Optimization of Essential Oil Solubilization by Sophorolipids through High-Throughput Research

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Abstract

Background: Market trends are moving towards biosurfactants. For widespread adoption, these surfactants must match or improve upon the properties of surfactants derived from petrochemical sources. A key feature of many cosmetic formulations is solubilization of essential oils and fragrances. As such, the major thrust of our research was to identify optimal sophorolipid surfactant formulations for solubilization of essential oils. The performance of the sophorolipid surfactants was benchmarked against widely used ethoxylated, hydrogenated castor oil surfactants, which are currently ethoxylated using petrochemically derived ethylene oxide (EO).

Methods: This study describes the use of a high-throughput research (HTR) workflow to optimize essential oil solubilization by sophorolipid surfactants. Specifically, an automated liquid handling robot was used to prepare samples, and a custom imaging robot was used to characterize the phase behavior in the samples. The method allows preparation and analysis of over 1,000 samples per month.

Results: It was observed that sophorolipid surfactants were more efficient solubilizers of essential oils than a widely used ethoxylated, hydrogenated castor oil surfactant with an average degree of ethoxylation of 40 within the concentration ranges studied. The optimal concentration of lactone relative to the acid form of the sophorolipid surfactant was dependent on the essential oil.

Conclusion: Sophorolipid surfactants are highly efficient solubilizers of essential oils. Access to both acid form-rich and lactone form-rich sophorolipids surfactants will allow cosmetic formulators to develop tailored blends of the forms to optimize performance for a product formulation. Future studies will elucidate the impact of sophorolipid surfactant micelle microstructure on solubilization capacity.

Keywords: Sophorolipid, Surfactant, Essential Oil, Solubilization, Cosmetic

Introduction. A key consideration in the design of aqueous cosmetic formulations is solubilization of essential oils and fragrances [1, 2]. These mixtures of fragrant molecules provide an aesthetically pleasing experience to many consumers. For example, floral scents are often associated with cleanliness. When a water clear formulation is the desired product format, essential oils and fragrances need to be solubilized to prevent light scattering from emulsified droplets which can result in a hazy or turbid appearance. Solubilization also prevents phase separation of the oils from water, which is especially important in products that need to be sprayed to prevent uneven application of fragrant molecules.

The primary tool for solubilization of essential oils and fragrances is surfactants [1]. Much research exists on essential oil solubilization using petrochemical surfactants. Nonionic surfactants are particularity efficient at solubilizing essential oils and fragrances [1, 3-8]. For example, alkylphenol ethoxylate surfactants are highly efficient solubilizers [8]. However, alternatives to these materials are desired because they have the potential to be endocrine disruptors for aquatic life [9, 10]. Alkanol ethoxylate surfactants are widely used to solubilize essential oil and fragrances [3-7]. These materials have a better toxicology profile than alkylphenol ethoxylate surfactants.

Perhaps the most widely used surfactants for essential oil and fragrance solubilization are ethoxylated, hydrogenated vegetable oils. For example, ethoxylated, hydrogenated castor oil (**Figure 1A**) has been shown to be a highly efficient solubilizer [11-14]. Ethoxylated, hydrogenated vegetable oils are highly complex mixtures of surface-active materials. For example, 90% of the fatty acids that make up castor oil are ricinoleates [15]. The remaining

10% is a mixture of fatty acids containing oleic acid, linoleic acid, and other fatty acids. Ethoxylation of hydrogenated castor oil results in a mixture of surfactant structures with between one and three hydrophobic tails [15].

It is hypothesized that this complex mixture of surfactant species provides an array of micelle microstructures that enable solubilization of the complex mixture of molecules that make up essential oil and fragrances. Essential oils are composed of up to thirty different molecules of varying hydrophobicity [16]. One way of classifying the hydrophobicity of a fragrant molecule is the use the logarithm of the partition coefficient of the molecule between octanol and water ($LogP_{ow}$). It has been shown that the hydrophobicity of the fragrant molecule impacts where it partitions in surfactant micelles [17]. The most hydrophobic molecules will partition to the hydrophobic core of a micelle, whereas molecules of intermediate hydrophobicity will partition to the palisade layer (the layer just below the hydrophilic head group of the surfactant). The most hydrophilic molecules will associate with the hydrophilic head groups of a surfactant or are readily soluble in water. The partitioning of fragrant molecules to different areas of micelles will alter their microstructures and ability to solubilize the essential oil or fragrance. The complex nature of ethoxylated, hydrogenated vegetable oils may allow them to accommodate the microstructural changes better than other alkanol ethoxylates.

