ORIGINAL RESEARCH

The Impact of Surfactant Structures and High-Speed Mixing Dynamics in Achieving Nano-Sized Emulsions with Simple High-Speed Homogenization

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Purpose: This study investigates the impact of various mixing parameters and surfactant combinations on the physical characteristics of nanoemulsions produced using high-speed homogenization. Nanoemulsions are explored for their capacity to enhance transdermal drug delivery in pharmaceutical and cosmetic contexts.

Methods: Employing a standard high-speed homogenizer typical in the cosmetic industry, we tested different combinations of Polysorbate (Tween®) and Sorbitan ester (Span®) surfactants under single and intermittent process configurations. Key parameters assessed included particle size, size distribution, Oswald ripening, and creaming index.

Results: Nanoemulsions synthesized had particle sizes below 200 nm and appeared as white liquids with slight creaming. Extended mixing times were associated with smaller droplet sizes and reduced creaming. Both processing methods yielded similar nanoemulsion properties, indicating minimal impact from the type of process used. The nanoemulsions exhibited polydispersity indices ranging from 0.3 to 0.5, suggesting a uniform size distribution, and showed negligible Oswald ripening, which indicates stable droplet sizes over time.

Conclusion: The study confirms that precise control of mixing parameters and the selection of appropriate surfactant pairs are more critical than the choice of process in producing stable nanoemulsions. With efficient use of standard high-speed homogenizers, stable nanoemulsions suitable for large-scale production can be achieved, providing a cost-effective method for pharmaceutical and cosmetic industries.

Keywords: surfactant combinations, nanoemulsion stability, Ostwald ripening, creaming index

Introduction

Nanoemulsions have gained considerable attention as advanced drug delivery systems due to their ability to enhance the transdermal permeation of active ingredients.[1] These colloidal dispersions, typically characterized by droplet sizes below 500 nm,[2] offer significant advantages in pharmaceutical and cosmetic applications, appearing in various forms such as liquids, creams, sprays, gels, aerosols, and foams.[3] The preparation of nanoemulsions traditionally relies on high-energy methods,[4] requiring specialized equipment like microfluidizers, high-pressure homogenizers, or ultrasonicators. While these methods are effective in producing nanoemulsions with desirable properties, they come with notable drawbacks. The high cost of equipment and the technical complexity of these processes present significant challenges, particularly for large-scale manufacturing. Furthermore, the scalability of these methods for industrial production is limited, making it difficult to meet the demands of mass production efficiently and economically.

Graphical Abstract
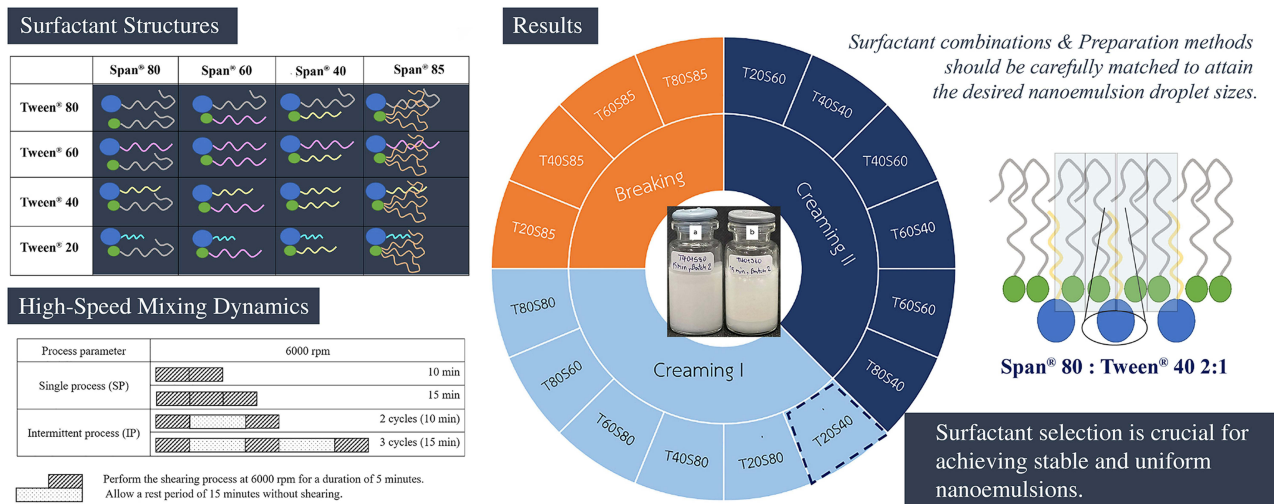
In contrast, low-energy methods such as phase inversion temperature, spontaneous emulsification, membrane emulsification, and emulsion inversion point methods, although less demanding in terms of equipment, are intricate and less commonly employed. Among these methods, homogenization stands out in the cosmetic and food processing industries for its ability to blend immiscible liquids into a unified phase, thereby reducing creaming and coalescence. Utilizing a high-speed homogenizer, a widely accessible tool in the cosmetics industry, this study aims to investigate the effects of process parameters and surfactant pairings on the formulation of nanoemulsions.

As reported in our earlier work,[5] optimizing the hydrophilic-lipophilic balance (HLB) in nanoemulsions is critical for enhancing stability. In this study, we extend that work by examining the impact of surfactant structures and high-speed mixing dynamics using simpler high-speed homogenizers. This research focuses on optimizing nanoemulsion formulations with various combinations of Polysorbate (Tween®) and Sorbitan ester (Span®) surfactants. By comparing single and intermittent process configurations, key parameters such as Ostwald ripening, creaming index, particle size, and distribution were assessed. While our previous research[5] employed a microfluidizer to optimize surfactant balance, this study explores a more accessible and cost-effective high-speed mixer to achieve similar nano-sized emulsions.

The objective is to develop a scalable, cost-effective method for producing stable nanoemulsions with desirable physical properties using conventional high-speed homogenizers. The findings are expected to offer valuable insights into high-speed homogenization for broader applications in the pharmaceutical and cosmetic industries.

Material and Methods
Materials
Soybean oil was purchased from Tip® Vegetable Oil Co. Ltd. (Bangkok, Thailand). Polysorbate 80 (polyoxyethylene sorbitan monooleate) and Polysorbate 20 (polyoxyethylene sorbitan monolaurate) was purchased from Sino-Japan Chemical Co. Ltd. (Taipei, Taiwan). Polysorbate 60 (polyoxyethylene sorbitan monostearate) was purchased from Oleo and Specialty Chemicals Co. Ltd. (Bangkok, Thailand). Polysorbate 40 (polyoxyethylene sorbitan monopalmitate) was purchased from Shanghai Zhanyun Chemical Co. Ltd. (Shanghai, China). Sorbitan monooleate was purchased from Guangdong Runhua Chemistry Co. Ltd. (Guangzhou, China). Sorbitan monostearate, Sorbitan monopalmitate, and Sorbitan trioleate were purchased from Chanjao Longevity Co. Ltd. (Bangkok, Thailand). Ultrapure water was used in this study.

