Effects of Ultrasonic Operating Parameters and Emulsifier System on Sacha Inchi Oil Nanoemulsion Characteristics

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Abstract: The objective of this study was to investigate the effect of ultrasonic operating conditions, i.e., ultrasonic mode, amplitude, total ultrasonic duration time, and emulsifier system in producing an optimum oil-in-water of sacha inchi oil nanoemulsions (SIO-NEs). Physicochemical characteristics (including average droplet size, polydispersity index (PDI), zeta potential, and viscosity) were the evaluated response variables. Smaller droplet size was obtained from SIO-NEs prepared by ultrasonic pulse application (15s ON and 10s OFF) with an ultrasonic amplitude level of 60%. In contrast, excess energy produced by ultrasonication amplitudes of more than 60% resulted in larger average droplet size and PDI. A decrease in the absolute value of zeta potential and a lower viscosity of SIO-NEs were also observed in conjunction with the high amplitude level of the ultrasonication process. An ultrasonication duration time of longer than 10 minutes did not significantly reduce the droplet size. Five emulsifier systems were evaluated in this study, including Pluronic® L-31, Brij® C-10, Tween® 80, Tween® 80/Pluronic® L-31, and Brij® C-10/Pluronic® L-31. The results revealed that the Brij® C-10/Pluronic® L-31 mixture produced the smallest droplet size (148 nm) with the lowest PDI (0.210), viscosity (3.35 cPs), and zeta potential (−31.09 mV). The concentration of the Brij® C-10/Pluronic® L-31 mixture, used as an emulsifier of SIO-NEs, varied from 1.5% to 9%. Based on the present findings, the most suitable concentration of mixed emulsifier used was deemed as 3% (w/v). The selected SIO-NEs were stored under room temperature to determine their droplet size stability, with the constant slightly increasing within 90 days of storage.

Key words: ultrasonic emulsification, emulsifier system, sacha inchi oil, nanoemulsion

1 Introduction

Sacha inchi oil (SIO) is extracted from the seeds of Plukenetia volubilis L., also known as the sacha peanut, jungle peanut, or Inca peanut. It is a rich source of omega-3 and omega-6 fatty acids, protein, and other substances such as phytosterols, tocopherols, beta-carotenoids and phenolic compounds. SIO exhibits antibacterial, anti-inflammatory, antioxidant, and antiproliferative activities, and offers many health benefits for preventing several diseases like arthritis, cancer, cardiovascular disease, diabetes, hypertension, attention-deficit/hyperactivity disorder, and inflammatory skin diseases. Recently, it has gained international attention for food, pharmaceutical, and cosmetic applications as well.

Nanoemulsions (NEs) or submicron-sized emulsions are dispersed systems consisting of at least two immiscible liquids stabilized using an appropriate emulsifier, such as a surfactant. The diameter of the droplet in NEs may vary from about 50 to 500 nm, which means that their appearance may range from transparent to opaque. NEs are a class of kinetically stable, nonspontaneous systems, typical of either the oil-in-water (o/w) or the water-in-oil (w/o) type. They show improved properties over conventional emulsions, such as high stability, high oil-in-water interfacial tensions, bioavailability, nontoxicity, and nonirritant behavior. NEs have been applied in different fields and products, such as food processing, and cosmetic and pharmaceutical industries. In the latter, NEs are utilized as drug delivery systems to achieve alternative administration routes and have been explored as drug carriers for lipophilic compounds, potentially offering improved drug loading capacity for lipophilic active ingredients, controlled drug release, and protection of active ingredients from chemical and enzymatic degradation.
The preparation of NEs can be broadly categorized as involving high-energy or low-energy methods[10]. Ultrasonic emulsification is a high energy emulsification method that involves processing very fine tiny droplets through two mechanisms. At first, the applied sound field produces interfacial waves, and the dispersion of the oil phase in the continuous phase takes place in the form of droplets. The second mechanism, the application of ultrasound, causes sound cavitation, which generates the formation and collapse of microbubbles by the pressure fluctuations of the simple sound wave. Moreover, it creates extreme levels of highly localized turbulence, which promotes the primary droplets to break up into submicron sizes[22, 23]. NEs produced using the ultrasonication method have broader and bimodal size distributions[4, 25].

The objective of this study was to produce SIO-NEs with desirable physicochemical properties such as a small average droplet size (<200 nm), low polydispersity index (PDI) value, high zeta potential, and attractive viscosity using the emulsion-sonication method for pharmaceutical and cosmetic application. The impact of ultrasonication mode, ultrasonic amplitude level (%), and ultrasonication duration time (min), as well as the type of emulsifiers system and emulsifiers concentration, were also studied.