While ethoxylated, hydrogenated vegetable oils are widely used for essential oil and fragrance solubilization, the materials rely on petrochemically-derived ethylene oxide (EO) as the surfactant hydrophile. There is an industry push to develop processes for bioderived EO. However, few such surfactants are commercially available. Alternatively, several fermentation-based biosurfactants are coming to market that could be used for essential oil and fragrance solubilization [18]. As such, this study focuses on investigation of sophorolipid surfactants for essential oil solubilization. Most sophorolipids on the market are mixtures of the acid and lactonic form of the native sophorolipid (**Figure 1B**) [18]. The acid form exhibits a pH-dependent protonation state. The complexity of these surfactants has been shown to result in unique micellar microstructures that we hypothesized would lead to improved essential oil solubilization [19, 20]. Below, we present the results of a study that utilized a

high-throughput research (HTR) workflow to demonstrate that sophorolipids are promising alternatives to ethoxylated, hydrogenated vegetable oils for solubilization.

Materials and Methods.

Materials. Lavender oil, rosemary oil, citronella oil, and thyme oil were obtained from the Wellington Fragrance Company (Livonia, Michigan, United States). EcoSense™ SL-60 HA Surfactant and EcoSense™ SL-60 HL Surfactant were the sophorolipid surfactants explored, and were obtained from Dow (Midland, Michigan, United States). EcoSense™ SL-60 HA Surfactant is composed of ≥ 80 % acid form and ≤ 20 % lactonic form, while EcoSense™ SL-60 HL Surfactant is composed of ≤ 47 % acid form and ≥ 53% lactonic form. Kolliphor® RH 40 surfactant was used as the benchmark ethoxylated, hydrogenated castor oil surfactant, and was obtained from Sigma Aldrich (St. Louis, Missouri, United States). It contains average of 40 EO units in its structure and is referred to as polyethylene glycol-40 hydrogenated castor oil (PEG-40 HCO) below for brevity. Deionized water was used for all formulations. The pH values of solutions were adjusted using 1 normal (1 N) hydrochloric acid (HCl) or sodium hydroxide (NaOH) from Fisher Scientific (Waltham, Massachusetts, United States). Formulations were prepared in 7.5 milliliter (mL) Qorpak™ clear borosilicate sample vials with polytetrafluoroethylene (PTFE)-lined caps. The vials were also purchased from Fisher Scientific.

HTR Method for Characterization of Essential Oil Solubilization. The HTR methodology used to screen essential oil solubilization by sophorolipids is shown in **Figure 2**. Design of experiment (DOE) strategies were pursued using JMP® Pro software version 16.0.0 from SAS (Cary, North Caroline, United States). The designs were programed into Library Study software version 8.6 from Unchained Labs (Pleasanton, California, United States). Once the designs were complete, formulations were prepared using a Hamilton® Microlab STAR liquid handling system from Hamilton Company (Reno, Nevada, United States). The samples were prepared from deionized water, 25 weight percent (wt%) surfactant stock solutions, and essential oils. The order of addition was water, followed by surfactant stock, and, finally, essential oil. Prior to imaging, the samples were agitated for 30 minutes using an Eberbach model E6010 fixed-speed reciprocal shaker from Fisher Scientific. The samples were imaged

using a proprietary imaging robot called the Phase Identification and Characterization Apparatus (PICA). The robot illuminates the samples with an LED light source held at a 90 ° angle relative to a high-resolution camera. Custom image analysis software is used to measure the grayscale intensity in a region of interest (ROI) in a sample. The intensity scale can be correlated to Nephelometric Turbidity Units (NTUs). For this study, the essential oil was classified as solubilized when the grayscale intensity of a sample matched that of water. The experiments were conducted to determine the concentration of surfactant (wt%) required to solubilize a fixed concentration of essential oil (wt%). Overall, the HTR method allowed preparation and analysis of over 1,000 samples per month.