Table 1 Studied Surfactant Mixtures to Achieve an HLB of 8

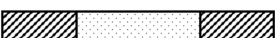| Types of Surfactants	Sorbitan Monooleate (HLB 4.3)		Sorbitan Monostearate (HLB 4.7)		Sorbitan Monopalmitate (HLB 6.7)		Sorbitan Trioleate (HLB 1.8)	
Polysorbate 80 (HLB 15.0)		4.91%		5.10%		6.33%		3.98%
	2.59%		2.40%		1.17%		3.52%	
Polysorbate 60 (HLB 14.9)		4.88%		5.07%		6.31%		3.95%
	2.62%		2.43%		1.19%		3.55%	
Polysorbate 40 (HLB 15.6)		5.04%		5.23%		6.40%		4.13%
	2.64%		2.27%		1.10%		3.37%	
Polysorbate 20 (HLB 16.7)		5.26%		5.44%		6.52%		4.38%
	2.24%		2.06%		0.98%		3.12%	

Abbreviation: HLB, hydrophilic and lipophilic balance.

Preparation of Nanoemulsions

The preparation of nanoemulsions was guided by the theoretical framework of hydrophilic-lipophilic balance (HLB) values, which significantly influence the interfacial properties and stability of oil-surfactant systems.[6] While this study employed high-speed homogenization as the primary method, microfluidizers are frequently utilized in nanoemulsion production due to their ability to generate fine droplets through high-pressure forces, as highlighted by Mahdi Jafari, He and Bhandari[7] and our previous work.[5]

The oil phase consists of 10% w/w soybean oil and 7.5% w/w nonionic surfactant (Polysorbate and Sorbitan ester) mixture at HLB 8, required HLB for soybean oil[8] or HLB of 8 (Table 1). Nanoemulsions containing Sorbitan monopalmitate or Sorbitan monostearate were prepared using a hot process given their solid form. As a result, it was heated to a temperature range of 65–70 °C to facilitate melting. The remaining formulations were prepared using a cold process or without heat.

The nanoemulsions were prepared using a high-speed homogenizer (Homogenizing Mixer Mark II 2.5, PRIMIX, Japan), selected for its accessibility, scalability, and cost-effectiveness. In the preparation process, the nanoemulsion is homogenized in a beaker with a diameter of 60 mm and a height of 88 mm, and the homogenizer head height was set the same across all preparations so that all nanoemulsions would have the same shear area. The process parameters, including process type and total shearing time, were studied at a constant RCF 2012.4 g (or 6000 rpm, 5 cm - rotor). The parameter details are presented in Figure 1. This method was chosen for its widespread availability in both laboratory and industrial settings, allowing for easy scalability while maintaining efficiency and simplicity. The resulting nanoemulsions were left overnight at ambient temperature, protected from light, to reach equilibrium before being

| Process parameter		6000 rpm
Single process (SP)		10 min
		15 min
Intermittent process (IP)		2 cycles (10 min)
		3 cycles (15 min)

Notes:

Perform the shearing process at 6000 rpm for a duration of 5 minutes.

Allow a rest period of 15 minutes without shearing.

Figure 1 Studied process parameters.

characterized for droplet size, polydispersity index (PDI), and stability which are key parameters for evaluating stabilization mechanisms.[9] These findings demonstrate that high-speed homogenizers can produce nanoemulsions of quality comparable to those achieved with advanced techniques.

Determination of Size and Size Distribution

The mean droplet sizes and size distribution (polydispersity index; PDI) were determined using dynamic light scattering (Zetasizer Nano ZS™ instrument, Malvern Instruments, Malvern WR14 1XZ, UK) in triplicate. The nanoemulsions were diluted 1:100 with ultrapure water before the measurement. The intensity-based average diameter (Z-average diameter) was obtained by a correlation function provided by the software.

Physical Stability Studies

Centrifugation Test

All nanoemulsion formulations were centrifuged at Radius Centrifuge Force (RCF) 1369.55 g (or 3500 rpm, 10 cm - rotor) and 25 °C for 30 minutes to observe phase separation by using a high speed refrigerated micro centrifuge (Tomy MX - 300, Tokyo, Japan). Only the formulations with no cracking were further evaluated.

Oswald Ripening Study

The nanoemulsions were stored at room temperature, and on a weekly basis, samples were collected to measure droplet size and PDI for a duration of 21 days. Before taking measurements, the samples were properly redispersed. The Oswald ripening rates (ω) were determined by analyzing the slope of the relationship between the cube of the emulsion radius (r^3_N) and time (t) as referred from Equation 1.[10]

$$\omega = dr^3_N/dt \tag{1}$$

Creaming Index Measurement

Each nanoemulsion sample was meticulously transferred into a glass test tube with an internal diameter of 15 mm and a height of 150 mm. The test tubes were then securely sealed and stored at room temperature. During the storage period, careful monitoring of each nanoemulsion sample was conducted to detect any signs of separation, particularly the formation of an opaque cream layer at the top. To quantify the extent of gravitational separation, measurements were taken of both the total height of the emulsions (H_t) and the height of the cream layer (H_c). The degree of gravitational separation was evaluated using the creaming index (CI) as defined by Equation 2, with a lower creaming index indicating higher emulsion stability.[11]

$$\% \text{ CI} = H_c/H_t \times 100 \tag{2}$$

Statistical Analysis

Each experiment was conducted in triplicate, and the results are presented as the mean ± standard deviation. To assess the differences among the studied variables, including mean values of size and size distribution, creaming index, and Oswald ripening rate, a one-way analysis of variance (ANOVA) was employed, followed by Tukey's test for post hoc analysis using IBM SPSS Statistics for Windows, Version 28.0. (IBM Corp SPSS software, Armonk, NY, USA). Statistical significance was determined when the p-value was less than 0.05.

Results

Preparation of Nanoemulsions

In their freshly prepared state, all emulsions exhibited an opaque white appearance. The emulsions were composed of different pairs of Polysorbates (Polysorbate 80, Polysorbate 60, Polysorbate 40 and Polysorbate 20) and Sorbitan esters (Sorbitan monooleate and Sorbitan trioleate) surfactants, which were allowed to reach equilibrium over a 24-hour period. During this time, a slight creaming effect was observed on the top surface of the emulsions. In contrast, when Sorbitan monostearate or Sorbitan monopalmitate were combined with Polysorbates and prepared using a hot process, a high level of foam was produced during mixing. Some air bubbles persisted until the end of the 24-hour equilibrium period,

resulting in semi-solid creams. The exception was the emulsion containing Sorbitan monostearate and Polysorbate 80, which exhibited a slightly creamy layer like the emulsions with Polysorbates (Polysorbate 80, Polysorbate 60, Polysorbate 40 and Polysorbate 20) and Sorbitan esters (Sorbitan monooleate and Sorbitan trioleate) surfactants.