2 Materials and Methods

2.1 Materials

SIO was purchased from Khonkaen Kasettagram Hitec Center, Nai Muang, Muang, Khonkaen, Thailand. Nonionic surfactants, polyoxyethylene sorbitan monooleate (Tween®80; Mw = 1310), Brij®C-10 (Mw = 685), and Pluronic®L-31 (Mw = 1100) were purchased from Sigma Aldrich (St. Louis, MO). Fresh distilled water was prepared in our laboratory and used throughout the experiment.

2.2 Preparation of SIO-NEs

An emulsion containing 5% (w/v) of SIO, 3% (w/v) of Brij®C-10/Pluronic®L-31 in a ratio of 60%:40% (w/w) as the emulsifier, and 92% purified water (w/v) as a water phase were used throughout the ultrasonic operating parameter studies. Initially, the coarse emulsion was developed by adding the emulsifier to SIO (oil phase) and then mixing the components using a magnetic stirrer for 15 minutes. The surfactant-oil mixture phase and water phase were separately warmed on the water bath to 40–45°C. The water phase was later added into the oil phase and pre-emulsified using a digital homogenizer (Ultra-Turrax® T 25; IKA, Königswinter, Germany) at 4,200 rpm for 10 minutes to form the coarse emulsion before further treatment with ultrasonication. All emulsions were carried out at room temperature.

2.3 Ultrasonic operating parameters

A Vibra-Cell® Ultrasonic Liquid Processor (VCX 130; Sonics & Materials Inc., Newtown, CT, USA), with 130 W of output power and a frequency of 20 kHz, operating together with a 6-mm probe, were used. To prevent overheating and temperature rise during the ultrasonication process; thus, an ice bath was used to maintain the temperature difference (before and after sonication) at 5°C or less. The ultrasonic operating parameters of interest for the present study included ultrasonic mode, amplitude level, and duration time.

2.3.1 Ultrasonic mode and amplitude level effects

The ultrasonic probe was used to generate the o/w NEs from the prepared coarse emulsion. Continuous-mode or pulsed treatment (15 seconds ON and 10 seconds OFF) for 10 minutes was applied to compare the effects on the physical characteristics of the obtained SIO-NEs. These comparisons were made at two amplitude levels of 30% and 60%. All samples were prepared in triplicate, and then the droplet size and polydispersity index were measured.

2.3.2 Ultrasonic duration time and amplitude level effects

The pulse ultrasonic treatment of eight amplitude levels (20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%) at various sonication times of 5, 10, 15, and 20 minutes were evaluated. All samples were prepared in triplicate, and the effects of the ultrasonic operating parameters on physical characteristics, including droplet size and polydispersity index, zeta potential, and viscosity were evaluated after undergoing the above processes.

2.4 The emulsifiers system and emulsifier concentration studies

The NEs with 5% (w/v) of SIO, 92% (w/v) of water and 3% (w/v) of emulsifiers were incorporated. There are no literature data reports about the single or combined effects of hydrophilic–lipophilic balance (HLB) of the surfactants or the preparation and the properties of SIO-NEs. In this study, the experiments were conducted at different HLB values for getting the homogeneous system and minimum droplet size. The nonionic surfactants polyoxyethylenenedecyl ether, also commercially known as Brij®C10 (HLB = 12); polysorbate 80 or polyoxyethylene(20)sorbitan mono- noleate, also commonly known as Tween®80 (HLB = 15); and polyethylene oxide (PEO)-polipropylene oxide (PPO) -polyethylene oxide (PEO)block copolymers (PEO₆-PPO₇₆-PPO₆) supplied commercially as Pluronic®L-31 (HLB = 4.3) were used as emulsifiers.

For mixed surfactant (Smixed), the mass ratios of Tween®80/Pluronic®L-31 and Brij®C-10/Pluronic®L-31 were kept at ratio 60:40% (w/w), and the HLB was defined by the following equation:

\[
HLB = \frac{\left(m_A \times HLB_A + m_B \times HLB_B\right)}{\left(m_A + m_B\right)}
\]

where \(m_A\) and \(m_B\) is the mass of surfactant A and B, respec-
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Relatively, HLB A and HLB B is the HLB number of surfactant A and B, respectively.