Results. Past research has shown that sophorolipid surfactants can adopt a wide array of micelle microstructures in water above their critical micelle concentration (cmc). The microstructures are highly dependent on the ratio of the lactone relative to the acid form of the sophorolipid surfactant. For the acid form of a sophorolipid, the microstructures are highly dependent on the protonation state of the carboxylic acid group [19]. At concentrations below 5 wt%, micelles of the acid form of a sophorolipid surfactant have been shown to undergo a transition from spheroidal to obloidal micelles as the carboxylic acid group begins to deprotonate. Above a threshold deprotonation level, the micelles transition to a tubule microstructure. At concentrations above 5 wt%, crowding of the micelles can trigger a spherical-to-rodlike transition. By contrast, past reports on ethoxylated, hydrogenated castor oil surfactants largely report formation of spheroidal micelles [21]. Based on past studies, we hypothesized that the complex microstructures accessible by sophorolipid surfactants (*e.g.*, rodlike and tubular micelles) would promote increased micellar volume for essential oil solubilization relative to ethoxylated, hydrogenated castor oil surfactants.

To test our hypothesis, we first measure the solubilization capacity of PEG-40 HCO surfactant micelles. This was done by leveraging our HTR workflow (**Figure 2**) to prepare aqueous formulations of PEG-40 HCO surfactant containing 0.5 wt% essential oil. This concentration was selected because it is commonly used in cosmetic formulations [1, 2]. **Figure 3** shows that 5 wt% PEG-40 HCO surfactant was required to solubilize 0.5 wt% rosemary oil or thyme oil, and 10 wt% surfactant was required to solubilize lavender oil.

Citronellal oil could not be solubilized at the highest surfactant concentration studied (*i.e.*, over 10 wt% surfactant was required).

With the performance of the PEG-40 HCO surfactant benchmarked, we next studied the solubilization capacities of a lactone form-rich sophorolipid surfactant (EcoSense™ SL-60 HL Surfactant; ≤ 47 % acid form and ≥ 53% lactonic form) and an acid form-rich sophorolipid surfactant (EcoSense™ SL-60 HA Surfactant; ≥ 80 % acid form and ≤ 20 % lactonic form). The pH values of the samples were adjusted to 7 to ensure a similar protonation state of the acid form of the sophorolipid across the samples. Assuming a pKa value similar to that of oleic acid (*i.e.*, pKa = 4.8) [19, 22, 23], the acid form of the sophorolipid is expected to be > 99% deprotonated at pH = 7. **Figure 4** reveals that the acid form-rich sophorolipid surfactant had a higher solubilization capacity than PEG-40 HCO surfactant for each of the essential oils studied at 0.5 wt% essential oil. The lactone form-rich sophorolipid surfactant was observed to have a higher solubilization capacity than the acid form-rich surfactant for citronellal oil.

To further understand the solubilization behavior of sophorolipid surfactants, we next studied the solubilization capacity of the surfactant micelles in the presence of 1.0 wt% essential oil. Again, the pH values of the samples were adjusted to 7. **Figure 5** shows that at the higher essential oil loading, the solubilization capacity of the lactone form-rich sophorolipid surfactant was higher than the capacities of both the acid form-rich sophorolipid surfactant and the PEG-40 HCO surfactant for all four essential oils studied. To highlight this effect, we re-plotted the data in **Figure 4** and **Figure 5** to show the mass ratio of surfactant to essential oil required to solubilize 0.5 wt% or 1.0 wt% essential oil in **Figure 6**. Except for citronella oil, both the acid form-rich sophorolipid surfactant and the PEG-40 HCO surfactant required a higher mass ratio of surfactant at 1.0 wt% essential oil compared to 0.5 wt%. The trend was the opposite for the lactone form-rich sophorolipid surfactant. A lower mass ratio of this surfactant was required for lavender, rosemary, and thyme oil.

