Preparation of Tamanu Oil Nanoemulsions by Phase Inversion Temperature

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Abstract. In this study, the phase inversion temperature (PIT) method was used to prepare a stable tamanu oil nanoemulsion at the lowest surfactant content. The factors affecting the formation of nanoemulsions such as type of surfactant, surfactant to oil ratio (SOR), and water content were investigated. The results showed that Tween 80 is suitable for making a stable nanoemulsion. The SOR is more than or equal to 1.5:1 produced emulsions with particle size less than 100 nm. The particle size decreased as SOR, and water content increased. A higher quantity of surfactant caused higher turbulence and affinity towards the aqueous phase, resulting in smaller droplets. Decreasing droplet size by increasing water content may result from a decreased viscosity of the surfactant at the interface that promotes fluidity and the easy movement of the oil phase towards the aqueous phase. The SOR of 3:1 and water content of 80% were selected due to cost, taste, and toxicity concerns. The impact of storage condition on nanoemulsion stability was also investigated. After one month, samples were stored in the refrigerator had an insignificant increase in absorbance. Particle size analysis also showed a similar result (< 20 nm).

1. Introduction
Nowadays, the requirement to use products derived from natural is gaining much attention. Calophyllum inophyllum is grown throughout the tropical regions of the world, including Vietnam. Tamanu oil products are widely used in diesel energy to produce biodiesel, pharmaceutical products, and cosmetics [1,2]. In short, tamanu oil can be used in many fields thanks to its excellent biological properties.

Xanthones and coumarins in tamanu oil showed antioxidant activity and inhibition of lipid peroxidation [3]. Tamanu oil contains several compounds (4-phenylcoumarins) that are valuable as potential cancer chemotherapeutic agents [4]. The tamanu oil is also known to be rich in phospholipids and unsaponifiable compounds. They are characterized by the antioxidant, anti-aging, anti-inflammatory, and therapeutic activity. It is used as an emollient in cosmetic [5]. Tamanu oil also contains many compounds with these biological activities [6]. It is still employed in traditional medicine to treat cuts, scratches, burns, insect bites, acne, scars, psoriasis, ulcerative diabetes, anal fissures, dry skin, blisters, herpes, eczema, reduce foot and body odor [7]. The main disadvantage of using tamanu oil is hydrophobicity. Therefore, it is difficult to apply in water-based cosmetics and pharmaceuticals.

Oil-in-water nanoemulsions are the colloidal systems suitable for encapsulating essential oil [8]. They consist of small oil droplets dispersed in water by an emulsifier. In contrast to the traditional emulsions, the particles in nanoemulsion have an average diameter of less than 200 nm. The small size has some advantages for commercial applications: stability in gravity and droplet agglomeration, high transparency, and increased biological activity [8,9]. In a recent study, cinnamon oil nanoemulsions have an antimicrobial activity tested on E.coli bacteria [10]. The other research on sage essential oil showed that the sage nanoemulsion has significantly different antimicrobial activities against E.coli,
Shigella dysentery, Salmonella typhi [11]. In Brazil, nanoemulsions formed by vegetable oil were investigated and showed these formulations have some advantages for the systematic distribution of cosmetics and pharmaceuticals [12].

In common, nanoemulsion can be formed by both low and high-energy methods [8]. The high-energy method uses specially designed machinery that creates powerful forces that break up and blend the oil and aqueous phases [13]. The typical automatic homogenizers for nanoemulsion formation are high-pressure homogenizers, sonicators, and microfluidizers [14,15]. The low energy method is based on the spontaneous formation of microscopic oil particles in an oil-surfactant-water mixture when the system's constituent or medium changes in a particular technique [15]. The low energy method's principal benefits are easy performance and no need for any specially designed machinery [16,17]. The different low energy methods include phase inversion temperature, spontaneous emulsification, and emulsion inversion point [14]. Each method has individual strengths and weaknesses and should be optimized to create nanoemulsion with suitable stability, physicochemical characteristics, and functional efficiency [17].

Previous studies have demonstrated that nanoemulsion can be created by employing high energy [18] and low energy methods [16,19,20]. This research aimed to use the PIT method because of nanoemulsion formation with small stable droplets [19,21]. Recent research showed that oregano nanoemulsion was created by heating the mixture of oregano oil, sunflower oil, nonionic surfactant, and water. These nanoemulsions had vigorous antibacterial activity on *S.aureus* and *E.coli* in the flesh [19]. Similar results were obtained for orange [9] and cinnamon essential oil [8].