Therefore, the HLB values of mixed systems of Tween®80/Pluronic®L-31 and Brij®C-10/Pluronic®L-31 were 10.4 and 8.6, respectively. The NE was prepared under the same conditions deemed optimal from the above study. The compositions of NE formulations are described in Table 1.

Furthermore, the effects of emulsifier concentration on average droplet size, PDI, zeta potential, and viscosity were also evaluated. These were done by increasing the concentration of the chosen emulsifier system in the formulation from 1.5% to 9% (w/v) whereas the amount of oil (SIO) used was fixed at 5% (w/v) throughout the study with different surfactant-to-oil ratios as described in Table 2.

2.5 Droplet size, polydispersity index, and zeta potential measurement

The mean droplet diameter (Z-average), polydispersity index (PDI), and zeta potential of SIO-NEs were determined by photon correlation spectroscopy and electrophoretic light scattering, using the NanoPlus analysis system (Micromatics/NANO Plus 3). Samples were diluted with deionized water at a ratio of 1:10 (v/v) to avoid multiple dispersion effects and placed in the capillary cell for measurements. The size reported corresponded to intensity-weighted average hydrodynamic diameter or Z-average diameter obtained through cumulant analysis. PDI is a dimensionless measure of the width of size distribution calculated from the cumulant analysis, which ranges from 0 to 1.0. Small PDI value (≤0.08) indicates a nearly monodispersed population, while large PDI values (≥0.7) indicate a very broad distribution of droplet size. All experiments were performed in triplicate, and the average and standard deviation (SD) values obtained were recorded.

2.6 Viscosity determination

The viscosity of the NE was determined without dilution using Brookfield DV-H+ Viscometer (Brookfield®, Middleboro, MA, USA) using spindle No.61 at 25 ± 0.3°C.

2.7 Long term stability of NEs

The long-term stability of NEs was evaluated by measuring the droplets’ size, PDI, zeta potential, and viscosity as a function of time. The selected sample prepared at the optimum conditions was stored at room temperature for up to six months, and droplet sizes were measured at different intervals of time during the storage period.

2.8 Statistical analysis

All values obtained were reported as mean values ± SDs. Significant differences in the measured mean physical characteristic parameters were analyzed by one-way analysis of variance (ANOVA) and followed by Tukey’s honestly significant difference (HSD) Post Hoc test. The significance level for all tests (α) was assessed at 0.05. The correlation between the ultrasonication operating parameter variables and the droplet physical properties was evaluated using multiple linear regression analysis. SPSS (IBM Corp., Armonk, NY, USA) was the statistical computation program.

Table 1  SIO-NEs composition with different surfactant systems of fixed concentration (3%).

| Code | SIO (%w/v) | Pluronic®L-31 (%w/v) | Brij®C-10 (%w/v) | Tween®80 (%w/v) | Water (%w/v) |
|------|------------|----------------------|------------------|----------------|-------------|
| Rx 1 | 5          | 3                    | -                | -              | 92          |
| Rx 2 | 5          | -                    | 3                | -              | 92          |
| Rx 3 | 5          | -                    | -                | 3              | 92          |
| Rx 4 | 5          | 1.7                  | -                | 1.3            | 92          |
| Rx 5 | 5          | 1.2                  | 1.8              | -              | 92          |

Table 2  SIO-NEs composition with a mixed surfactant system of Brij®C-10/Pluronic®L-31 at different concentration.

| Code   | SIO (%w/v) | Brij®C-10 (%w/v) | Pluronic®L-31 (%w/v) | Water (%w/v) |
|--------|------------|------------------|----------------------|--------------|
| S mixed 1.5% | 5          | 0.9              | 0.6                  | 93.5         |
| S mixed 3%    | 5          | 1.8              | 1.2                  | 92           |
| S mixed 5%    | 5          | 3                | 2                    | 90           |
| S mixed 7%    | 5          | 4.2              | 2.8                  | 88           |
| S mixed 9%    | 5          | 5.4              | 3.6                  | 86           |

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used throughout this study.

3 Results and Discussion

When the SIO-NEs were developed, the mixture of Brij®-C-10/Pluronic®L-31 was used as a preliminary emulsifier in the early operating process evaluation experimental section. The coarse emulsion was prepared by adding a dispersed phase of SIO mixed with an emulsifier to the continuous phase (purified water) and performing a pre-emulsification using a homogenizer. The coarse emulsion droplets were broken down by applying high-energy ultrasoundation, and then the NEs were formed. This is a usual method adopted to produce NEs in which the high-energy shockwaves produced from ultrasonication create turbulence, which ruptures the droplets, yielding a globule size at the nanometric scale. However, the size reduction process condition is critical and should be continued until the droplet size becomes constant. In this study, the effects of operating parameters and compositional variables of NEs prepared by ultrasonication on droplet size, PDI, zeta potential, and viscosity were evaluated.

