 1:10 (in Bottle Numbers 26-30, respectively), a 5:5 mixture of fingerroot oil and Tween80 provided unstable dispersion while other ratios (lower surfactant concentrations) provided milky emulsions. Mixtures of fingerroot oil and Tween80 at ratios of 7:3, 7.5:2.5, 8:2, 8.5:1.5 and 9:1 (in Bottle Numbers 36-40, respectively) were yellowish clear liquids. After water dilution at the ratios of 1:50 (in Bottle Numbers 46-50, respectively) and 1:100 (in Bottle Numbers 56-60, respectively), they provided unstable dispersions (two-layer liquids).

Both sweet basil oil and fingerroot oil had small microemulsion regions in the phase diagrams when

directly mixing with water and Tween80 at various component ratios as shown in Figure 3.

#### *Effect of surfactant and cosolvent type on SEDDS/SMEDDS of sweet basil oil and fingerroot oil:*

In Table 1, it was found that CremophorRH40:PEG400 and CremophorRH40:PG at the ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 could provide clear liquids when mixed with either sweet basil oil or fingerroot oil. However, only high ratios of Tween80:PEG400 and Tween80:PG could provide clear liquids when mixed with each volatile oil. Additionally, these clear samples were still clear after 1000 folds of water dilution. Regarding the cosolvents, PEG and PG gave similar results when used as cosolvents in the systems. Moreover, the samples containing the lowest concentration of surfactant while having clear appearance were designated in Table 1 as A1 to A8 whose particle size and PI are shown in Table 2.



**Figure 3** Phase diagrams showing microemulsion regions (shaded areas) in systems of Tween80, water and sweet basil oil (left) or fingerroot oil (right)

**Table 1** Appearance of mixtures of sweet basil oil and fingerroot oil and various mixtures of surfactant (S) and cosolvent (C)

S:C	Sweet basil oil				Fingerroot oil			
	CremophorRH40		Tween80		CremophorRH40		Tween80	
	PEG400	PG	PEG400	PG	PEG400	PG	PEG400	PG
9:1	clear colorless	clear colorless	clear yellowish	clear yellowish	clear colorless	clear colorless	clear yellowish	clear yellowish
8:2	clear colorless	clear colorless	clear yellowish	clear yellowish	clear colorless	clear colorless	clear yellowish	clear yellowish
7:3	clear colorless	clear colorless	clear yellowish	clear yellowish	clear colorless	clear colorless	clear yellowish	clear yellowish
6:4	clear colorless	clear colorless	clear yellowish	clear yellowish	clear colorless	clear colorless	clear yellowish	clear yellowish
5:5	clear colorless	clear colorless	clear yellowish	clear yellowish	clear colorless	clear colorless	clear yellowish <sup>7</sup>	clear yellowish
4:6	clear colorless	clear colorless	clear yellowish <sup>3</sup>	clear yellowish <sup>4</sup>	clear colorless	clear colorless	hazy	clear yellowish <sup>8</sup>
3:7	clear colorless	clear colorless	hazy	hazy	clear colorless	clear colorless	hazy	hazy
2:8	clear colorless	clear colorless	hazy	hazy	clear colorless	clear colorless	hazy	hazy
1:9	clear colorless <sup>1</sup>	clear colorless <sup>2</sup>	hazy	hazy	clear colorless <sup>5</sup>	clear colorless <sup>6</sup>	hazy	hazy

Note: 1: A1, 2: A2, 3: A3, 4: A4, 5: A5, 6: A6, 7: A7, and 8: A8.

**Characteristics and stability of the selected SEDDS/SMEDDS of sweet basil oil and fingerroot oil:**

CremophorRH40 was selected as a surfactant, and either PEG400 or PG was used as a cosolvent for preparation of SEDDS/SMEDDS of each volatile oil which contained as high as possible concentration of the volatile oil, i.e. 18% v/v for sweet basil oil and 40% v/v for fingerroot oil. It was found that 5:1 CremophorRH40:PG or 5:1 CremophorRH40:PEG400 was suitable to prepare SEDDS/SMEDDS of sweet basil oil which could form o/w microemulsion after diluted in 300 folds of water to obtain an effective concentration of 0.6 µl/ml. It could be observed that 2:1 CremophorRH40:PG and 6:1 CremophorRH40:PEG400 were required to prepare

