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Ultrasonic modification for stabilizing *Lantana camara* nanosuspension emulsification

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ABSTRACT

The preparation of emulsification by low-energy phase inversion is an appropriate method for providing a nanosuspension of Lantana camara Ethyl Acetate Fraction (EAF) dispersed in water. In the formulation, the challenge faced is preventing the re-agglomeration of nanosuspension and stabilizing the formula over the storage period. This study reports the preparation of the *L. camara* EAF in water media using a low-energy phase inverse emulsion method with the modification of ultrasonic applications. The low-energy ultrasonic application effectively breaks the larger clusters of *L. camara* EAF suspension into a nanosuspension, allowing for a 77% increase in the distribution of primary size particles (8.3 ± 1.3 nm) and receiving a higher fraction of nano sized particles dispersed in the water solvent. It showed by the increase of zeta potential and the reduction of index polydispersity (Z = –8.5 mV, PI = 0.665) after sonicated. Optimal ultrasonication of the prepared *L. camara* EAF nanosuspension is achieved with a 50% amplitude vibration maintained for 60 minutes, which can effectively control the nano-size particle dispersion as well as improve the stability of the suspension. The *L. camara* EAF nanosuspension also maintained stability over the 60 days of storage with re-stirring and re-ultrasonic agitation. The effective ultrasonic application was critical in controlling the size distribution and stability of the prepared *L. camara* EAF nanosuspension through low-energy emulsification.

Keywords: *Emulsification, Lantana camara EAF, Low energy-phase inversion, Nanosuspension, Ultrasonication*

1. INTRODUCTION

Emulsification techniques are employed in biopesticide formulations to ensure optimal efficacy [1–2]. Among these techniques, ultrasonic homogenization is considered an energy-efficient method for dispersing solid or insoluble bioactive compounds within an aqueous system via reverse emulsion [3–4]. Compared to high-pressure homogenizers, the low-energy method utilizing high-intensity ultrasonication is favored due to its environmental friendliness and cost-effectiveness. Moreover, the mechanical agitation induced by ultrasonication facilitates the creation of fine and stable emulsion droplets [2–3]. In a previous study, we employed low-energy phase inversion emulsification for *L. camara* EAF [5]. Tween 80 served as the surfactant, with appropriate variations in the organic-phase composition, resulting in a higher fraction of nanosized particles dispersed in the aqueous system. To address this issue, it is essential to investigate suitable ultrasonication treatments to enhance the stability of the emulsion.

Studies on nanofluid preparation using various ultrasonication processes have identified specific treatments to achieve optimal emulsion stability, such as optimizing the duration and amplitude of ultrasonication [6]. It has been emphasized that controlling the ultrasonication period is crucial for enhancing mechanical performance by breaking agglomerations and improving particle dispersity [6–7]. The effectiveness of a biopesticide formula is also influenced by its storage stability [8]. During storage, the formula may encounter various instability mechanisms, such as re-agglomeration, flocculation, and phase separation, which can affect its performance in emulsion-based products [2]. Therefore, efficient emulsifiers and mechanical agitation are necessary to produce a stable emulsion [9]. Additionally, it is important to monitor the stability of the *L. camara* EAF nanosuspension throughout the storage period.

Therefore, studies were conducted to investigate the particle distribution and stability of *L. camara* EAF nanosuspension under various ultrasonication treatments to determine the optimal conditions for reverse emulsification. Additionally, the research aimed to assess the particle distribution and stability of *L. camara* EAF nanosuspension over time during storage. Ensuring the stable performance of *L. camara* EAF nanosuspension in an aqueous system could enhance its efficacy against target insect pests and support the formulation of an efficient and cost-effective biopesticide.

2. MATERIALS AND METHODS

2.1. Materials

Lantana camara was obtained from the Arboretum of Universitas Padjadjaran, Jatinangor, West Java, Indonesia. Ethanol was purchased from Merck (EMSURE®, 99.9%), and n-hexane, ethyl acetate, and Tween 80 were purchased from Bratachem (Bandung, Indonesia).

The crude extract was prepared from *L. camara* leaves, which were macerated in 95% ethanol for 3–5 days. The filtrate was then evaporated using a vacuum evaporator (Buchi®-R-300) at 45°C and 100 atm to obtain a paste-like crude extract. The *L. camara* Ethyl Acetate Fraction (EAF) was obtained from the ethyl acetate partition (semi-polar solvent), which had previously been partitioned with non-polar compounds using *n*-hexane.