3.1 Effects of ultrasonication operating parameters

The different ultrasonic operating modes (continuous or pulse sonication) were compared regarding their performance in reducing the droplet size. The experiments were carried out at the amplitude levels of 30% and 60% for both the continuous and pulse sonication modes. The results are depicted in Fig. 1. Significantly smaller droplet size and PDI were obtained from NEs realized by using pulse ultrasonic application (15s ON and 10s OFF) as compared with continuous treatment for both amplitude levels (p < 0.05). This is explained by the cavitation intensity of the pulse ultrasonic condition prompting an increasing force sufficient to disintegrate large droplets into smaller ones.

Due to the smaller droplet size and lower PDI obtained with pulse sonication, it was then chosen to prepare SIO-NEs in the remaining experiments. To evaluate the effects of ultrasonication operating parameters, amplitude (X1), and time (X2); four responses including droplet size (Y1), PDI (Y2), zeta potential (Y3), and viscosity (Y4) were assessed. The ultrasonic amplitude, designated as the independent variable or predictor 1 (X1), was assessed using eight amplitude levels of 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% to give different energy levels into the system. The ultrasonic duration time, independent variable, or predictor 2 (X2) was assessed using four levels of 5, 10, 15, and 20 minutes. The response outcomes of four variables correlated with two predictors were analyzed using multiple linear regression to determine the strength of the predictor effect on each response variable. X1, the ultrasonic amplitude, was divided into two levels, designated further as X1L and X1H for the ultrasonication amplitude level of 20% to 60% and 60% to 90%, respectively.

The effects of both predictors on the four response variables are presented in Fig. 2. Multiple linear regression was employed to determine the effects of independent variables (predictors) on each response variable, as can be seen in the results shown in Table 3.

In Table 3, the upper section includes the results determined at the low amplitude level. The model fitted for all variables was significant at a 95% confidence interval with the R² values ranging from 0.7 to 0.9. All predictor coefficients of all responses, including the constant, exhibited a p-value significance of less than 0.05; thus, the regression equation of each response variable (Yi) could be obtained as follows:

Size (nm):  
\[ Y_{1L} = 280.822 - 1.085 X_{1L} - 5.538 X_2 \]

PDI:  
\[ Y_{3L} = 0.144 + 0.001 X_{1L} + 0.006 X_2 \]

Zeta potential (mV):  
\[ Y_{4L} = -14.494 - 0.247 X_{1L} - 0.395 X_2 \]

Viscosity (cps):  
\[ Y_{4L} = 2.978 + 0.010 X_{1L} + 0.028 X_2 \]

where X1L = amplitude level ranging from 20% to 60% and X2 = time ranging from 5 to 20 minutes.

At the high amplitude level, in the lower section of Table 3, different correlations were obtained. The model fitted for all variables was significant at a 95% confidence interval with R² values ranging from 0.5 to 0.8. The coefficients of constant values were not significant for size, and the PDI responses and the coefficients of the time variable were not significant for zeta potential and viscosity. All of the insignificant coefficients were thus excluded from the regression equation as follows:

Fig. 1 Effects of ultrasonication operating mode on droplets size and PDI of SIO-NEs at different amplitudes level. Values are presented as means ± SDs (n = 3).
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Fig. 2  Effect of ultrasonication amplitude ($X_1$) and ultrasonication duration time ($X_2$) on A) Droplet size, B) PDI, C) Zeta potential, and D) Viscosity. Values are presented as means ± SDs (n = 3).

Table 3  Model summary and predictor coefficients obtained from multiple linear regression at the low amplitude level predictor ($X_{1L}$) and high amplitude level predictor ($X_{1H}$).