Nanoemulsions were successfully formulated using a high-speed homogenizer with varying process designs and parameters. Following preparation and overnight equilibrium, the nanoemulsions appeared as white liquids with minimal creaming. Although, this slight creaming effect indicated the oil droplets' tendency to rise and accumulate at the top layer of the liquid,[12] this effect was minor, resulting in relatively high stability of the nanoemulsions. Agitation or shaking could easily redisperse the nanoemulsions, uniformly redistributing the oil droplets within the aqueous phase. Based on these observations, all nanoemulsion formulations, despite the slight creaming, were selected for further investigation due to their promising characteristics and suitability for subsequent studies or applications.

The acceptance criteria for the nanoemulsions in this study included maintaining a droplet size below 200 nm during the storage period and ensuring no occurrence of emulsion breaking. Before characterization, the prepared emulsion samples underwent screening through centrifugation to categorize them based on the results of physical stability studies. Figure 2 illustrates the appearance of the emulsions after a 24-hour equilibrium period, showing two distinct types based on visual observation. "Slightly creamy emulsions" were challenging to discern due to their opaque nature, whereas "Two-layer emulsions" were easily noticeable.

Preliminary Stability Screening by Centrifugation

The surfactant pairings (eg, Sorbitan monooleate with Polysorbate 80, 60, 40, and 20) were chosen to examine the effects of combining surfactants with different structures and hydrophilic-lipophilic balance (HLB) values on the interfacial arrangement and stability of the nanoemulsion system. The goal was to maintain a consistent overall HLB value across formulations while exploring how structural differences between Polysorbates and Sorbitan esters influence packing density, interfacial film properties, and droplet stabilization mechanisms. These differences could lead to variations in physical properties (eg, viscosity, droplet size distribution) and stability profiles of the emulsions.

Figure 2 The physical appearance of emulsion after left 24-hr equilibrium: (**a**) Slightly creamy emulsions (**b**) Two-layer emulsions.

The prepared emulsions underwent preliminary stability testing via centrifugation and were categorized into Breaking, Creaming I, and Creaming II. After centrifugation, emulsions formed by pairing Sorbitan monooleate with Polysorbate 80, 60, 40, 20, and Sorbitan monostearate – Polysorbate 80 exhibited creaming layers and were categorized in the Creaming I category. Exceptions included emulsions with Sorbitan monopalmitate - Polysorbate 20, which initially formed a two-layer emulsion but were later classified in the same category due to reduced droplet particle size in the nano-range.

Creaming was attributed to droplet density lower than the medium, resulting in the formation of cream layers due to gravity.[13] This effect could be reversed by shaking the emulsion, allowing large and small droplets to remix. Conversely, emulsions containing Sorbitan trioleate formed distinct oil layers upon centrifugation, indicating emulsion breaking. This led to the complete separation of the oil phase from the aqueous phase, classifying them in the Breaking category due to the highly lipophilic nature and low HLB value of Sorbitan trioleate, which likely hindered the formation of a stable interfacial film around the droplets.

These findings highlight important considerations for the use of Sorbitan trioleate in nanoemulsion formulations. The tendency for Sorbitan trioleate emulsions to break more easily suggests potential limitations in their application, particularly in systems requiring long-term stability or subjected to mechanical stress (eg, centrifugation or shaking). However, this characteristic may also have practical implications for applications where phase separation is desirable or acceptable, such as in biphasic delivery systems. Understanding these behaviors aids in selecting appropriate surfactants for specific formulation requirements, emphasizing the importance of HLB balance and structural compatibility in achieving stable nanoemulsions.

Emulsions with Sorbitan monostearate and Sorbitan monopalmitate, except for the surfactant pairs Sorbitan monostearate - Polysorbate 80 and Sorbitan monopalmitate - Polysorbate 20, exhibited cream and translucent layers upon centrifugation, classifying them in the Creaming II category. Analyzing droplet size, especially in formulations prone to flocculation or coalescence, is imperative for exploring emulsion properties and stability. This analysis is crucial for understanding the impact of different surfactant combinations and processes on droplet size and stability.

Determination of Size and Size Distribution

In the Creaming I category, the average droplet sizes ranged from 138 to 253 nm after 24 hours of equilibrium, as shown in Figure 3, and from 133 to 200 nm during storage, fitting within the nano-sized range. Extending the mixing time significantly reduced particle size, irrespective of whether a single continuous process or an intermittent process was employed. Notably, there were no significant differences in droplet size and size distribution between the two process designs in the Creaming I category when the same mixing time was applied, except for the surfactant pairs Sorbitan monostearate - Polysorbate 80 and Sorbitan monopalmitate - Polysorbate 20. Additionally, the surfactant pair Sorbitan monopalmitate - Polysorbate 20 exhibited larger droplet sizes in the intermittent process than in the single continuous process when the same mixing time was applied. In the single continuous process, droplet sizes ranged from 184 to 200 nm. In contrast, the intermittent process, which involved alternating between mixing and pausing, resulted in particle sizes from 238 to 286 nm. This led to a temperature decrease, causing the emulsion to transition from liquid to semi-solid. Despite this change, the emulsion was still classified under Creaming I, as the particle size remained within the acceptable range.

The solid forms of Sorbitan monopalmitate (melting point 43–48 °C) and Sorbitan monostearate (melting point 53–57 °C) regained their solidity, which increased the viscosity of the emulsion. This transition to a semi-solid state could be caused by the specific structural properties of Sorbitan monopalmitate and Sorbitan monostearate that contain fatty acids such as palmitic acid and stearic acid in their hydrophobic chains.[14] During the homogenization process, the hot liquid cools and transforms from a liquid to a semi-solid state. This decrease in temperature during homogenization deforms the emulsion structure, resulting in larger droplet sizes. Similar behavior observed in other emulsions containing Sorbitan monostearate and Sorbitan monopalmitate led to their classification in the Creaming II category (Figure 4).

