

# Effect of Centrifugation on Nanoemulsion Stability with Tween, PEG, Poloxamer 188, and Transcutol-p

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## ABSTRAK

Penelitian ini bertujuan mengevaluasi pengaruh variasi kecepatan sentrifugasi terhadap stabilitas fisik basis nanoemulsi berbasis grapeseed oil (LCT) yang distabilkan kombinasi Tween 80-PEG 400, Poloxamer 188, dan Transcutol-P untuk potensi aplikasi topikal maupun oral. Metode yang digunakan adalah deskriptif-eksperimental; basis nanoemulsi disiapkan melalui metode titrasi air diikuti sonikasi 60 menit. Empat formula (F1-F4) mengandung grapeseed oil 5% dan Poloxamer 188 2%, dengan variasi konsentrasi Tween 80/PEG 400 serta Transcutol-P (0,5-2%). Uji stabilitas dipercepat dilakukan menggunakan sentrifugasi pada 2000, 4000, 6000, dan 8000 rpm selama 30 menit, kemudian dievaluasi secara organoleptik, tipe emulsi, pH, dan viskositas. Hasil menunjukkan seluruh formula stabil pada 2000 rpm; pada 4000 rpm F1-F2 mulai menunjukkan incipient creaming (lapisan tipis) sedangkan F3-F4 tetap stabil. Pada 6000 rpm, F1-F2 semakin keruh dan mulai terbentuk lapisan minyak, sementara F3-F4 tidak menunjukkan pemisahan fase. Pada 8000 rpm, F1-F2 mengalami pemisahan fase total, F3 membentuk lapisan minyak ringan, dan F4 paling stabil dengan turbidity minimal tanpa pemisahan total. Seluruh formula bertipe oil-in-water (O/W); pH berada pada 6,0-7,0 (rerata 6,45 ± 0,42) dan viskositas 176-220 cP (rerata 196,25 ± 18,89). Disimpulkan bahwa peningkatan kecepatan sentrifugasi memperjelas perbedaan ketahanan stabilitas fisik antar formula, dengan F4 sebagai formula paling resisten terhadap stres mekanik hingga 8000 rpm.

**Kata Kunci:** Nanoemulsi; Sentrifugasi; Tween 80; PEG 400; Poloxamer 188.

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## ABSTRACT

This study evaluated the effect of centrifugation speed on the physical stability of a nanoemulsion base formulated with grapeseed oil (long-chain triglyceride, LCT) and stabilized using Tween 80-PEG 400, Poloxamer 188, and Transcutol-P, as a preliminary platform for topical and oral pharmaceutical applications. A descriptive-experimental design was employed. Nanoemulsion bases were prepared by the aqueous titration method followed by 60-minute sonication. Four formulations (F1-F4) contained 5% grapeseed oil and 2% Poloxamer 188, while Tween 80/PEG 400 levels and Transcutol-P (0.5-2%) were varied. Accelerated physical stability was assessed by centrifugation at 2000, 4000, 6000, and 8000 rpm for 30 minutes, followed by organoleptic observation, emulsion type determination, pH measurement, and viscosity testing. All formulations remained visually stable at 2000 rpm. At 4000 rpm, F1-F2 showed increased turbidity with a thin top layer (incipient creaming), whereas F3-F4 remained stable without phase separation. At 6000 rpm, F1-F2 became more turbid with the onset of a surface oil layer, while F3-F4 maintained stability. At 8000 rpm, F1-F2 exhibited complete phase separation; F3 showed a slight oil layer; and F4 was the most stable, showing only minimal turbidity without total phase breaking. All formulations were classified as oil-in-water (O/W). The pH ranged

from 6.0 to 7.0 (mean  $6.45 \pm 0.42$ ) and viscosity from 176 to 220 cP (mean  $196.25 \pm 18.89$ ). In conclusion, increasing centrifugation speed effectively differentiated formulation robustness, with F4 demonstrating the highest resistance to mechanical stress up to 8000 rpm.

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**Keywords:** Nanoemulsion; Centrifugation; Tween 80; PEG 400; Poloxamer 188.