Discussion. Overall, a higher essential oil solubilization capacity was observed for the sophorolipid surfactants than for the PEG-40 HCO surfactant. These observations were

consistent with our hypothesis that the unique surfactant micelle architectures accessible by the sophorolipids offer enhanced solubilization capacity relative to ethoxylated, hydrogenated castor oil alternatives that predominately adopt spherical microstructures at the aqueous concentrations studied. While the solution self-assembly of the acid form of the sophorolipids has been rigorously characterized [19, 20], the microstructures adopted by the lactone form and mixtures of the lactone and acid forms need further investigations.

The results presented above, suggest 5.0 to 15.0 wt% of the acid form-rich sophorolipid surfactant is required to solubilize 0.5 to 1.0 wt% essential oil. The surfactant micelles are expected to adopt obloidal or tubular micelles at these concentrations in the absence of oil. These micelle microstructures are anticipated to have a higher solubilization capacity than spheroidal micelles. This is consistent with the observation that the acid form-rich sophorolipid surfactant solubilized essential oils more efficiently than PEG-40 HCO. However, further investigation is required to understand impact of the essential oils on the microstructures of the surfactant micelles.

Similarly, further investigation is required to understand the aqueous self-assembly behavior of the lactone form-rich sophorolipid surfactant in both the absence and presence of sophorolipid. Here we speculate that the lactone form of the sophorolipid will promote the transition to a tubular microstructure at lower concentrations than the acid form. If this is indeed the case, we expect that the tubules will have a higher solubilization capacity than obloidal micelles. This could be the reason the lactone form-rich sophorolipid surfactant was a highly efficient solubilizer of 1.0 wt% essential oil.

Conclusion. In this study, an HTR workflow for characterizing essential oil solubilization by surfactants was developed and leveraged to demonstrate that sophorolipid surfactants are more efficient solubilizers than widely used ethoxylated, hydrogenated castor oil surfactants. Thus, sophorolipid surfactants represent a promising class of 100% bio-based, biodegradable alternatives to petrochemical surfactants for solubilization of essential oils and fragrances in cosmetic formulations. While more work is required to test the hypothesized origins of the improved solubilization capacity (*e.g.*, a high solubilization capacity by the tubular micelles

formed by sophorolipids), the results of this study can be readily applied to design of cosmetic products. For example, the results presented here suggest that access to both acid form-rich and lactone form-rich sophorolipids surfactants will allow cosmetic formulators to develop tailored blends of the forms to optimize solubilization of an essential oil or fragrance of interest.

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Conflict of Interest Statement. NONE.

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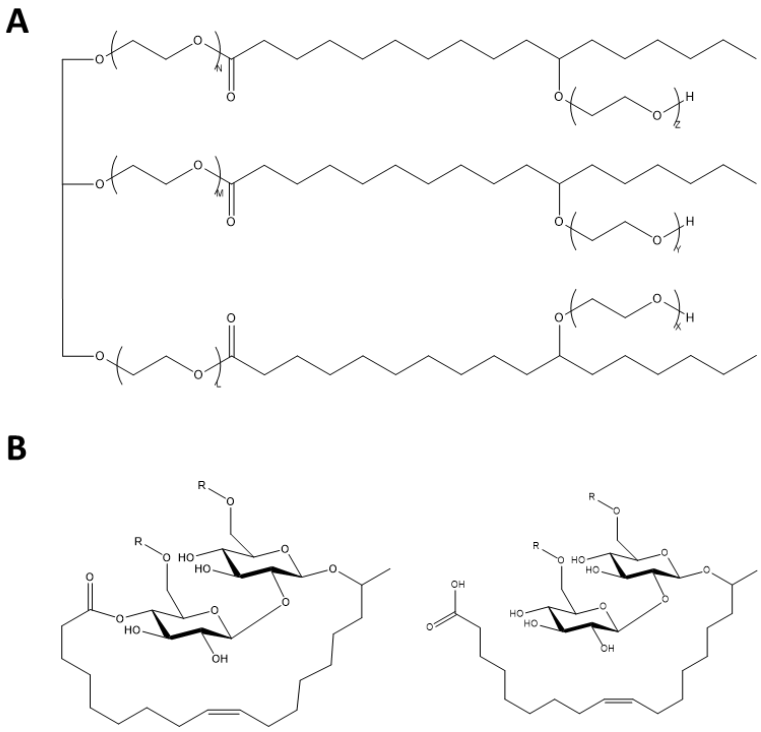