Surprisingly, emulsions containing Sorbitan monostearate and Polysorbate 80, when homogenized, resulted in droplet sizes ranging from 206 to 253 nm, which were classified in the Creaming I category. The effect of temperature on the surface tension of Polysorbate 80, Polysorbate 60 and Polysorbate 20 was investigated by Szymczyk, Zdziennicka and
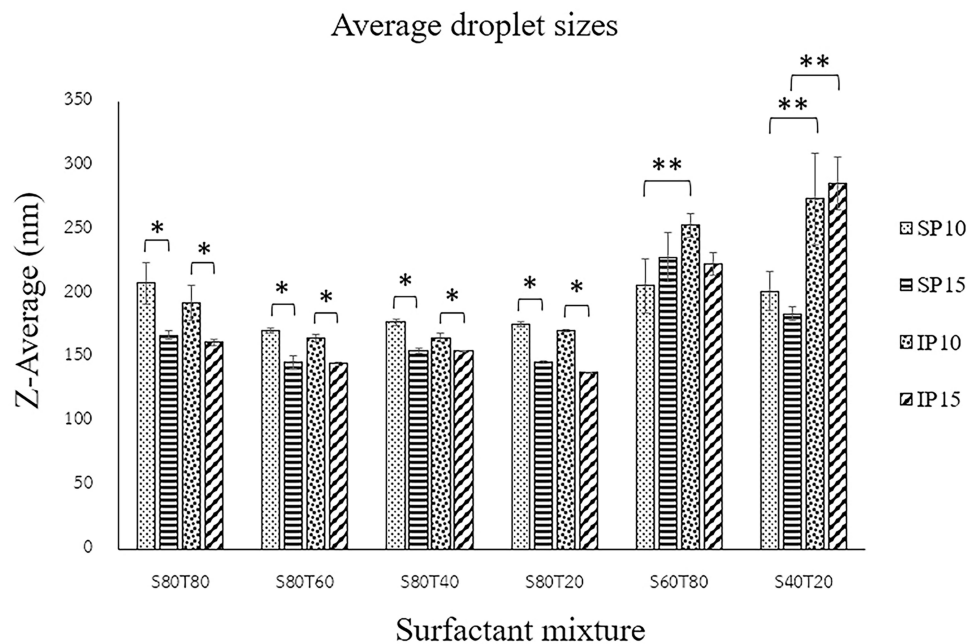
Average droplet sizes

Figure 3 The droplet sizes of nanoemulsions in the Creaming I category, prepared under different processing conditions, were measured after being left to equilibrate for 24 hours.
Notes: *Statistically significant difference (p < 0.05) in mixing time. **Statistically significant difference (p < 0.05) in process parameters.
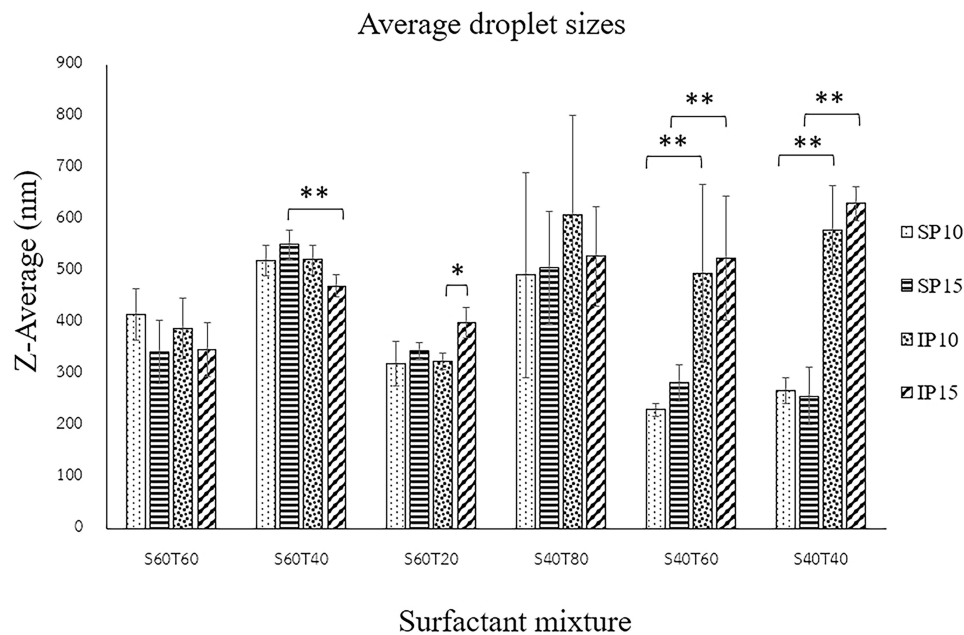
Average droplet sizes

Figure 4 The droplet sizes of nanoemulsions in the Creaming II category, prepared under different processing conditions, were measured after being left to equilibrate for 24 hours.
Notes: *Statistically significant difference (p < 0.05) in mixing time. **Statistically significant difference (p < 0.05) in process parameters.

Jańczuk,[15] revealing that Polysorbate 80 demonstrated the lowest critical micelle concentration (CMC), suggesting greater ease of emulsification. Moreover, its emulsifying capacity was found to enhance with rising temperatures.

In the Creaming II category, most droplet sizes were larger, ranging from 231 to 631 nm, as shown in Figure 4. No significant differences were observed between the single continuous process and the intermittent process, except for the

surfactant pairs Sorbitan monopalmitate - Polysorbate 60 and Sorbitan monopalmitate - Polysorbate 40 with both mixing times, and Sorbitan monostearate - Polysorbate 40 with a 15-minute mixing time, where the intermittent process led to significantly larger droplet sizes due to the temperature decrease during the rest period. Additionally, these formulations exhibited larger droplet sizes than the nanoscale range (more than 200 nm), classifying them under Creaming II.

Despite appearing as a two-layer emulsion, the combination of Sorbitan monopalmitate and Polysorbate 20 fell under the Creaming I category. This classification was attributed to its remarkable capability to achieve nanoscale droplet sizes in a single preparation process within 15 minutes. The emulsion was homogenized while the solution was still hot, allowing sufficient mixing time to effectively reduce droplet size.

When comparing the polydispersity indexes (PDIs) of nanoemulsions within the Creaming I category, those prepared using a high-speed homogenizer exhibited slightly higher PDIs ranging from 0.3 to 0.5 (Figure 5a). Conversely, nanoemulsions prepared with a high-pressure homogenizer showed PDIs around 0.1, indicating a narrower particle size distribution with uniform droplet sizes.[16] In contrast, emulsions categorized under Creaming II displayed higher PDIs ranging from 0.4 to 1.0 (Figure 5b), signifying a broader size distribution with a wider range of droplet sizes. The preparation process significantly influences these differences, particularly noticeable in emulsions containing Sorbitan monopalmitate, which often exhibited PDIs greater than 0.7, particularly in intermittent processes. Such high PDIs indicate a substantially wide particle size distribution with multiple peaks, making it unsuitable to rely solely on the reported Z-average for representing droplet size averages when using light scattering methods, leading to the rejection of the emulsion formulation. It's important to recognize that the PDI does not directly convey absolute droplet size values or individual droplet sizes but offers insights into the relative distribution of sizes within the sample, enhancing our understanding of the system's droplet size characteristics in conjunction with mean droplet size measurements.