The *L. camara* EAF nanosuspension was prepared using the phase inversion emulsion method [5, 11]. The composition of the surfactant (Tween 80) and the *L. camara* EAF (insoluble liquid) represents the respective surfactant and organic phase (SOR 9, 11, 12, 14). Variations in SOR composition and the preparation procedure of *L. camara* EAF nanosuspension, based on the reverse emulsion method [5].

2.2. Characterizations

The particle size distribution and dispersion stability of the *L. camara* EAF nanosuspension were characterized using dynamic light scattering for particle size and zeta potential analyze (HORIBA® SZ-100) at 25°C. Ultrasonication treatment was performed using a Fischer®-100 device with a power input of 240 W, 2 A, and a frequency of 50 kHz. The effect of sonication on the nanosuspension was examined at various ultrasonic vibration amplitudes (0%, 25%, and 75%) and at different sonication times (0, 30, 60, 120, and 180 minutes). The optimal sonication treatment was identified for standard application. Furthermore, the *L. camara* EAF nanosuspension was stored in a container shielded from light exposure at room temperature (25°C). The size distribution and stability of the *L. camara* EAF nanosuspension were monitored at 0, 15, 30, 45, and 60 days of storage to assess its stability against sedimentation. Re-sonication was performed to re-disperse the nanosuspension stored for 30 and 60 days, treated with additional sonication to break up and re-disperse the agglomerated suspension.

3. RESULTS AND DISCUSSION

3.1. Comparison of Particle Distribution and Stability of *L. camara* EAF Suspension Before and After Ultrasonic Agitation

Ultrasonic agitation was essential for dispersing the suspension during emulsification. In this study, *L. camara* EAF SOR 11 and ultrasonic agitation were applied according to the reverse emulsion method [5]. The particle size distribution was observed before and after ultrasonic treatments. The results indicate that ultrasonic treatment effectively breaks up the agglomerate suspension of secondary particle clusters of *L. camara* EAF post-emulsion, leading to a 77% increase in the distribution of primary-sized particles (8.3 ± 1.3 nm) and improved dispersion of particles in the nanosuspension (PI = 0.589) (Figure 1).

3.2. Distribution of Particles and Stability of *L. camara* EAF Nanosuspension with Varying Ultrasonication Agitation

The test results of various sonication amplitude treatments were 0%, 25%, and 50% (Figure 2), showing ultrasonication at 50% vibration amplitude is optimal for breaking up agglomerates, resulting in the smallest mean diameter size (79.43 ± 17.85 nm) and the highest observed zeta potential (Z = –8.3 ± 1.07 mV) compared to other amplitudes. However, the mean diameter size rises again at 75% vibration amplitude.

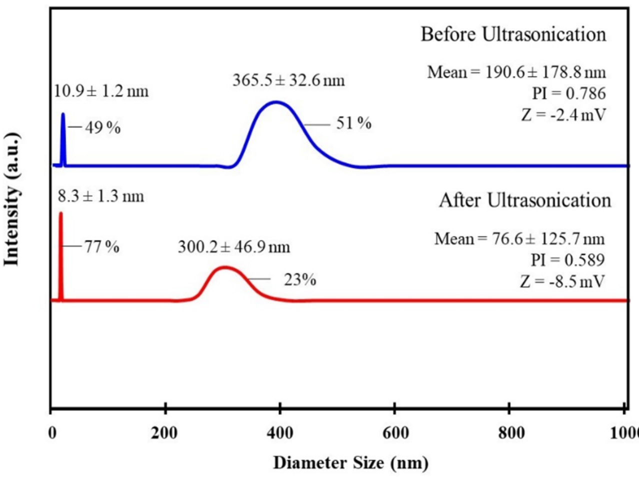

Figure 1. Comparison of the mean diameter size distribution and polydispersity index (PI) of *L. camara* EAF SOR 11 before and after ultrasonic treatments

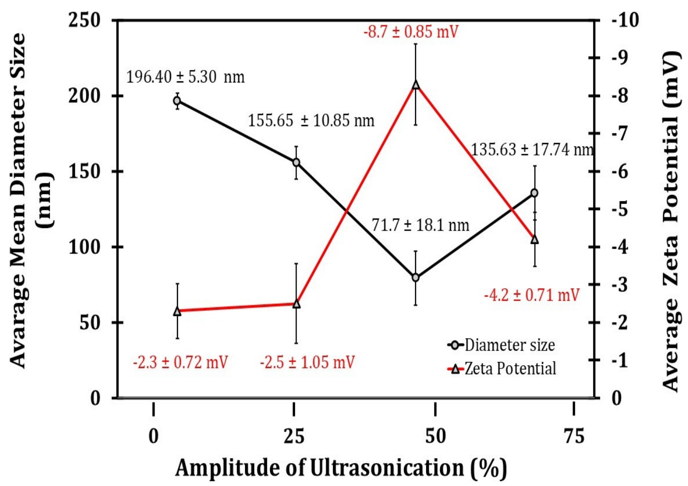

Figure 2. Mean particle diameter and zeta potential of EAF. *L. camara* SOR 11 with varying vibration amplitudes modified by ultrasonic treatments