Figure 1. Idealized chemical structures of surfactants used in this study. (A) Idealized structure of ethoxylated, hydrogenated castor oil. L, M, N, X, Y, Z indicate the lengths of ethylene oxide segments. The actual structure will be a complex mixture of ethoxylated, hydrogenated triglycerides in which 90 % of the fatty acids are ricinoleates and ethoxylated free hydrogenated fatty acids. (B) Idealized structures of the lactonic (left) and acid (right) forms of sophorolipid surfactants. R can be a hydrogen atom or an acetyl group.

Figure 2. High-throughput research (HTR) workflow used to characterize essential oil solubilization by surfactants. Design of experiment strategies were used to plan samples. Automated liquid handling was used to dispense water, surfactants, and essential oils into glass vials. A custom sample imaging robot was used to collect images of samples. The images were analyzed to construct ternary phase diagrams which reveal the ratio of surfactant to essential oil required for solubilization.

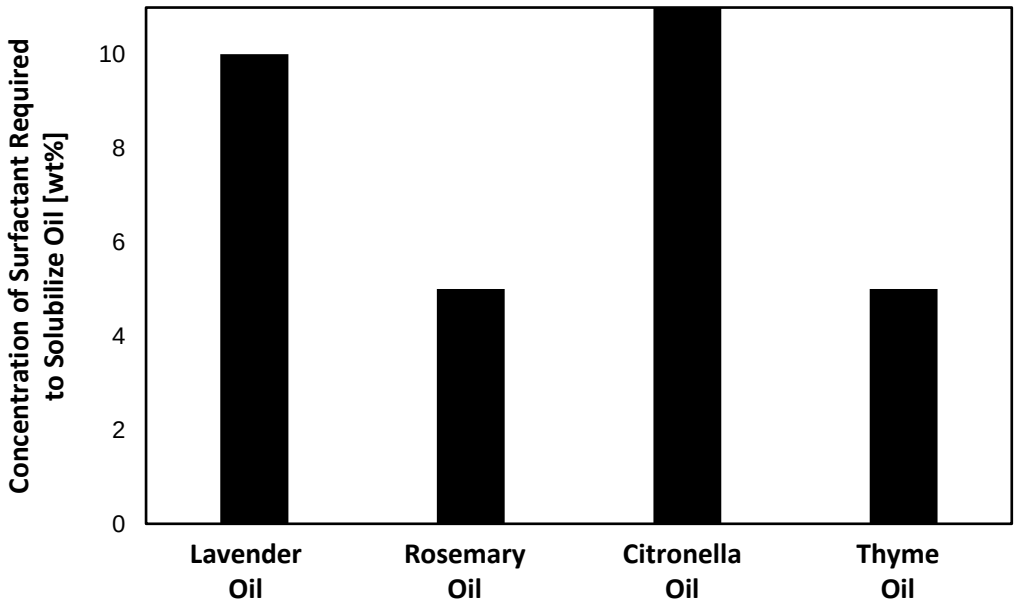

Figure 3. Concentration of PEG-40 HCO surfactant required to solubilize 0.5 wt% of the indicated essential oil in water. Citronella oil required > 10 wt% surfactant.