The basis of the PIT method is variation in nonionic surfactant's hydration as the temperature varies. The surfactant's hydrophilic head is significantly hydrated at low temperatures but gradually dehydrated at high temperatures. This variation in hydration changes the surfactant's water-solubility and surfactant monolayer's optimal curvity [22]. The PIT method is easy to carry out and no need for complicated machinery in industrial applications [8,21]. Oil, surfactant, and water are blended at room temperature, heated to PIT later, and finally fast cooled. This process dependably creates nanoemulsion consisting of microscopic particle size and concentrated particle size distribution [23]. The PIT of almost nanoemulsions was found that is below 90°C [8,19,21].

This study's objective was to make a stable tamanu oil nanoemulsion at minimum surfactant content due to expense, flavor, and virulence. These results can be used for applications in the cosmetics or pharmaceutical industry.

2. Materials and methods

2.1. Materials

Deodorized tamanu oil was offered by Dong May Man joint-stock company. The nonionic surfactants (Tween 20, 40, 80) with a purity of 99.5% were purchased from Bach Khoa limited liability company. Twice distilled water was used in all experiments.

2.2. Methods

2.2.1. Preparation of tamanu oil nanoemulsions. Tamanu oil nanoemulsions were prepared using Rao and McClements’ method with some minor adjustments [21]. Initially, tamanu oil and nonionic surfactants (oil phase) were mixed at room temperature for 15 minutes. At the same time, distilled water (aqueous phase) was heated to 40°C. The oil phase was then dripped into the aqueous phase with a constant stirring speed of 700 rpm. All components were mixed for 1.5 hours and heated up to the PIT (62-70°C) to create an emulsion system. Then the system was rapidly cooled down to 5°C to form nanoemulsions.

2.2.2. Determination of PIT. PIT is the temperature at which the system has minimum absorbance (i.e., the absorbance reduced significantly during heating) [13,21]. The absorbance measurement was performed at 600 nm using a UV-Vis spectrophotometer with distilled water as a blank [8,19,21]. The nanoemulsions were kept at a specific temperature for 10 minutes before measurement.
2.2.3. Particle size measurement. Particle size analysis was performed at the Institute of Applied Materials Science by dynamic light scattering (DLS) using the SZ-100 nanoparticle series instruments (HORIBA Ltd., Japan). The particle size is proportional to the absorbance, so it can be based on the absorbance at first to select samples for DLS measurement.

2.2.4. Determination of nanoemulsion stability. For testing the stability of nanoemulsions, we centrifuged the samples at 4000 rpm in 1 hour. In another test, nanoemulsions were put in the ultrasonic tank in 2 hours at 30°C [24]. Then, sampling at the center of nanoemulsions to measure the absorbance at 600 nm. The nanoemulsion stability was also tested by measuring particle size change after 30 days of storage at 15°C.

3. Results and discussion

3.1. The impact of surfactant type on nanoemulsion formation
The effect of nonionic surfactant type on the generation of tamanu oil nanoemulsion was studied. Tamanu oil nanoemulsions were made up using various surfactants (Tween 20, 40, 80) with the method described in 2.2.1. Figure 1 displayed the impact of surfactant types on the turbidity of nanoemulsion. We observed that tamanu oil nanoemulsion was formed by using Tween 40 and 80. Besides, nanoemulsions created by Tween 80 has better transparent when compare with Tween 40. In previous studies, the hydrophilic-lipophilic balance (HLB) of the surfactants has an essential role in forming the transparent systems and small droplets [25,26]. It was shown that both surfactants with high (Tween 20, 16.7) and low HLB numbers were impossible to create nanoemulsions. On the other hand, the surfactants with medium HLB numbers (Tween 40, 15.6, and Tween 80, 15) could form nanoemulsions with small droplet sizes [26,27]. However, tamanu oil nanoemulsions created with Tween 40 had a layer separation after stored in the fridge for three days. The previous studies showed that the surfactant with an HLB value from 12 to 16 is generally considered an oil in water emulsifier [27]. Although Tween 40 and 80 have HLB numbers approximately 15, Tween 80 has the nonpolar unsaturated structure. The surfactant structure considerably affects the encapsulation at the oil-water interface, thereby affecting the particle creation. The unsaturated nonionic surfactants have been published to be more appropriate to create nanoemulsions with smaller particle sizes [25–27]. From the above results, we selected Tween 80 for further experiments.