This increase in particle size at 75% amplitude may be attributed to the excessive cavitation energy generated at higher vibration intensities. Although ultrasonic cavitation is essential for de-agglomeration, an excessively high amplitude can induce unstable acoustic streaming and localized overheating, which promote particle collisions and re-aggregation. Such conditions may also disrupt interactions between particles and stabilizing agents, reducing overall dispersion stability. Consequently, the efficiency of particle size reduction becomes sub-optimal beyond the optimal amplitude threshold [11].

Furthermore, ultrasonic treatment at a 50% vibration amplitude was conducted to observe the effect of various sonication times (0, 30, 60, 120, and 180 minutes) on the mean particle diameter of the suspension. The results indicate that a sonication time of 60 minutes was optimal for breaking up agglomerates, resulting in the smallest mean diameter size (71.7 ± 18.1 nm) and the highest observed zeta potential (Z = –8.7 ± 0.85 mV) compared to others. However, the mean diameter size slightly increased at 120 minutes of sonication. Therefore, it is noted that higher vibration amplitudes (>50%) and sonication times longer than 60 minutes can lead to re-agglomeration of the EAF nanosuspension (Figure 3).

The fact that sonication amplitudes above 50% and durations longer than 60 minutes lead to re-agglomeration indicates the existence of an optimal threshold in the ultrasonication process of *L. camara* EAF nanosuspensions. Excessive ultrasonic energy may disrupt particle stability, promote collisions, and weaken interactions with stabilizing agents, resulting in particle re-agglomeration. This highlights the importance of carefully optimizing processing parameters to produce stable nanoparticles efficiently for formulation purposes.

Ultrasonic vibration is one of several techniques used to break up particle clusters when preparing a stable colloidal system [12]. Particle clusters need to be reduced to finer sizes (nano-size) because the nanoscale particles are more stable due to their low mass. However, if the particles aggregate, they will rapidly sediment due to gravitational attraction [13]. Therefore, ultrasonication at 50% amplitude for 60 minutes was effectively applied to prepare the *L. camara* EAF nanosuspension. This process successfully controlled the particle size distribution and slightly increased the zeta potential of the nanosuspension.

3.3. Stability of *L. camara* EAF Nanosuspension After Ultrasonication During Storage Time

The *L. camara* EAF nanosuspension, modified by emulsification with SOR and ultrasonication, improved particle dispersion and wettability on the surface of cabbage leaves. Another challenge is to confirm that the formula remains stable for re-use after prolonged storage. Therefore, particle size distribution and dispersion were characterized after a shelf-life period. In this study, the optimal formula of *L. camara* EAF SOR 11 was used, and the results are presented in Figures 4 and 5.

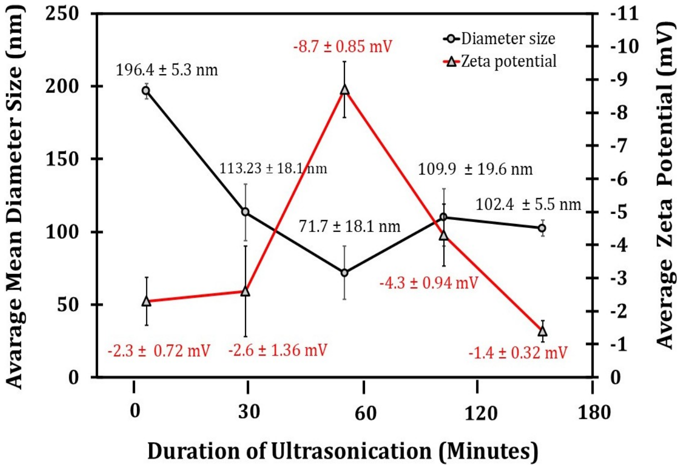

Figure 3. The mean particle diameter and zeta potential of EAF. *L. camara* SOR 11 with varying duration time modified by ultrasonic treatments