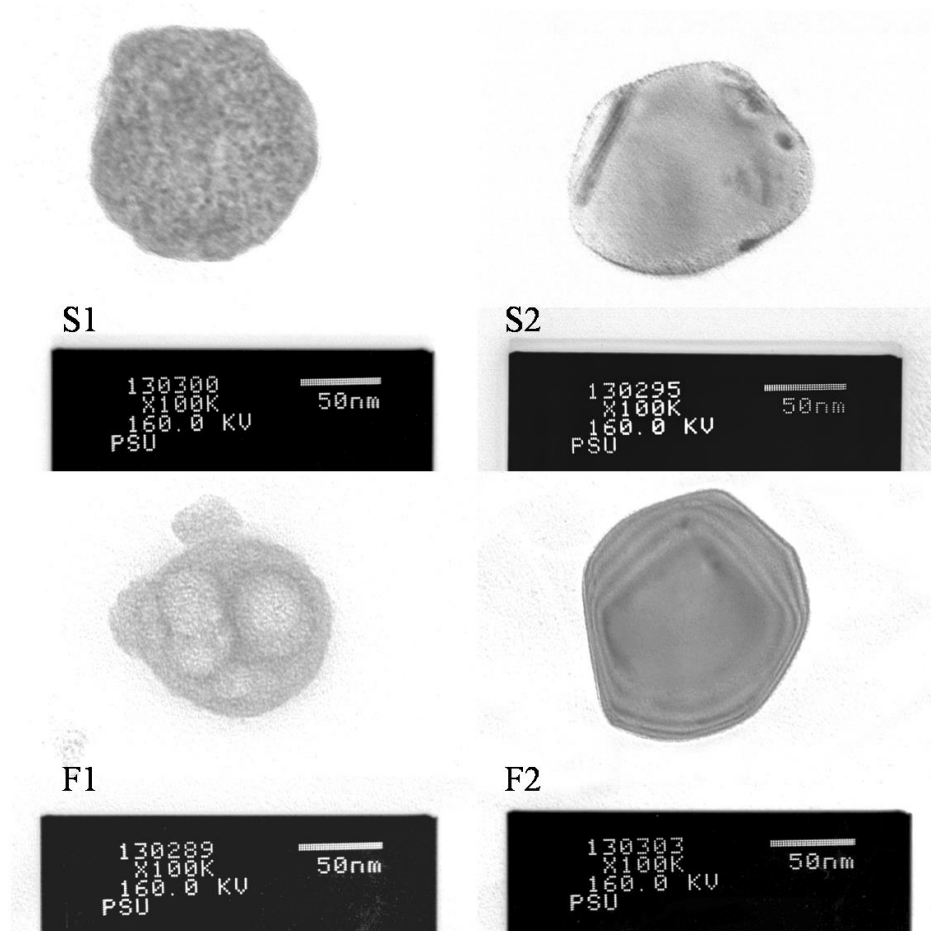
SEDDS/SMEDDS of fingerroot oil which could form o/w microemulsion after diluted in 1000 folds of water to reach an effective concentration of 0.4 µl/ml.

Two formulations of SEDDS/SMEDDS of sweet basil oil (S1, S2) and two formulations of SEDDS/SMEDDS of fingerroot oil (F1, F2) were further investigated for z-ave, PI and zeta potential as exhibited in Table 3. Additionally, their morphological shape observed under TEM was spherical as demonstrated in Figure 4.

From the validation results, the retention times of methyl chavicol in sweet basil oil and methyl cinnamate in fingerroot oil were 17.865 and 64.346 min, respectively, as shown in Figure 5. The peak areas at

**Table 2** Particle size and PI of selected mixtures of sweet basil oil and fingerroot oil and various mixtures of surfactant and cosolvent (see details for Formulation Numbers in Table 1)

Formulation Numbers	Particle size (nm)	PI
A1	52.4±1.3	0.357
A2	36.6±1.0	0.276
A3	13.1±0.1	0.147
A4	12.3±0.1	0.187
A5	42.4±0.6	0.313
A6	22.3±0.3	0.221
A7	13.8±0.1	0.202
A8	14.3±0.5	0.126



**Figure 4** Morphological shape under TEM of SEDDS/SMEDDS of sweet basil oil (S1-S2) and SEDDS/SMEDDS of fingerroot oil (F1-F2)

these retention times were used to assay the amounts of the studied volatile oils. Additionally, linearity ( $r^2 > 0.99$ ) was found in the standard curve of peak areas of methyl chavicol and sweet basil oil concentrations ( $y = 2011070.5846x + 1455918.8577$ ,  $r^2 = 0.9971$ ) and that of peak areas of methyl cinnamate and fingerroot oil concentrations ( $y = 54133.3133x - 79189.4667$ ,  $r^2 = 0.9955$ ). It was observed in Table 4 that all formulations were more stable when stored at  $5 \pm 3^\circ\text{C}$  than those at  $30 \pm 2^\circ\text{C}$  and  $45 \pm 2^\circ\text{C}$ , respectively.