![Figure 1. The impact of surfactant types on nanoemulsion formation.](image)

3.2. The impact of surfactant to oil ratio (SOR) on nanoemulsion formation
Tamanu oil nanoemulsions were prepared using different SORs (1:1; 1.25:1; 1.5:1; 1.75:1; 2:1; 3:1; 4:1) while keeping the oil phase content (20% v/v). Figure 2 shows that SORs above 1.5:1 can form the tamanu oil nanoemulsions. The mechanism of forming nanoemulsions by using the PIT method was described in previous studies [8,23]. The monolayer of the surfactant has a nearly identical optimal curvity at the PIT. The surface tension is comparably small, and the surfactant has an akin affection for both water and oil phases. The result is a microemulsion with a cramped colloidal structure, and low
light scattering was created. Below PIT, the optimal curvity aids in forming the oil particles. The surface tension rises, and the surfactant has a bigger affection for water phase. The result is microscopic oil particles diffused in water were created. Another study showed that the main factor for nanoemulsion creation is chaos at the interface between the oil and water phase. A greater surfactant volume generates greater chaos and affection to water phase. As a result, the smaller particles will be created [28].

Figure 3 shows that all tamanu oil nanoemulsions with SORs above 1.5:1 had average droplet sizes below 100 nm. The higher the SOR, the smaller the particle size. These nanoemulsions systems were also stable when centrifuged at 4000 rpm for 1 hour and ultrasound at 30°C for 1 hour. However, after one month of storage in the fridge, tamanu oil nanoemulsions with SOR of 1.5:1 had been separated. So we chose SORs above 1.5:1 for the next experiments.

Figure 2. The impact of SOR on nanoemulsion formation.

Figure 3. The average droplet diameter of tamanu oil nanoemulsion at different SORs (a) 1:5, (b) 2:1, (c) 3:1, and (d) 4:1.
3.3. The impact of water phase content
Tamanu oil nanoemulsions were prepared using SORs above 2:1 with water phase content of 80, 85, and 90% (v/v). Figure 4 shows that the absorbance decreased as water phase content increased, and thus the particle size also reduced. The results are similar for all SORs. Decreasing particle size by rising water phase content may be due to decreased surfactant viscosity at the interface, promoting easy flow and straightforward motion of the oil phase to water phase [28]. At the same water phase content, larger surfactant content assisted the creation of the continuous nanoemulsion. The nanoemulsion created at the PIT is clearer at larger surfactant content [8].

In the survey conducted by the Personal Care Products Council in 2014, the maximum concentration of Tween 80 in cosmetics is 18.1%. If SOR is 3:1, the maximum concentration of tamanu oil approximately will be 6%. That leads to the maximum concentration of the oil phase is 24%, or the minimum aqueous phase content is 76%. Therefore, nanoemulsions with water phase content above 76% can be applied in cosmetics, as long as the mean particle size and storage time are satisfied.

3.4. Storage stability
The stability of nanoemulsion was tested by centrifugation and ultrasonic methods. Figure 5a shows that the samples had a slight increase in absorbance at all SORs. It proved that the samples are stable under centrifugal force and ultrasonic waves.

There was a considerable increase in absorbance at room temperature after one month of storage (Figure 5b). This shows that the nanoemulsion was unsteady for particle development at room temperature. This result may be due to the rate of particle conglomerate in nanoemulsion rises at temperatures below PIT. Increased particle conglomerate happens in this range because of partial dehydration of hydrophilic heads, making the surfactant monolayer's optimal curvity close to unity. The result is the surface tension turned into nearly small, and the monolayer turned into very flexible, promoting the combining of oil particles as colliding. So, the samples should be stored at low temperatures.

However, after one-month storage in the fridge, the sample with SOR of 2:1 had a significant increase in particle size than the rest (Figure 6). It could be due to the surfactant content was insufficient to prevent droplet coalescence. Therefore, SOR of 3:1 and water content of 80% were selected due to cost, taste, and toxicity concerns.
Figure 5. The absorbance of nanoemulsions under (a) the influence of external forces and (b) the different storage conditions.

Figure 6. Mean particle diameter of tamanu oil nanoemulsions after one-month storage in the fridge with SOR of (a) 4:1, (b) 3:1, and (c) 2:1.

4. Conclusion
In this research, the PIT method was employed to prepare a stable nanoemulsion at the lowest surfactant content. This method has never been applied before to make tamanu oil nanoemulsion. Factors such as the type of surfactant, SOR, and water phase content influenced nanoemulsion's formation and stability. Among surfactants, Tween 80 was the most suitable for forming stable nanoemulsions and small droplets. The higher the SOR, the lower the absorbance and particle size. The increase in water phase content also leads to similar results. The SORs above 1.5:1 could make nanoemulsions whose droplet size is less than 100 nm.

The storage condition also affected the stability of nanoemulsions. The samples should be stored at a low temperature instead of room temperature. However, the sample with SOR of 2:1 was unstable after one-month storage in the fridge. The particle size analysis indicated a significant increase. Therefore, the SOR of 3:1 and water content of 80% were chosen due to stability, cost, taste, and toxicity in cosmetics.

Acknowledgment
This research was funded by The Student Scientific Research Program of Nguyen Tat Thanh University (Project no. TPMT 2019.14).

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