Physical Stability Studies
Oswald Ripening Study

Ostwald ripening is a destabilization phenomenon in emulsions where smaller droplets dissolve and redeposit onto larger droplets over time. This process is driven by differences in Laplace pressure between droplets of varying sizes, ultimately resulting in droplet growth and phase separation, which compromise emulsion stability. This phenomenon is particularly concerning in polydisperse nanoemulsions, where diffusional mass transfer facilitates droplet growth and further destabilizes the system.[17] Surprisingly, in the formulations studied here, Ostwald ripening rates were nearly zero or negative for both single continuous and intermittent processes in the Creaming I category, indicating effective suppression of particle growth. Unexpectedly, slight size reductions occurred in all process designs during storage (Figure 6), contrary to ripening expectations, warranting further investigation.

The nanoemulsions containing Sorbitan monostearate and Polysorbate 80 exhibited negative Ostwald ripening rates when particle size measurements were taken after 24 hours of equilibrium, with droplet sizes ranging from 206 to 253 nm. However, after a week, the droplet sizes ranged from 168 to 200 nm, indicating a slow equilibrium in this formulation. As the droplet sizes remained below 200 nm during storage, they were deemed acceptable Conversely, the emulsions containing Sorbitan monopalmitate and Polysorbate 20 in an intermittent process were rejected due to their inability to form the desired nanoemulsions in terms of appearance and particle size.

In the Creaming II category, variations in Ostwald ripening rates were observed between the single continuous process and the intermittent process. For instance, when using Sorbitan monopalmitate – Polysorbate 40 in a single process, a slightly positive Ostwald ripening rate was observed, while the intermittent process showed a significantly negative value. The ripening rate values in the intermittent process were inconsistent, indicating more complex behavior. The Ostwald ripening rates in this category exhibited a wide range of both positive and very negative values, scattered across different processes and formulations, as shown in Figure 7. In contrast, the Creaming I category displayed Ostwald ripening rates closer to zero. In the Creaming II category, droplet size was determined by averaging the measured particle sizes from multiple peaks in the sample, as explained in the droplet size and size distribution study results. However, using the Z-Average of an extremely wide size distribution to calculate the Ostwald ripening rate did not yield accurate results. Consequently, these values were considered unreliable, leading to the rejection of the Ostwald ripening rate values for the Creaming II category.
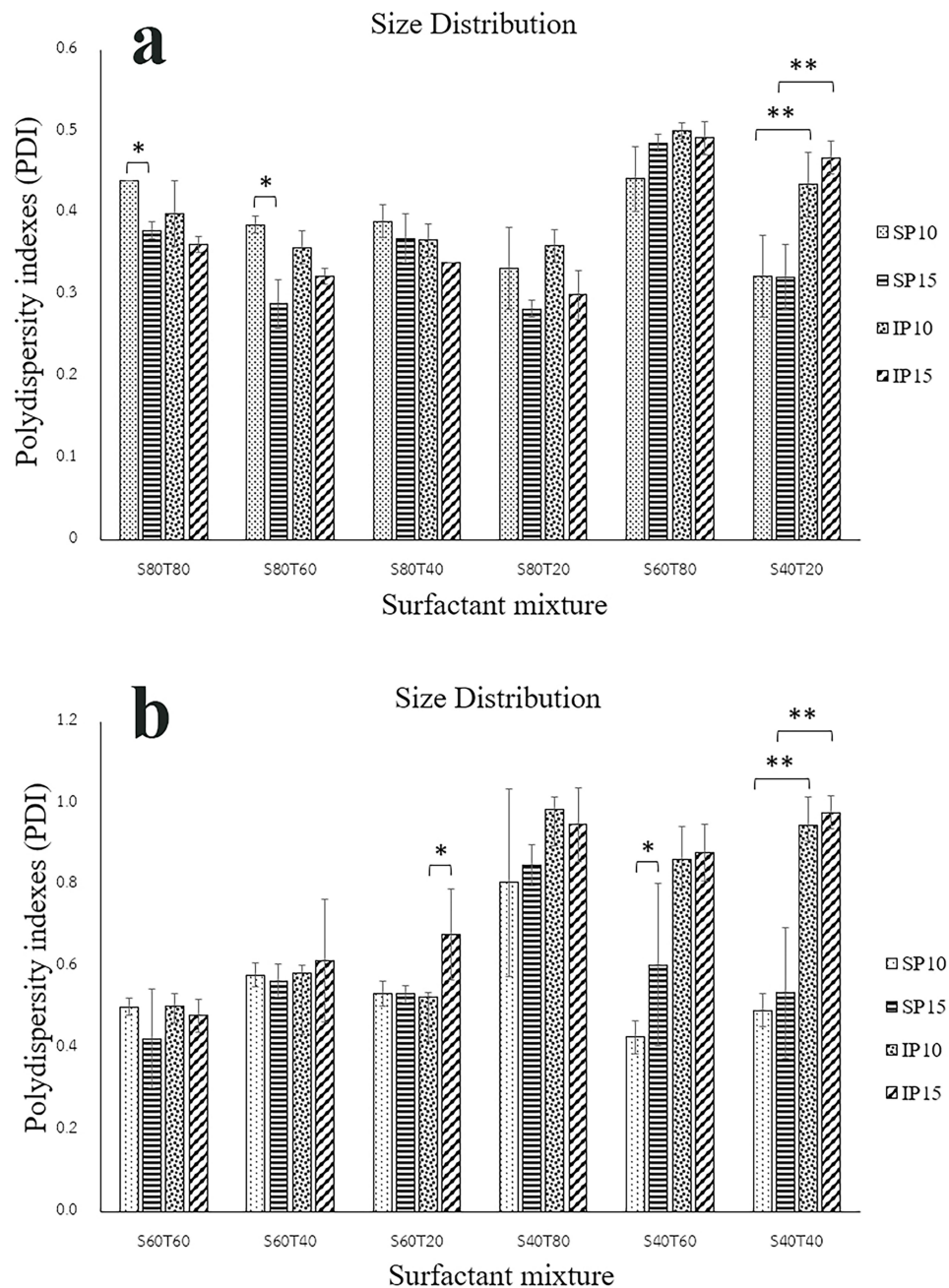
Figure 5 The size distributions of nanoemulsions in the Creaming I (**a**) and Creaming II (**b**) categories, prepared under different processing conditions, were measured after being left to equilibrate for 24 hours.
Notes: *Statistically significant difference ($p < 0.05$) in mixing time. **Statistically significant difference ($p < 0.05$) in process parameters.