### Discussion

**Preparation of SEDDS/SMEDDS of sweet basil oil and fingerroot oil:** It was found that that all studied ratios of mixtures of sweet basil oil and Tween80 were

yellowish clear liquids; however, the appearance after water dilution at various ratios was different depending to amounts of the surfactant in the system as shown in Figure 1. This phenomenon was also observed when fingerroot oil was used instead of sweet basil oil as exhibited in Figure 2. Although possibility of emulsion formation could be found, both sweet basil oil and fingerroot oil provided small microemulsion regions in the phase diagrams as shown in Figure 3 due to low microemulsification ability for volatile oils of Tween80. Thus, changing a surfactant type and/or adding a cosolvent might be able to increase the possibility of SEDDS/SMEDDS formation of both studied volatile oils.

**Table 3** Formulations and properties of SEDDS/SMEDDS of sweet basil oil (S1-S2) and SEDDS/SMEDDS of fingerroot oil (F1-F2)

	S1	S2	F1	F2
Sweet basil oil (% v/v)	18	18	-	-
Fingerroot oil (% v/v)	-	-	40	40
Surfactant:cosolvent mixture (% v/v)	to 100	to 100	to 100	to 100
Ratio of CremophorRH40:PG	5:1	-	2:1	-
Ratio of CremophorRH40:PEG400	-	5:1	-	6:1
Appearance of SEDDS/SMEDDS	clear colorless	clear colorless	clear colorless	clear colorless
Water dilution (folds)	300	300	1000	1000
Final concentration of volatile oil ( $\mu\text{l/ml}$ )	0.6	0.6	0.4	0.4
Appearance of diluted mixtures	clear colorless	clear colorless	clear colorless	clear colorless
Z-ave (nm)	$25.4 \pm 0.1$	$29.8 \pm 0.4$	$45.6 \pm 0.1$	$201.1 \pm 19.6$
PI	0.262	0.295	0.285	0.378
Zeta potential (mV)	$-3.91 \pm 0.16$	$-7.76 \pm 0.07$	$-8.42 \pm 0.05$	$-8.02 \pm 0.06$

**Table 4** Chemical stability of SEDDS/SMEDDS of sweet basil oil (S1-S2) and SEDDS/SMEDDS of fingerroot oil (F1-F2) when kept at various conditions for four months