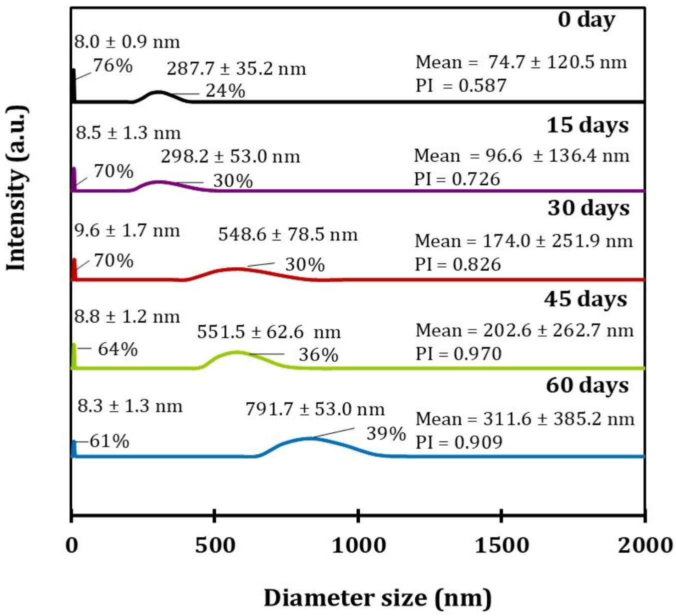

Figure 4. Distribution of mean particle diameter and Polydispersity Index (PI) of *L. camara* EAF nanosuspension (SOR 11) at 15-day intervals over a 60-day storage period

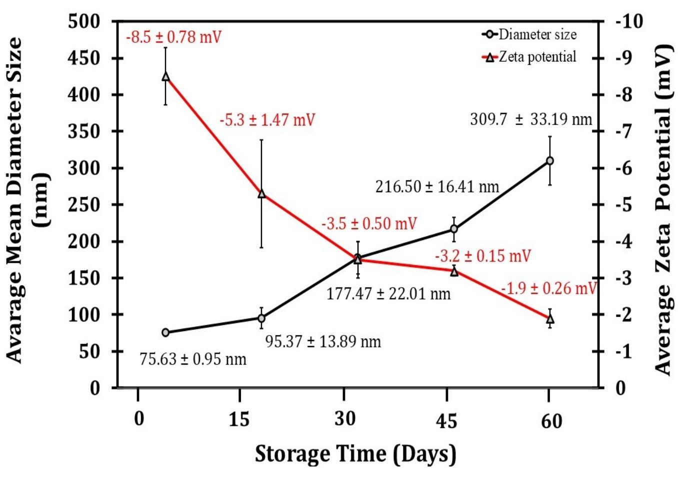

Figure 5. Plot of mean particle diameter size and zeta potential of *L. camara* EAF nanosuspension (SOR 11) during storage duration

Agglomeration of the *L. camara* EAF SOR 11 nanosuspension occurred, particularly in the clusters of secondary particles, after 60 days of storage. However, the primary particles remained relatively stable within the nano-size range (8.0–9.6 nm). The percentage of primary particle distribution gradually decreased by 9% (from 70% to 61%), and the secondary particles increased by 15% (from 24% to 39%), indicating re-agglomeration of nano-sized particles into clusters of secondary particles (micro-sized suspension) (Figure 4).

However, the particle size plot shows that the average mean diameter of *L. camara* EAF remains below the sub-micron size (< 0.5 µm) even after 60 days of storage (Figure 5).

In general, the surface area of particles contributes to the total surface energy, causing particles to agglomerate to minimize this energy [14]. This tendency to agglomerate aims to achieve stability, as evidenced by the decreasing average zeta potential (from −8.5 ± 0.78 mV at 0 days to −1.9 ± 0.78 mV at 60 days). Particle dispersions with a zeta potential of less than ± 25 mV are likely to agglomerate eventually [7]. Ultrasonic vibration is an effective method for maintaining the stability of nanosuspension [12]. Therefore, treatments involving stirring and ultrasonication are necessary to ensure formula stability during storage.

3.4. The Effect of Stirring and Ultrasonication on the Stability of Particle Diameter Size in *L. camara* EAF Nanosuspension at Different Storage Times

After 60 days of storage, *L. camara* EAF SOR 11 showed a tendency for larger particle clusters to agglomerate, as evidenced by an increase in the polydispersity index. After 60 days of storage, *L. camara* EAF SOR 11 showed a tendency for larger particle clusters to agglomerate, as evidenced by an increase in the polydispersity index. To overcome this, mechanical agitation, such as re-stirring or re-ultrasonication, is necessary to redisperse the agglomerated particles. Re-ultrasonication treatment was conducted using the optimal setting of 50% vibration amplitude for 60 minutes to restore particle dispersion.