| Variable ($Y_{1L}$) | $R^2$ | Unstandardized coefficient | Standardized coefficient | $p$-value | $p$-value | $p$-value | $p$-value |
|---------------------|-------|----------------------------|--------------------------|-----------|-----------|-----------|-----------|
|                     |       | Constant                   | Amplitude $X_{1L}$       | Time $X_2$| Amplitude $X_{1L}$| Time $X_2$|           |
| $Y_{1L}$            | 0.701 | 280.822                    | -1.085                   | -5.538    | -0.373    | -0.750    |           |
| $Y_{1H}$            | 0.905 | 0.144                      | 0.001                    | 0.006     | 0.330     | 0.892     |           |
| $Y_{2L}$            | 0.820 | -14.494                    | 0.000                    | 0.395     | -0.765    | -0.485    |           |
| $Y_{2H}$            | 0.662 | 2.978                      | 0.010                    | 0.028     | 0.555     | 0.595     |           |

| Variable ($Y_{1H}$) | $R^2$ | Unstandardized coefficient | Standardized coefficient | $p$-value | $p$-value | $p$-value | $p$-value |
|---------------------|-------|----------------------------|--------------------------|-----------|-----------|-----------|-----------|
|                     |       | Constant                   | Amplitude $X_{1H}$       | Time $X_2$| Amplitude $X_{1H}$| Time $X_2$|           |
| $Y_{1H}$            | 0.508 | 89.676                     | 1.600                    | -3.645    | 0.470     | -0.535    |           |
| $Y_{2H}$            | 0.863 | -0.016                     | 0.003                    | 0.008     | 0.566     | 0.736     |           |
| $Y_{3H}$            | 0.837 | -80.896                    | 0.750                    | -0.106    | 0.912     | -0.065    |           |
| $Y_{4H}$            | 0.641 | 4.798                      | -0.016                   | 0.013     | -0.742    | 0.302     |           |
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amplitude level higher than 60 ˋ, a positive correlation of droplet size was increased on droplet size was obtained, where the mean variable. A higher absolute value of a standardized beta coefficient indicated a stronger effect of that predictor. A positive value of the coefficient indicated a positive correlation, wherein there is an inverse relationship between two variables: when the predictor increased, the response decreased, and vice versa. The interpretations of the results obtained from Table 3 are provided in Table 4.

Table 4 Interpretation of results obtained from Table 3.

| Response           | Low-level amplitude       | High-level amplitude       |
|--------------------|---------------------------|---------------------------|
|                    | Amplitude | Time | Amplitude | Time |
| Size               | Negative correlation     | Negative correlation*     | Positive correlation | Negative correlation* |
| PDI                | Positive correlation     | Positive correlation*     | Positive correlation | Positive correlation* |
| Zeta               | Negative correlation*    | Negative correlation      | Positive correlation* | Negative correlation  |
| Viscosity          | Positive correlation     | Positive correlation*     | Negative correlation* | Positive correlation  |

* Indicated a stronger effect of the predictor to variable response

Size (nm): $Y_{ih} = 1.600 \times X_{ih} - 3.645 \times X_2$

PDI: $Y_{ih} = 0.003 \times X_{ih} + 0.008 \times X_3$

Zeta potential (mV): $Y_{ih} = -80.896 + 0.750 \times X_{ih}$

Viscosity (cps): $Y_{ih} = 4.798 - 0.016 \times X_{ih}$

where $X_{ih} =$ amplitude level ranging from 60% to 90% and $X_2 =$ time ranging from 5 to 20 minutes.

Due to the different units of two predictors; thus, in this study, a standardized beta coefficient was used to compare the strength of each predictor effect on each response variable. A higher absolute value of a standardized beta coefficient indicated a stronger effect of that predictor. A positive value of the coefficient indicated a positive correlation between two variables, wherein both variables move in the same direction (i.e., when the predictor increased, the response increased and vice versa). In contrast, the negative value of the coefficient indicated a negative correlation, wherein there is an inverse relationship between two variables: when the predictor increased, the response decreased, and vice versa. The interpretations of the results obtained from Table 3 are provided in Table 4.

In conclusion, the application of the ultrasonication amplitude level of more than 60 ˋ produced a much higher PDI, an undesired characteristic for the NEs. Hence, the unsatisfactory PDI results may be influenced by excess energy input of too high of an amplitude level and also with longer ultrasonication times.

Particular energy input is required to initiate cavitation and then reduce the droplet size of the coarse emulsion. The results from this study indicated that the ultrasonic sonication time exhibited a stronger effect on size reduction when compared with the ultrasonic amplitude level. Emulsion ultrasonicated at different amplitudes revealed that the mean droplet size lessened with sonication time. However, after 10 minutes of ultrasonication, the size was not significantly reduced ($p > 0.05$), as shown in Fig. 2A. Conversely, when the sonication amplitude increased from 20% to 60%, there was a decrease in droplet size; this could be attributed to the cavitation effect and because the intensity of the cavitation activity, which is proportional to sonication amplitude. However, when the ultrasonication amplitude level higher than 60%, a positive correlation of amplitude on droplet size was obtained, where the mean droplet size was increased ($p < 0.05$) with a rise in the amplitude. The largest droplet size was obtained at the 90% amplitude level. The excess energy supply was the cause of intense turbulence and promoting a higher rate of collision between droplets, coalescence, and larger droplets sizes were the results. The rates of droplet collision in this amplitude range are higher than the drop breakage, resulting in a more significant drop-drop coalescence and an expansion in droplet size.