Creaming Index (CI)

Despite the suppression of Ostwald ripening, as indicated by lower or negative ripening rates, all nanoemulsions experienced creaming after equilibrium, highlighting the distinct and independent nature of these instability mechanisms. Ostwald ripening is a slow, diffusion-driven process influenced by the solubility of oil in water and the Laplace pressure gradient, whereas creaming occurs more rapidly due to the density mismatch between the oil and water phases, further influenced by the viscosity of the continuous phase. In this study, the significant density difference between soybean oil and water, combined with insufficient viscosity to counter gravitational forces, facilitated droplet migration and led to creaming within 24 hours of equilibrium. These findings emphasize that while ripening rates provide insight into
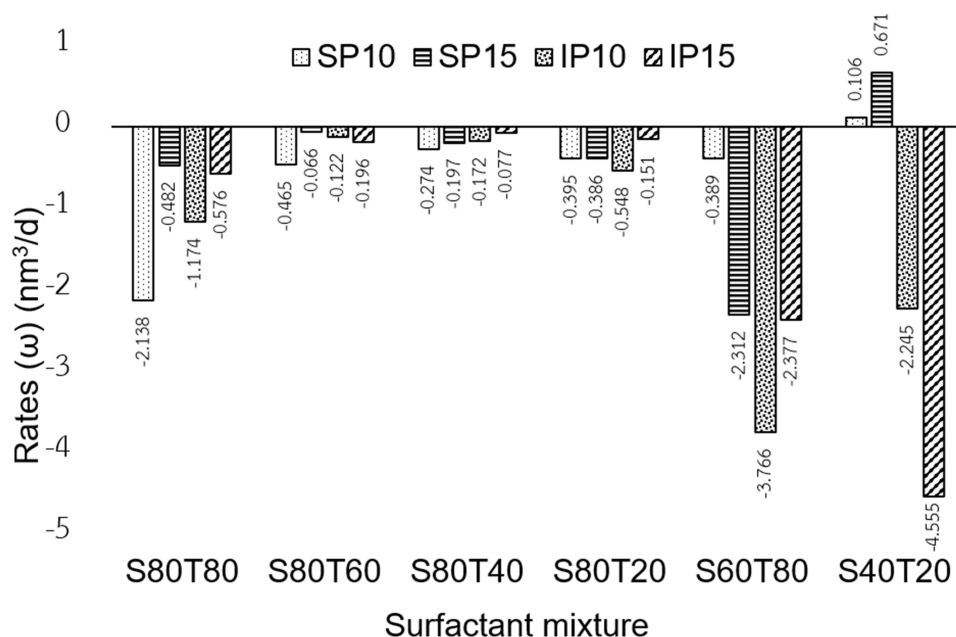
Figure 6 The Ostwald ripening rates of nanoemulsions in the Creaming I category.
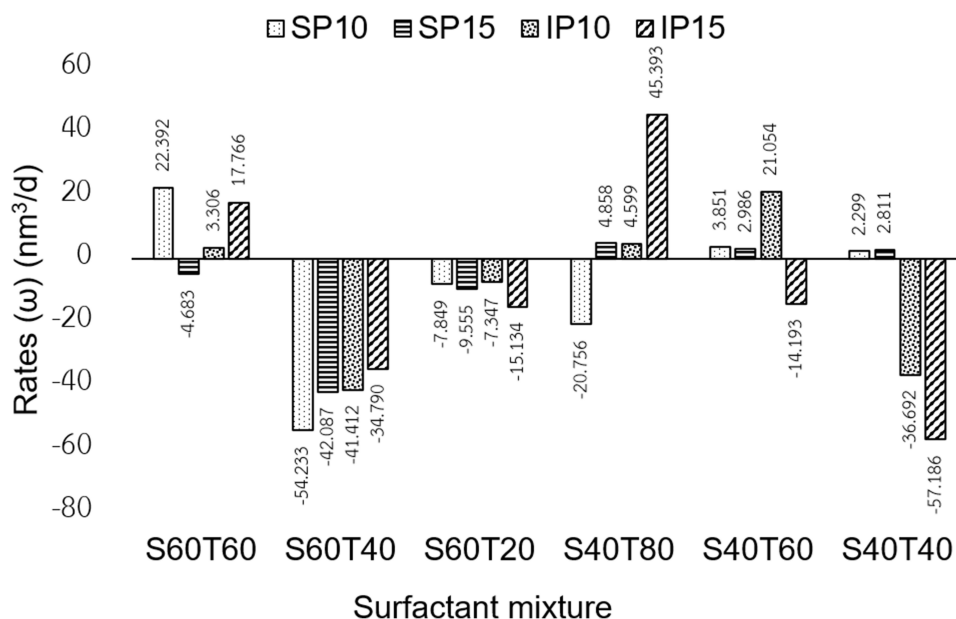

Figure 7 The Ostwald ripening rates of nanoemulsions in the Creaming II category.

molecular-level stability, addressing emulsion behavior requires a comprehensive approach that accounts for both time-dependent factors and physical properties like density and viscosity to effectively mitigate both instability mechanisms.

In the Creaming I category, only 5 out of the 6 emulsions had measurable creaming heights. Emulsions containing Sorbitan monopalmitate – Polysorbate 20 were an exception due to air bubbles, which led to the formation of a two-layer emulsion without a visible cream layer. In this formulation, the upper layer contained dispersed air bubbles, disrupting the typical migration and coalescence of oil droplets at the surface. The buoyancy of these air bubbles likely hindered the accumulation of oil droplets, resulting in a distinct upper layer that could not be quantified as a cream layer. This interference affected the calculation of the creaming index (CI), as the height of the cream layer (H_c) was not

representative of the typical gravitational separation dynamics. These findings underscore the importance of minimizing air bubble inclusion during sample preparation to ensure reliable CI measurements and an accurate assessment of emulsion stability.

The creaming index used to assess creaming extent after 24 hours (week 0) ranged from 5.42 to 10.85 (Table 2). Among the formulations, Sorbitan monostearate – Polysorbate 80 showed the highest index of 10.85, gradually increasing during the second and fourth weeks, indicating susceptibility to creaming over time. This trend offers valuable insights into long-term stability challenges in nanoemulsions. A rising creaming index over time indicates the gradual migration of oil droplets to the surface due to the density difference between the oil and aqueous phases, highlighting a tendency for destabilization over extended storage or mechanical stress despite initial stability. This behavior poses challenges for formulations requiring long shelf life or industrial robustness, as even slow creaming can affect product

Table 2 The Creaming Indexes of Nanoemulsions After 1, 2, 3, and 4 weeks of Storage at Ambient Temperature