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## 1. Introduction

Nanoemulsions are colloidal dispersion systems composed of two immiscible phases, oil and water, stabilized by a combination of surfactants and co-surfactants, producing droplet sizes ranging from 20 to 200 nm. This system has gained significant interest due to its capability to enhance the solubility of lipophilic drugs, improve bioavailability, and promote better penetration of active compounds into target tissues (1,2). Compared to conventional emulsions, nanoemulsions exhibit smaller droplet sizes that render them transparent or semi-transparent, providing higher kinetic stability and more appealing aesthetics for both pharmaceutical and cosmetic applications (3).

Although kinetically stable, nanoemulsions remain thermodynamically unstable and prone to several physical instabilities such as creaming, flocculation, coalescence, and Ostwald ripening, which may lead to phase separation over time (4,5). Among these mechanisms, Ostwald ripening is recognized as the predominant cause of droplet growth. It occurs due to differences in Laplace pressure between small and large droplets, resulting in the diffusion of oil molecules from smaller (high-pressure) to larger (low-pressure) droplets, thereby increasing droplet size progressively (6). The rate of this process depends on the oil's solubility in water, the chain length of fatty acids, and the type of surfactant and co-surfactant employed (7).

The choice of the oil phase plays a crucial role in controlling Ostwald ripening. Long-chain triglycerides (LCTs) such as olive oil or grapeseed oil exhibit lower solubility in water, thus minimizing oil molecule diffusion and enhancing the physical stability of the system (8,9). Furthermore, nonionic surfactants such as Tween 80, when combined with hydrophilic co-surfactants like PEG 400, effectively reduce interfacial tension and facilitate the formation of stable nano-sized droplets. Nonionic surfactants are also preferred for their low toxicity and high biocompatibility compared to ionic surfactants (10).

Additional stabilizers, such as Poloxamer 188, are often incorporated to reinforce the interfacial film through steric stabilization, thereby reducing the likelihood of droplet flocculation and coalescence during storage or mechanical stress (11). Meanwhile, Transcutol-p (diethylene glycol monoethyl ether) acts as a cosolvent and penetration enhancer, improves the solubility of lipophilic drugs, and enhances system dispersion (11). However, excessive amounts of Transcutol-p may alter system polarity and accelerate Ostwald ripening if the surfactant-to-co-surfactant ratio is not optimized (12).

In addition to formulation parameters, processing conditions also significantly influence nanoemulsion stability. Centrifugation testing is one of the most widely used accelerated methods for evaluating a system's physical robustness. This test simulates multiple gravitational accelerations to rapidly predict the tendency of creaming or phase separation that might occur during storage (13).

Therefore, the present study aims to evaluate the effect of varying centrifugation speed on the physical stability of a nanoemulsion base formulated with Tween 80, PEG 400, Poloxamer 188, and Transcutol-P using long-chain triglycerides

(LCT) as the oil phase. The findings are expected to provide deeper insights into the relationship between processing conditions and system stability, as well as to serve as a foundation for developing stable and effective nanoemulsion formulations for both topical and oral pharmaceutical applications.

## 2. Research Methodology

This study employed a descriptive experimental approach to formulate and evaluate the physical stability of a nanoemulsion base. The nanoemulsion was prepared using Tween 80 and PEG 400 as surfactant and cosurfactant, with Poloxamer 188 as a stabilizer and Transcutol-P as a cosolvent, while grapeseed oil served as the oil phase. The nanoemulsion base was produced by the aqueous titration method followed by sonication to obtain a homogeneous nanoscale dispersion.

### Materials

All materials used were of pro-analytical (PA) grade, including distilled water, 95% ethanol, Poloxamer 188 (Sigma-Aldrich), Polysorbate 80 (Tween 80, Sigma-Aldrich), grapeseed oil, Polyethylene glycol 400 (PEG 400, Sigma-Aldrich), and Diethylene glycol monoethyl ether (Transcutol-p, Chemical Bull).

### Preparation of Nanoemulsion Base

**Table 1.** Composition of the nanoemulsion base

Materials	Concentration (%)			
	F1	F2	F3	F4
<i>Grapeseed oil</i>	5	5	5	5
Tween 80	27,5	27,5	30	30
PEG 400	30	30	32,5	32,5
Poloxamer 188	2	2	2	2
Transcutol-p	0,5	1	1	2
Aquadest	Ad 50 ml			

The preparation began by weighing the required amount of Poloxamer 188 and dissolving it in 3 mL of 95% ethanol. Tween 80 and PEG 400 were measured at the specified concentrations, mixed in a beaker, and stirred for 10 minutes until homogeneous. Grapeseed oil was then added and stirred for another 10 minutes. Distilled water was added dropwise from a burette while continuously stirred until approximately half of the total water volume had been incorporated. The Poloxamer 188 solution was added gradually, followed by the slow addition of Transcutol-p using a micropipette after three-fourths of the total water volume had been introduced.