Time and amplitude exhibited positive correlations with PDI, mainly when a high degree of amplitude level was applied. From the regression equation of the PDI variable, the coefficient of $X_1$ increased when the amplitude level increased from low to high. This indicated that an ultrasonication amplitude level of more than 60% produced a much higher PDI, an undesired characteristic for the NEs. Hence, the unsatisfactory PDI results may be influenced by excess energy input of too high of an amplitude level and also with longer ultrasonication times.

Generally, zeta potential values that exceed 30 mV regardless of whether they are positive or negative prefix are considered as stable NEs. The zeta potential of SIO-NE droplets is negative, but they have a higher absolute value indicating better stability. The ultrasonic amplitude displayed a strong negative correlation with zeta potential at $X_{ih}$, but interestingly a strong positive correlation was obtained with amplitudes at $X_{ih}$. Time exhibited a lesser influence on the zeta potential when either $X_{ih}$ and $X_{ih}$ were applied. This implied that a decrease in the negative value of the zeta potential was obtained with increasing ultrasonic amplitude levels but not more than 60%.

Low amplitude level ($X_{ih}$) and time exhibited approximately equal-strength positive correlations with SIO-NEs viscosity. However, the stronger negative correlation to SIO-NEs viscosity was the result of a high amplitude level ($X_{ih}$) being applied. This suggested that a sonication amplitude of higher than 60% yielded lower viscosity NEs. Time had less effect on viscosity when the high amplitude level was applied. An increase in sonication amplitude resulted in increasing the ultrasonic power dissipation and overprocessing. In this situation, nearby droplets that were close to each other and then coalesced caused an increase in droplet size. Larger dispersed droplets led to a decrease in the dispersed phase volume fraction and a reduction in the viscosity of the NEs.

In conclusion, the application of the ultrasonication am-
plitude level exceeds the appropriate value; this may cause the alteration in physical droplet characteristics, producing a larger droplet size. Higher PDI, lower (absolute) zeta potential, and lower viscosity were the consequences of larger droplet size. This can be explained by the larger size that resulted in a lower particle surface area. Even though sonication time influenced SIO-NEs droplet size reduction, times longer than 10 minutes did not produce a significantly smaller droplet. Therefore, applying an ultrasonication amplitude level of 60% for 10 minutes was deemed as the optimal condition in the present study, as it produced a small droplet size, low PDI, the ideal range of the negative zeta potential and desired viscosity.

3.2 Effects of different emulsifier systems

Initially, NEs were prepared with three single nonionic surfactants including Pluronic® L-31 (Rx 1), Brij® C-10 (Rx 2), Tween® 80 (Rx 3) and two combination surfactants including Tween® 80/Pluronic® L-31 (Rx 4) and Brij® C-10/Pluronic® L-31 (Rx 5). The surfactants used in this study possess different HLB values. The emulsifier system has an impact on NEs formation as the obtained products shown in Fig. 3. The Rx 1 and Rx 3 exhibited a nonhomogeneous system as phase separation and creaming occurred within 10 minutes of initial emulsion formation; thus, they were excluded from the experimental. Conversely, the formulation of Rx 2, Rx 4, and Rx 5 demonstrated a homogeneous system. The results here suggest that the stabilization effects of Brij® C-10, Tween® 80/Pluronic® L-31, and Brij® C-10/Pluronic® L-31 were much more significant than those of Pluronic® L-31 and Tween® 80 alone. The HLB and chemical structure of these surfactants, as shown in Table 5, also affect the formation and stabilization of the NEs.