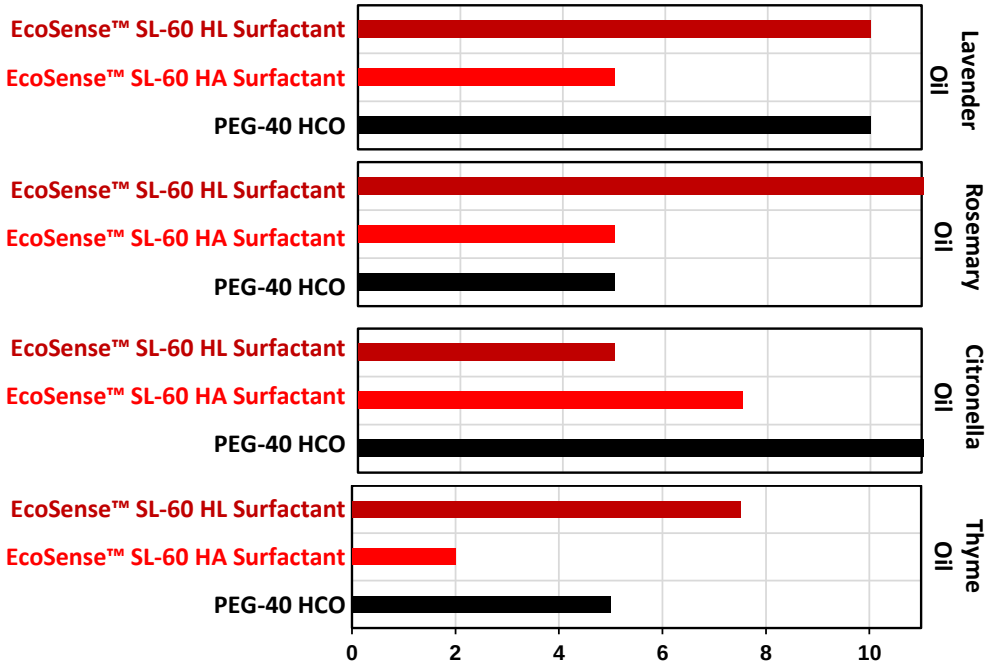

Figure 4. Concentration of EcoSense™ SL-60 HL Surfactant, EcoSense™ SL-60 HA Surfactant, or PEG-40 HCO surfactant required to solubilize 0.5 wt% of the indicated essential oil in water. Rosemary oil required > 10 wt% EcoSense™ SL-60 HL Surfactant and citronella oil required > 10 wt% PEG-40 HCO surfactant.