Condition	Formulation	Active amount (mg/ml) [%labeled amount]				
		Initial	1 month	2 months	3 months	4 months
$5 \pm 3^\circ\text{C}$	S1	-	$20.70 \pm 0.62$ [111.18 $\pm$ 3.35]	$18.63 \pm 0.04$ [100.03 $\pm$ 0.24]	$12.17 \pm 0.22$ [65.36 $\pm$ 1.18]	$17.31 \pm 0.64$ [92.97 $\pm$ 3.45]
	S2	-	$22.05 \pm 0.43$ [106.65 $\pm$ 2.10]	$14.61 \pm 0.46$ [70.68 $\pm$ 2.24]	$11.44 \pm 0.04$ [55.32 $\pm$ 0.19]	$14.15 \pm 0.03$ [68.47 $\pm$ 0.13]
	F1	-	$35.79 \pm 0.95$ [88.73 $\pm$ 2.36]	$31.01 \pm 1.09$ [76.87 $\pm$ 2.71]	$24.34 \pm 0.37$ [60.33 $\pm$ 0.92]	$33.36 \pm 0.56$ [82.76 $\pm$ 1.38]
	F2	-	$40.67 \pm 1.39$ [100.27 $\pm$ 3.43]	$29.59 \pm 0.52$ [72.95 $\pm$ 1.27]	$23.32 \pm 0.52$ [57.51 $\pm$ 1.28]	$33.36 \pm 1.76$ [82.26 $\pm$ 4.34]
	S1	$18.62 \pm 0.30$ [100 $\pm$ 0.00]	$21.82 \pm 3.69$ [117.17 $\pm$ 19.87]	$19.29 \pm 0.86$ [103.59 $\pm$ 4.64]	$17.59 \pm 0.11$ [94.47 $\pm$ 0.57]	$17.07 \pm 0.42$ [91.66 $\pm$ 2.26]
	S2	$20.67 \pm 0.33$ [100 $\pm$ 0.00]	$20.72 \pm 0.08$ [100.25 $\pm$ 0.41]	$16.75 \pm 0.50$ [81.01 $\pm$ 2.42]	$16.96 \pm 0.48$ [82.07 $\pm$ 2.34]	$16.41 \pm 0.72$ [79.40 $\pm$ 3.48]
	F1	$40.34 \pm 2.71$ [100 $\pm$ 0.00]	$38.35 \pm 0.66$ [95.08 $\pm$ 1.65]	$35.22 \pm 1.57$ [87.31 $\pm$ 3.88]	$34.78 \pm 1.70$ [86.21 $\pm$ 4.22]	$32.34 \pm 0.82$ [80.17 $\pm$ 2.04]
	F2	$40.56 \pm 1.63$ [100 $\pm$ 0.00]	$37.66 \pm 1.28$ [92.86 $\pm$ 3.16]	$35.14 \pm 2.21$ [86.64 $\pm$ 5.44]	$32.86 \pm 0.96$ [81.02 $\pm$ 2.37]	$30.05 \pm 0.57$ [74.09 $\pm$ 1.40]
$30 \pm 2^\circ\text{C}$ $75 \pm 5\% \text{RH}$	S1	-	$21.15 \pm 0.68$ [113.58 $\pm$ 3.64]	$18.59 \pm 0.09$ [99.82 $\pm$ 0.49]	$16.23 \pm 0.28$ [87.16 $\pm$ 1.49]	$17.37 \pm 0.79$ [93.28 $\pm$ 4.27]
	S2	-	$21.79 \pm 0.18$ [105.41 $\pm$ 0.88]	$15.79 \pm 0.11$ [76.37 $\pm$ 0.54]	$15.88 \pm 0.67$ [76.82 $\pm$ 3.26]	$16.79 \pm 0.75$ [81.22 $\pm$ 3.65]
	F1	-	$38.35 \pm 0.66$ [95.08 $\pm$ 1.65]	$30.73 \pm 0.48$ [76.18 $\pm$ 1.20]	$26.60 \pm 0.22$ [65.95 $\pm$ 0.54]	$30.96 \pm 0.32$ [76.75 $\pm$ 0.80]
	F2	-	$36.10 \pm 4.31$ [89.01 $\pm$ 10.64]	$30.25 \pm 0.23$ [74.59 $\pm$ 0.56]	$24.71 \pm 0.34$ [60.92 $\pm$ 0.84]	$31.34 \pm 0.64$ [77.29 $\pm$ 1.57]
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	S2	$20.67 \pm 0.33$ [100 $\pm$ 0.00]	$20.72 \pm 0.08$ [100.25 $\pm$ 0.41]	$16.75 \pm 0.50$ [81.01 $\pm$ 2.42]	$16.96 \pm 0.48$ [82.07 $\pm$ 2.34]	$16.41 \pm 0.72$ [79.40 $\pm$ 3.48]
	F1	$40.34 \pm 2.71$ [100 $\pm$ 0.00]	$38.35 \pm 0.66$ [95.08 $\pm$ 1.65]	$35.22 \pm 1.57$ [87.31 $\pm$ 3.88]	$34.78 \pm 1.70$ [86.21 $\pm$ 4.22]	$32.34 \pm 0.82$ [80.17 $\pm$ 2.04]
	F2	$40.56 \pm 1.63$ [100 $\pm$ 0.00]	$37.66 \pm 1.28$ [92.86 $\pm$ 3.16]	$35.14 \pm 2.21$ [86.64 $\pm$ 5.44]	$32.86 \pm 0.96$ [81.02 $\pm$ 2.37]	$30.05 \pm 0.57$ [74.09 $\pm$ 1.40]