Phuronic® L 31 is a liquid nonionic surfactant of triblock copolymers of two hydrophilic ethylene oxide units, 16 hydrophobic propylene oxide units, and two more ethylene oxide units. As a result of low HLB, it cannot stabilize oil-in-water emulsions. The chemical structure displays a large and long-tailed polymer. Due to its huge structure and not enough polyoxyethylene molecules, Phuronic® L-31 could not sharply cover the entire surface of the newly formed emulsion nanodroplet. Meanwhile, Tween® 80 (or Polysorbate 80, or Polyoxyethylene(20)sorbitan monooleate) is a nonionic surfactant that is widely used as an emulsifier in pharmaceuticals. The chemical structure of Tween® 80 exhibits a large and steric hydrophilic group with a long hy-

Table 5 Chemical structure of surfactants used as an emulsifier to form SIO-NEs.

| Generic name | Commercial name | HLB value | MW (g/mol) | Chemical structural and formula |
|--------------|----------------|-----------|------------|--------------------------------|
| Polyoxyethylene (20)sorbitan monooleate | Tween® 80 | 15 | 1310 | ![Chemical structure of Tween 80](image) |
| Polyoxyethylene-(10)-cetyl ether | Brij® C-10 | 12 | 1110 | ![Chemical structure of Brij C-10](image) |
| Polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymers (PEO-,PPO-,PEO2) | Pluronic® L-31 | 4.3 | 683 | ![Chemical structure of Pluronic L-31](image) |
drophobic tail. NEs produced using Tween®80 as a single emulsifier exhibited a rapid creaming phenomenon, resulting in undesired effects. This is due to the fact that the surfactant adsorption of large and steric structures of Tween®80 onto the interface was not enough to decrease the droplet size, and there was a density difference between the two phases, promoting phase separation due to gravitational forces.

The higher emulsification ability of Brij®C-10 could be due to its lower molecular weight and smaller molecular structure and, hence, smaller molecular volume when compared to the other included surfactants. These properties facilitate adsorption of the molecules quickly onto the newly formed oil-water interface during ultrasonication to prevent coalescence of the droplets.

However, a mixture of surfactants had a more effective packing in the oil-water interfacial layer and greater interfacial film rigidity. Using the combination of Brij®C-10/Pluronic®L-31 (Rx 5) as an emulsifier yielded a droplet size of 148.19 nm, smaller than the droplet size 302.53 nm that was obtained from Tween®80/Pluronic®L-31 (Rx 4). This is explained by the huge structure, and the highest molecular weight of Pluronic®L-31 and the bulky structure of Tween®80 contained in the system of Rx 4. A higher fraction of Pluronic®L-31 in addition to a combination with a high-molecular weight surfactant increased the hydrophobicity of the surfactant system of Rx 4, resulting in a weak packing of surfactant molecules around the interface due to an imbalance between hydrophobicity and hydrophilicity.

One of the main driving forces for NEs formation is an appropriate HLB value for the surfactant used in the formulation. HLB numbers allow for the ability of the surfactant to stabilize the emulsion to be predicted. Surfactants with HLB values of between 4 and 6 generally stabilize w/o emulsions whereas HLB values of between 8 and 18 would stabilize o/w emulsions. Nevertheless, in the field of emulsion formulation, another possibility to obtain finely dispersed and physically stable emulsions is the estimation of the “required HLB.” This means that the HLB of the surfactants should match the required HLB of the oil phase as close as possible. Furthermore, different oils have different HLB requirements; the experimental HLB value of SIO is 8.5. The HLB values of Brij®C-10/Pluronic®L-31 comes out around the required HLB of SIO and produces the interfacial complex condensed film, which efficiently is packed between each other. Thus, a tightly packed surfactant film explains why mixed surfactants are often more effective than single surfactants.

Different types of emulsifier systems have displayed a significant effect regarding droplet size, PDI, and viscosity (p < 0.05) but not for zeta potential, as illustrated in Fig. 4. The mixed surfactant Brij®C-10/Pluronic®L-31 produced the smallest droplet size and lowest PDI, with an average of 148.19 ± 5.52 nm and 0.210 ± 0.01, respectively. The highest SIO-NEs viscosity was also obtained from the mixed surfactant of Tween®80/Pluronic®L-31. In terms of zeta potential, the emulsifier system did not show a significant difference between groups (p > 0.05). Hence, the use of the mixed surfactant of Brij®C-10/Pluronic®L-31 showed advantages over other emulsifier systems used in this study. Particle size and size distribution of these droplets are critical criteria for the stability of the emulsion. In general, the smaller the average droplet size and size distribution, the more stable the emulsion. The mixed surfactant of Brij®C-10/Pluronic®L-31 was selected for further study as the NEs formation.