Stirring for 30 minutes was applied based on a standard method from a previous formulation study, where it was shown to be effective in re-dispersing similar nanosuspensions. Therefore, alternative stirring durations were not examined in this study. The results demonstrate that mechanical agitation, consisting of stirring and re-ultrasonication applied at 30-day intervals, can effectively restore the nanosuspension to its initial condition (day 0), as indicated by a reduction in mean particle diameter and polydispersity index (PI) following these treatments (Figure 6).

The formula's relatively stable condition is demonstrated by the average mean diameter size reduction and the zeta potential increase following stirring and ultrasonication on days 30 and 60 (Figure 7).

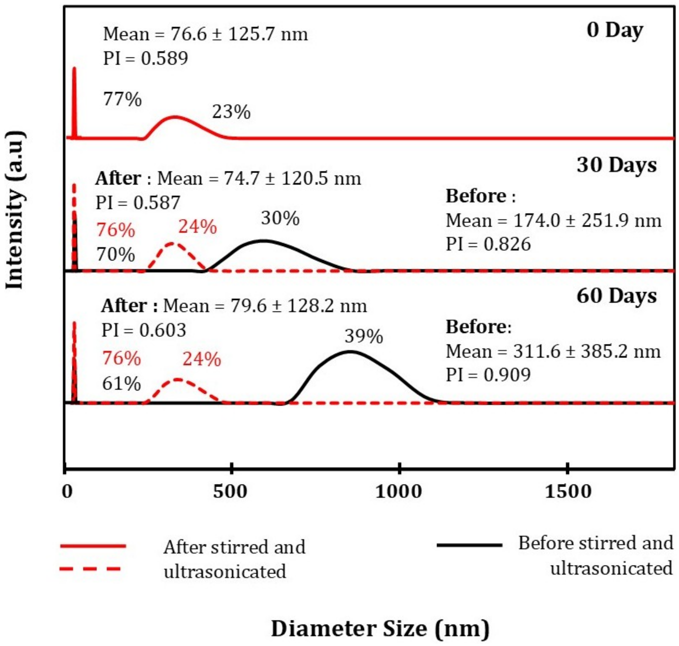

Figure 6. Comparison of mean diameter size distribution and polydispersity index (PI) of *L. camara* EAF suspension (SOR 11) before and after storage (0, 30, and 60 days) with re-stirring and re-ultrasonication treatments

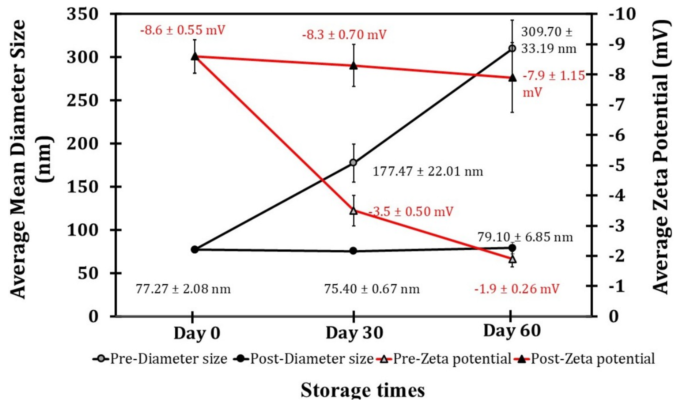

Figure 7. Plot of mean particle diameter size and zeta potential of *L. camara* EAF suspension (SOR 11) after storage times of 0, 30, and 60 days with re-stirring and re-ultrasonication treatments

Low-energy emulsification methods utilize lower internal chemical energy, generally producing small particle sizes with simple stirring [15]. Ultrasonication is an effective technique for achieving suspension stability, as it helps prevent the formation of particle clusters and ensures dispersed nanosuspension [12]. Therefore, in addition to the role of surfactants with suitable composition, stirring and ultrasonication are key factors in maintaining the stability of *L. camara* EAF nanosuspension.

4. CONCLUSION

Ultrasonication proved effective in regulating particle size distribution, promoting re-dispersion, and improving the overall stability of the nanosuspension. The treatment resulted in a 77% increase in the proportion of primary-sized particles (8.3 ± 1.3 nm) and a greater fraction of nanoparticles stably dispersed in the aqueous medium (Z = –8.5 mV; PI = 0.665). Mechanical agitation, consisting of 30 minutes of stirring followed by ultrasonication at 50% amplitude for 60 minutes, was identified as the optimal method for dispersing *L. camara* EAF nanosuspension. This combined approach effectively restored the agglomerated suspension to a dispersion state similar to that observed after 60 days of storage, as indicated by the reduction in particle size from 311.6 ± 385.2 nm (PI = 0.909) to 79.6 ± 128.2 nm (PI = 0.603).

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