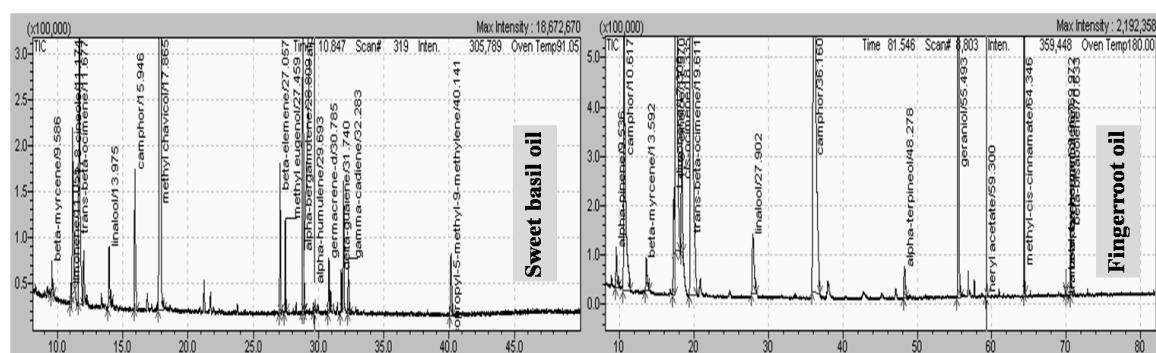


Figure 5 GC-MS chromatograms of sweet basil oil (left) and fingerroot oil (right)

#### Effect of surfactant and cosolvent type on SEDDS/SMEDDS of sweet basil oil and fingerroot oil:

In Table 1, it was seen that CremophorRH40 could microemulsify both sweet basil oil and fingerroot oil greater than Tween80 while PEG and PG could similarly act as cosolvents in the systems. In Table 1, it could be noted that all samples showed particle size in microemulsion range. Therefore, SMEDDS could be formed. CremophorRH40 provided bigger particle size than Tween80; however, the size remained in nanometer-range and the obtained products were clear, colorless and contained lower concentration of the surfactant. Thus, CremophorRH40 combined with PEG400 or PG was suitable for preparation of SEDDS/SMEDDS of both volatile oils. The concentrations of both volatile oils were also able to vary by adjusting the amount of water dilution.

#### Characteristics and stability of the selected SEDDS/SMEDDS of sweet basil oil and fingerroot oil:

It was found that the same ratios of CremophorRH40:PG and CremophorRH40:PEG400 at 5:1 were proper to prepare SEDDS/SMEDDS containing 18% v/v sweet basil oil which could form o/w microemulsion after diluted in 300 folds of water to obtain the effective concentration of 0.6  $\mu\text{l}/\text{ml}$ . However, the different ratios of CremophorRH40:PG and CremophorRH40:PEG400, i.e. 2:1 and 6:1, respectively, were required to prepare SEDDS/SMEDDS containing 40% v/v fingerroot oil which could form o/w microemulsion after diluted in 1000 folds of water to reach the effective concentration of 0.4  $\mu\text{l}/\text{ml}$ . The different suitable surfactant:cosolvent and water dilution ratios were due to different chemical components in both volatile oils (Jitviriyanon et al., 2012).

Table 3 and Figure 4 indicated that all selected formulations (S1, S2, F1, F2) could provide spherical microemulsion droplets with small size and narrow size distribution. Repulsive force between droplets could be observed from negative zeta potential. However, in this study, the aggregation of the droplets could be prevented by steric effects of nonionic surfactants, leading to physical stability.

In Figure 5, it was found that the GC-MS methods could provide good selectivity for methyl chavicol which was the marker of sweet basil oil and for methyl cinnamate which was the marker of fingerroot oil. Moreover, they could give linearity in both

standard curves of peak areas of the marker and volatile oil concentrations. Therefore, the GC-MS methods used in this study were suitable for quantitative analysis. Table 4 shows that all formulations were more stable when kept at low temperature than those at higher temperatures. This may be caused by volatilization of oils at higher temperatures, leading to lower %labeled amount. Besides, appearance of the samples stored at higher temperature became darker since the temperature could accelerate the oil degradation (Wongpoowarak et al., 2008). Hence the SEDDS/SMEDDS of both sweet basil oil and fingerroot oil should be stored in well-closed containers at low temperature.

In conclusion, among the studied formulations, mixtures of either sweet basil oil or fingerroot oil with CremophorRH40 and PEG400 or PG could be used as SEDDS/SMEDDS for diluting in drinking water for chickens. The particle size of the obtained products was in nanometer-range and they could be diluted with water without phase separation. Moreover, they possessed physicochemical stability when kept at low temperature. In further study, their effectiveness in the *in vivo* coccidiosis prevention and treatment in chickens will be investigated.