Surfactant Mixture	Process	Time (wk)	Creaming Index (% CI)	Surfactant Mixture	Process	Time (wk)	Creaming Index (% CI)
S80T80	SP10	0	8.29 ± 0.30	S80T60	SP10	0	9.39 ± 0.19
		2	9.34 ± 0.42			2	10.87 ± 0.26*
		4	11.76 ± 0.56*			4	11.15 ± 0.21*
	SP15	0	6.84 ± 0.33		SP15	0	8.25 ± 0.45
		2	6.83 ± 0.29			2	9.44 ± 0.08*
		4	8.21 ± 0.44*			4	11.07 ± 0.80*
	IP10	0	9.22 ± 0.45		IP10	0	9.10 ± 0.49
		2	10.66 ± 0.25*			2	10.24 ± 0.29
		4	10.64 ± 0.12*			4	11.02 ± 0.82
	IP15	0	8.02 ± 0.28		IP15	0	8.00 ± 0.68
		2	9.18 ± 0.11*			2	9.15 ± 0.59
		4	9.37 ± 0.35*			4	9.72 ± 0.20
S80T40	SP10	0	9.01 ± 0.26	S80T20	SP10	0	5.86 ± 0.62
		2	10.11 ± 0.64			2	7.48 ± 1.47*
		4	11.13 ± 0.71*			4	9.62 ± 1.06*
	SP15	0	8.12 ± 0.61		SP15	0	5.57 ± 0.21
		2	9.29 ± 0.59			2	6.83 ± 0.12
		4	9.60 ± 0.93			4	7.93 ± 0.37*
	IP10	0	9.36 ± 0.66		IP10	0	5.98 ± 0.47
		2	10.71 ± 0.94			2	8.65 ± 0.69*
		4	11.19 ± 1.00			4	9.21 ± 0.35*
	IP15	0	8.16 ± 0.32		IP15	0	5.42 ± 0.61
		2	8.92 ± 0.37			2	7.05 ± 0.72*
		4	9.86 ± 0.34*			4	8.61 ± 0.74*

(Continued)

Table 2 (Continued).

Surfactant Mixture	Process	Time (wk)	Creaming Index (% CI)	Surfactant Mixture	Process	Time (wk)	Creaming Index (% CI)
S60T80	SP10	0	5.52 ± 0.73	S60T80	IP10	0	8.53 ± 0.23
		2	6.95 ± 0.86*			2	13.87 ± 2.11*
		4	7.38 ± 0.51*			4	15.79 ± 0.60*
	SP15	0	10.85 ± 0.91		IP15	0	9.55 ± 0.49
		2	11.67 ± 0.49			2	16.23 ± 1.66*
		4	14.63 ± 0.68*			4	16.42 ± 0.64*

Notes: *Statistically significant difference ($p < 0.05$) compared to week 0.
Abbreviations: S, Sorbitan ester; T, Polysorbate; SP10 or SP15, single process 10 minutes or 15 minutes; IP10 or IP15, intermittent process 10 minutes or 15 minutes; wk, week; % CI, percentage of creaming index.

uniformity, appearance, and performance. These findings emphasize the need to optimize formulation parameters, such as surfactant ratios, oil type, and continuous phase viscosity, to minimize gravitational separation and enhance stability.

Longer mixing times, especially at 15 minutes, resulted in lower creaming indexes compared to 10 - minute durations, suggesting initial stability improvement. However, regardless of mixing time or process (single or intermittent), nanoemulsions showed stable creaming indexes over time, reflecting consistent creaming tendencies. Small creaming indexes determined by height measurement may indicate slight instability due to the gravity-driven movement of larger droplets.

Discussion

The nanoemulsion formulations in this study demonstrated the successful combination of Polysorbates and Sorbitan esters to achieve stability through a fixed HLB value of 8. However, a deeper understanding of the mechanisms influencing droplet size reduction and stabilization can further strengthen the formulation strategy.

Droplet size reduction during high-speed homogenization primarily relies on the interplay of shear forces, cavitation, and turbulence. While this study employed high-speed homogenization rather than high-pressure systems, the underlying principles remain relevant. As highlighted by Håkansson, Fuchs, Innings, Revstedt, Bergenståhl and Trägårdh,[18] high-pressure homogenizers utilize cavitation and turbulence to induce droplet breakup, making them effective for emulsification processes. Similarly, the energy input during high-speed homogenization likely influenced the breakup of oil droplets and their subsequent stabilization within the aqueous phase, providing a parallel to high-pressure techniques. These findings emphasize the importance of optimizing homogenization conditions to achieve uniform droplet size while maintaining formulation stability.

During high-speed homogenization (Figure 8), a pre-emulsion is drawn through circular holes, creating local high shear. Consequently, turbulence and possibly cavitation occur, leading to droplet break-up. This process is repeated in cycles, generating very intense flow fields that result in submicron droplets. It is expected to produce monodisperse emulsions. These mechanisms depend on the balance between interfacial tension and external disruptive forces.[4] This study focuses on using various non-ionic surfactant blends, with a fixed HLB value and concentration but different surfactant structures that play an important role in interfacial layer packing and emulsion stability. Additional process parameters, such as continuous and intermittent mixing, affect droplet formation.

Nanoemulsions possess nano-sized droplets, which result in high surface free energy due to the increased surface area.[19] This high surface free energy is energetically unfavorable and drives droplet coalescence. Stabilization against coalescence can be effectively achieved by creating a sufficient repulsive energy barrier between the droplets. To prevent instability, emulsifiers like nonionic surfactants containing alkyl polyoxyethylenes can adsorb at the particle surface in emulsion polymerization, preventing coalescence through steric stabilization. Tadros, Levecke and Booten[20] described
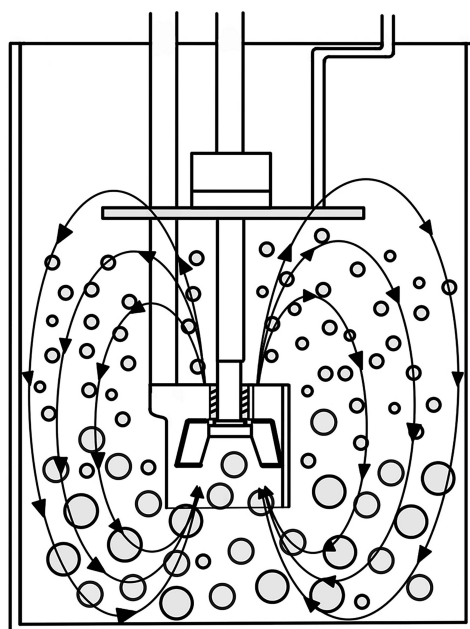
Figure 8 Schematic representation of emulsification method using Homogenizing Mixer Mark II 2.5, PRIMIX.

how steric stabilization mechanisms effectively suspend dispersed droplets in emulsions, particularly when polymeric or non-ionic surfactants are used. In our formulations, the combination of Polysorbates and Sorbitan esters likely provided both steric and interfacial stabilization. The favorable intermolecular interactions between these surfactants condensed the interfacial film, contributing to resistance against coalescence and phase separation. When two surfactants have compatible structures, the film of one surfactant can act as a better "solvent" for the second surfactant in the mixed film, allowing them to form a phase that resists collapse.[21] Conversely, if the two surfactants do not mix well, due to steric incompatibility, for example, the free volume per molecule increases and the film expands. The effectiveness of steric stabilization of Sorbitan esters and Polysorbates with different molecular structures (ie, lengths of hydrophobic and hydrophilic moieties) in o/w emulsions was investigated.