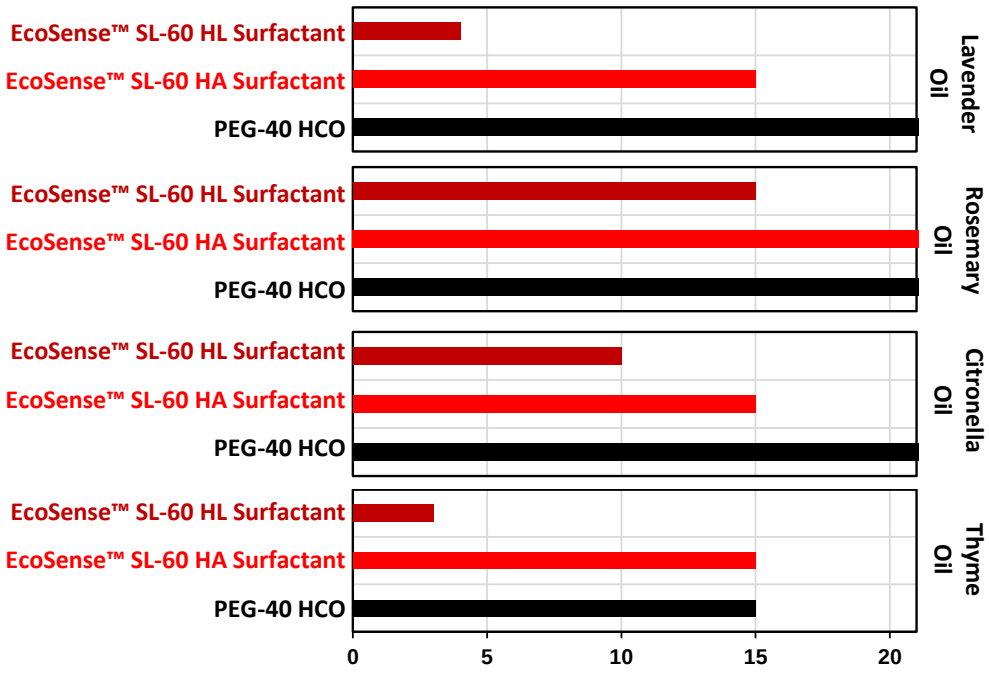

Figure 5. Concentration of EcoSense™ SL-60 HL Surfactant, EcoSense™ SL-60 HA Surfactant, or PEG-40 HCO surfactant required to solubilize 1.0 wt% of the indicated essential oil in water. Rosemary oil required > 20 wt% EcoSense™ SL-60 HA Surfactant. Lavender, rosemary, and citronella oil required > 20 wt% PEG-40 HCO surfactant.

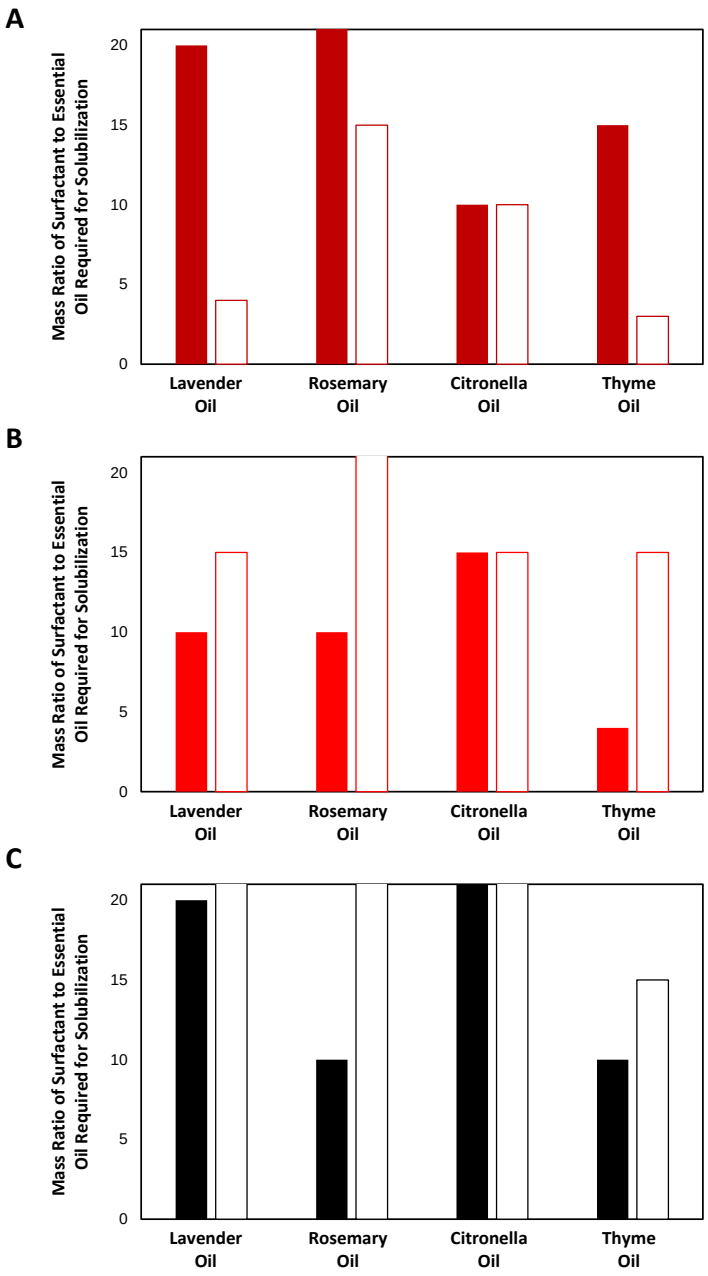

Figure 6. Mass ratio of surfactant to essential oil required to solubilize 0.5 wt% (filled bars) or 1.0 wt% (open bars) of the indicated essential oil. The surfactants were (A) EcoSense™ SL-60 HL Surfactant, (B) EcoSense™ SL-60 HA Surfactant, or (C) PEG-40 HCO surfactant.