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## บทคัดย่อ

### การพัฒนาตำรับ SEDDS/SMEDDS ของน้ำมันโหระพาและน้ำมันกระชาย เพื่อเพิ่มการละลายในน้ำสำหรับให้อาหารไก่

ประภาพร บุญมี<sup>1,2</sup> วราภรณ์ บุรพาชีพ จรรยาประเสริฐ<sup>3,4\*</sup>

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาระบบนำส่งยาในรูปแบบเซล์ฟเอมิัลซิฟายด์หรือระบบนำส่งยาในรูปแบบเซล์ฟไมโครอิมัลซิฟายด์ (SEDDS/SMEDDS) เพื่อทำให้เกิดการผสมอย่างทั่วถึงของน้ำมันโหระพาและน้ำมันกระชายในน้ำดื่มสำหรับไก่ เพื่อใช้ในการป้องกันและการรักษาโรคบิดในไก่ โดยศึกษาผลของสารลดแรงตึงผิวของน้ำมันโหระพาและตัวทำละลายร่วมต่อสูตรตำรับ จากนั้นศึกษาสมบัติทางกายภาพของสูตรตำรับที่ได้รับการคัดเลือก ส่วนความคงตัวของสูตรตำรับ ศึกษาด้วยแก๊สโครมาโทกราฟีแมสสเปกโตรเมทรี (GC-MS) หลังจากเก็บกักได้ 3 สภาวะที่แตกต่าง คือ  $5 \pm 3$  เซลเซียส (ในตู้เย็น)  $30 \pm 2$  เซลเซียส/  $75 \pm 5\%$  ความชื้นสัมพัทธ์ และ  $45 \pm 2$  เซลเซียส/  $75 \pm 5\%$  นาน 4 เดือน การศึกษาพบว่าทั้งน้ำมันโหระพาและน้ำมันกระชายสามารถผสมกับทวิน 80 ได้ของเหลวสีเหลืองใส ซึ่งเป็นอิมัลชันเมื่อเจือจางด้วยน้ำในความเข้มข้นที่เหมาะสม น้ำมันหอมระเหยทั้งสองชนิดให้พื้นที่ไมโครอิมัลชันที่มีขนาดเล็ก เมื่อผสมกับทวิน 80 และน้ำ แต่เมื่อใช้ครีโมฟอร์อาร์เอช 40 เป็นสารลดแรงตึงผิวสามารถผสมน้ำมันหอมระเหยทั้งสองชนิดในตำรับ SEDDS/SMEDDS ได้สูงกว่าทวิน 80 ในขณะที่พอลิเอธิลีนไกลคอล 400 (PEG400) และโพรพิลีนไกลคอล (PG) มีประสิทธิภาพในการเป็นตัวทำละลายใกล้เคียงกัน ในที่สุด SEDDS/SMEDDS ของครีโมฟอร์อาร์เอช 40 และ PEG400 หรือ PG ที่บรรจุน้ำมันโหระพาหรือน้ำมันกระชายสามารถพัฒนาให้ได้เป็นส่วนผสมเนื้อเดียวกันเมื่อผสมในน้ำดื่มสำหรับไก่ ตัวอย่างที่ได้รับการคัดเลือกทั้งหมดเป็นของเหลวใส มีอนุภาคทรงกลมในระดับนาโนเมตร (12.3-52.4 นาโนเมตร) ผลการทดลองชี้ให้เห็นว่าตัวอย่างทั้งหมดมีความคงตัวทางเคมี เมื่อเก็บในภาชนะบรรจุที่ปิดสนิทที่อุณหภูมิห้อง

**คำสำคัญ:** โรคบิด น้ำดื่มสำหรับไก่ น้ำมันกระชาย SEDDS/SMEDDS น้ำมันโหระพา

<sup>1</sup>ภาควิชาเทคโนโลยีเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หาดใหญ่ จ. สงขลา 90112 ประเทศไทย

<sup>2</sup>สถานวิจัยความเป็นเลิศระบบนำส่งยา คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หาดใหญ่ จ.สงขลา 90112 ประเทศไทย

<sup>3</sup>ภาควิชาเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ราชเทวี กรุงเทพฯ 10400

<sup>4</sup>ศูนย์ความเป็นเลิศด้านนวัตกรรมนำส่งยาและเวชศาสตร์นาโน คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ราชเทวี กรุงเทพฯ 10400

\*ผู้รับผิดชอบบทความ E-mail: varaporn.jun@mahidol.ac.th