In this study, nano-sized emulsions can be prepared with minor creaming after overnight equilibrium. However, after centrifugation, all formulations containing Sorbitan trioleate were severely unstable, leading to breaking, which is the complete separation of the oil phase from the aqueous phase. Sorbitan trioleate is a non-ionic surfactant with a large hydrophobic cross-sectional area relative to its headgroup, whereas Polysorbates® have a more balanced hydrophilic-lipophilic structure. Sorbitan trioleate tends to preferentially adsorb at the oil-water interface, displacing the Polysorbate molecules. Additionally, the mismatch in their preferred curvature and the hydrophilic-lipophilic balance (HLB) of the two surfactants can cause incompatibility, leading to emulsion instability when combined.[22,23]

Sorbitan monooleate, a single-chain surfactant, might be better suited to a positive curvature surface, such as a bubble or an oil-water interface, where the free volume between the acyl chains can be occupied by molecules of the oil phase.[21] According to simulations conducted by Kopanichuk, Vedenchuk, Koneva and Vanin,[24] Sorbitan monooleate tends to form nearly spherical reverse micelles, whereas Polysorbate 80 forms more elliptical shapes. The shape of the mixed Sorbitan monooleate/Polysorbate 80 in a 2:1 ratio, the same as our relative concentrations of the surfactants, produced a more spherical shape. Additionally, spherical droplet shapes are more stable than irregular shapes. Spherical droplets have the lowest surface area for a given volume, minimizing the interfacial area between the oil and water phases.[25] This reduction in free energy makes emulsion more thermodynamically stable Our results showed that all Sorbitan monooleate and Polysorbates combinations produced Cream I category nanoemulsions with good properties, including nano-sized droplets with low PDI, almost zero Ostwald ripening rate, and minor creaming, which is considered acceptable stability.

Several factors must be considered in emulsion production, a process that is far from trivial and often relies on trial-and-error for formulation and process optimization. For example, the flow conditions in the continuous phase determine

the droplet contact time, which influences whether droplet coalescence or recoalescence will occur. Common equipment, such as a high-speed homogenizer, used from lab bench to large-scale production, demonstrated its ability to produce nanoemulsions with good stability in this study. Both single and intermittent processes were examined, and while there was no significant difference between the two process types in most of the Cream I and II categories, it was observed that longer mixing times produced smaller droplet sizes in most cases. However, formulations containing Sorbitan mono-palmitate, which were prepared using a hot process, showed prominently different results between the two process types due to the cooling of the material. This may be due to the higher ratio of Sorbitan monopalmitate compared to Sorbitan monostearate in formulations prepared with the hot process, even though Sorbitan monopalmitate has a lower melting point than Sorbitan monostearate.

While the choice between single and intermittent mixing had a minimal impact on nanoemulsion properties, this finding carries important implications for the versatility and practicality of the mixing process. Unlike microfluidizers or high-pressure homogenizers, which often require multiple cycles to achieve uniform dispersion resulting in material loss, reduced yield, and potential degradation of active ingredients due to repeated high-pressure exposure. This study demonstrates that a simpler, cost-effective high-speed homogenizer can produce comparable results. Additionally, high-pressure systems necessitate effective cooling mechanisms to manage the heat generated during operation, further complicating the process.

In contrast, the negligible difference between single and intermittent mixing observed in this study suggests a more flexible method suitable for accommodating various formulation requirements, particularly for sensitive actives. The open system used in this study also allows for simultaneous temperature control, making it adaptable to processes that demand precise thermal management. Although the limitation of this approach lies in its production scale, it can be addressed by employing time-efficient methods to manage larger batch sizes effectively. These findings highlight the potential of high-speed homogenizers as a versatile and accessible alternative for nanoemulsion preparation in both research and industrial settings.

The stability observations, particularly the creaming index trends, provide valuable insights into the overall perfor-mance of nanoemulsions. While the formulations demonstrated strong molecular stability, as evidenced by the suppres-sion of Ostwald ripening, the occurrence of creaming highlights the need to address physical instability mechanisms to achieve complete stability. These findings suggest that further refinements, such as increasing the viscosity of the continuous phase or strengthening the interfacial film, could help mitigate gravitational separation. This study success-fully establishes the foundational stability of nanoemulsions, providing a solid platform for further optimization to enhance their suitability for long-term storage and commercial applications.

While the current study primarily focused on fixed process parameters, the use of statistical-based optimization methods, such as Response Surface Methodology (RSM), presents a promising avenue for future research. For example, Ngan, Basri, Lye, Fard Masoumi, Tripathy, Karjiban and Abdul-Malek[26] and Yakoubi, Kobayashi, Uemura, Nakajima, Isoda, Ksouri, Saidani-Tounsi and Neves[27] demonstrated the effectiveness of RSM in optimizing surfactant ratios, homogenization parameters, and other formulation variables to achieve enhanced droplet size control and stability indices. Applying these methodologies in future work could refine the current formulations, leading to improved scalability and long-term stability.

Conclusion

The primary aim of this study was to fabricate nanoemulsions using high-speed mixing techniques. Various surfactant combinations, including Sorbitan monooleate - Polysorbate 80, Sorbitan monooleate - Polysorbate 60, Sorbitan mono-oleate - Polysorbate 40, Sorbitan monooleate - Polysorbate 20, and Sorbitan monostearate - Polysorbate 80, were explored. The results indicated that the choice between single and intermittent mixing processes had minimal impact on droplet size reduction, while mixing time played a critical role. Notably, only the Sorbitan monopalmitate - Polysorbate 20 emulsion achieved nano-sized droplets within a 15-minute single process.

The molecular structures of the Sorbitan esters significantly influenced the preparation methods. Sorbitan mono-stearate and Sorbitan monopalmitate required pre-heating due to their solid state at room temperature, making them

unsuitable for intermittent processes. This highlights the importance of carefully matching surfactant combinations and preparation methods to achieve the desired nanoemulsion droplet sizes.

The nanoemulsions produced in this study exhibited droplet sizes around 200 nm, with polydispersity index (PDI) values ranging from 0.3 to 0.5, indicating commendable droplet size distribution. However, slight instability was observed, as indicated by an approximately 10% creaming index. This suggests the potential need for stabilizing agents, such as polymer additives, to further enhance emulsion stability by reducing droplet coalescence rates.

In comparison to our previous research,[5] which utilized a microfluidizer to optimize surfactant balance, this study focuses on a more accessible and cost-effective high-speed mixer to achieve similar nano-sized emulsions. This approach has significant commercial implications, offering an energy-efficient method suitable for large-scale production. The ability to achieve nanoemulsions through high-speed mixing makes production more scalable and economically feasible for broader industrial applications. Further research on scale-up procedures may be warranted to fully realize the potential of this technique in industrial settings.

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Disclosure

The authors report no conflicts of interest in this work.

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