3.3 Effect of emulsifier concentration

The emulsifier concentration is an additional crucial variable in NE preparation. A smaller droplet size produces a higher oil-water interface area, which requires more emulsifiers to completely cover the interfacial area to prevent the droplet coalescence through steric or electrostatic repulsion. In this study, the impact of a mixed surfactant (Brij®C-10/Pluronic®L-31) concentration in the range of 1.5% to 9% was investigated by preparing a series of emulsions with a fixed 5% (w/w) oil concentration and Smixed ratio of 60:40 (% w/w) as described in Table 2.

![Fig. 4 Influence of surfactants system of SIO-NEs on A) Average droplets size and PDI and B) Viscosity and zeta potential. Values are presented as means ± SDs (n = 3).](image-url)
As the results are shown in Fig. 5, increasing the concentration of the emulsifier from 1.5% to 5% w/v resulted in a significant reduction (p<0.05) of average droplet size, in agreement with previous studies. This circumstance is ascribed to several reasons, such as the reduction in interfacial tension with an increasing surfactant concentration and the surfactant diffusion into the aqueous phase facilitating the formation of fine droplets. Also, at low surfactant concentrations, the droplet size is independent of the energy input because the surfactant concentration is insufficient to stabilize smaller droplets. Further increasing the surfactant concentration to 7% or 9% influenced an increase in average droplet size and also caused heterogeneity in the overall droplet size; thus, leading to an increase in the PDI. It is possible that the high surfactant concentrations exceeded the certain surfactant level where a highly viscous liquid around the droplet phase was formed and could make spontaneous breakup of the oil-water interface more difficult. Consequently, the optimum surfactant concentration required to form uniformly dispersed droplets must be resolved for each oil, surfactant, and water composition.

The observed values of zeta potential were found to vary with increasing emulsifier concentration; it was in the range of −20.86 to −34.10 mV. This range in zeta potential is suitable to produce physically stable SIO-NE formulations. Therefore, it is essential to determine the appropriate surfactant concentration and the lowest concentration that should be used in the formulation. Furthermore, the use of the lowest suitable surfactant concentration also results in a low PDI. Based on the goal of NEs formation and given that a minimum droplet size with maximum stability is desired, the suitable concentration of the mixed surfactant used was deemed to be 3% (w/v). Thus, the stability of the NEs formulation was further studied and measured.

3.4 Physical stability of SIO-NEs

In the present study, SIO-NEs were prepared at a concentration of 3% (w/v) of Brij® C-10/Pluronic® L-31. The physical stability of NE was determined by monitoring the droplets' size, PDI, zeta potential, and viscosity over six months in a storage room. As shown in Fig. 6A, slight increases in droplet size and PDI were observed over 30 days. The observed constant droplet size and the slight in-
crease of other physical characteristics are a sign of their physical stability for the duration of 90 days. After the 90th day, the NEs were resized; that was most probably due to the interaction of NE molecules. Based on collision theory, the small droplets are collided and developed a reasonably large droplet size. Fortunately, the developed size of the droplets was within the range of the defined NE droplet size, and there was no phase separation after 180 days. As with the increase in the droplet size, decreasing the absolute values of the zeta potential and viscosity resulted, as shown in Fig. 6B. A slight decrease in the viscosity and zeta potential values was seen at the end of 45 days. However, all the physical characteristics remained relatively stable over the following days. This can be attributed to the presence of a surfactant that adsorbed onto the oil droplet surface, forming a protective layer that helped to hinder the droplets from being recoalesced through a steric repulsive force.

4 Conclusions
The objective of this study was to produce SIO-NEs with a small average droplet size, a low PDI value, a high zeta potential, and an attractive viscosity. To our knowledge, no prior research has been reported on the subject of the preparation of SIO-NEs, using the ultrasonic emulsification method. In this work, the impact of ultrasonication mode, ultrasonication amplitude level (%), the ultrasonication duration time, the type of emulsifier system, and the emulsifier concentrations were also studied. Determining the optimum range of all parameters was critical to producing the desired droplet properties. Excess ultrasonication energy applied or excess surfactant concentration present created adverse effects on the droplet properties. The emulsifiers system also had an impact on NEs formation. Thus, the suitable emulsifier systems and concentration should be obtained from individual experiments for each oil formulation. The mixed surfactant ratio and concentration showed an influence on the physical properties and stabilities of NEs. Therefore, further studies are needed to develop more stable NEs. This study revealed that using nonionic surfactants could be a good option when preparing SIO-NEs using an ultrasonic emulsification method. Further, alteration in a different type of emulsifier used when designing SIO-NEs as delivery systems could be considered.

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Conflict of Interest
There is no conflict of interest whatsoever in this study.

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