The resulting nanoemulsion base was subjected to sonication for 60 minutes to remove air bubbles formed during the mixing process. A sonication duration of 60 minutes has a significant effect on the stability of nanoemulsions. Nanoemulsions subjected to longer sonication times exhibited smaller droplet sizes and greater stability during storage compared to those treated with shorter sonication durations. This effect is attributed to more intense ultrasonic cavitation, which effectively disrupts droplet aggregates into finer droplets and enhances system homogeneity. Consequently, the resulting droplet structure becomes physically more stable immediately after production, thereby minimizing the risk of coalescence and phase separation during the early stages of storage (14). Afterward, samples were centrifuged at 8000 rpm for 30 minutes to assess initial stability prior to further physical evaluation. Centrifugation at high speeds is used as an accelerated physical stability test since it

amplifies the effects of gravitational force, allowing rapid screening for phase separation, creaming or sedimentation (15).

### **Centrifugation Procedure**

Each formulation was subjected to centrifugation at four different speeds : 2000, 4000, 6000, and 8000 rpm for 30 minutes each. This process served as an accelerated stability test, simulating increased gravitational forces to predict long-term stability and identify potential creaming or phase separation tendencies within the nanoemulsion system.

### **Evaluation of the Formulation**

#### **Organoleptic Test**

Organoleptic evaluation included the observation of color, odor, and the presence of phase separation in the nanoemulsion stored at room temperature (25°C) for two weeks (16).

#### **pH Test**

pH measurement was conducted to ensure that the nanoemulsion remained within a safe and stable range for formulation. The pH value was determined using a pH meter. For oral applications, an ideal nanoemulsion should have a pH range of 6.5–9.0, as formulations within this range remain clear and stable without flocculation, aggregation, phase separation, or turbidity. In contrast, formulations with a pH of 4.0–6.0 tend to exhibit reduced clarity and signs of physical instability (16).

The pH electrode was immersed in the sample at room temperature (approximately 25°C), and the pH value displayed on the instrument was recorded. For topical formulations, the optimal pH range is 4.5–6.5, which is compatible with the natural pH of the skin (17).

#### **Viscosity Test**

The viscosity of the nanoemulsion was measured using a Brookfield viscometer. The sample cup was removed from the instrument and filled evenly with the nanoemulsion, ensuring that no air bubbles were present to avoid measurement distortion. The cup was then reattached to the viscometer, and the instrument was operated until a stable viscosity reading was obtained (18). The displayed value was recorded as the viscosity of the formulation. In general, ideal semisolid formulations exhibit viscosity values in the range of 500–5000 cP (19).

#### **Emulsion Type Test**

The determination of emulsion or emulgel type was conducted to identify whether the system formed was an oil-in-water (O/W) or water-in-oil (W/O) emulsion. This analysis was performed using a dye method by adding a water-soluble dye, such as methylene blue, to the formulation. In O/W-type emulsions or emulgels, oil droplets are dispersed within the aqueous phase; therefore, the water-soluble dye interacts with the continuous phase, resulting in a homogeneous appearance of the sample (17).

## **3. Results and Discussion**

### **Centrifugation Results**

Centrifugation testing was employed as an accelerated method to evaluate the physical stability of the nanoemulsion system. The test simulates increased gravitational force over a short period, allowing the detection of instability phenomena such as creaming, flocculation, coalescence, or phase separation. The response of nanoemulsions to centrifugal stress is largely influenced by droplet size, phase density

difference, and the interfacial film strength formed by the surfactant-co-surfactant combination. High-speed centrifugation testing is effective as an accelerated stability method to predict the long-term stability of nanoemulsions, in which physically stable formulations do not exhibit phase separation after centrifugation (15). Nanoemulsions with smaller droplet sizes and stronger interfacial films tend to be more resistant to mechanical stress induced by centrifugation, thereby showing no significant physical changes (20).

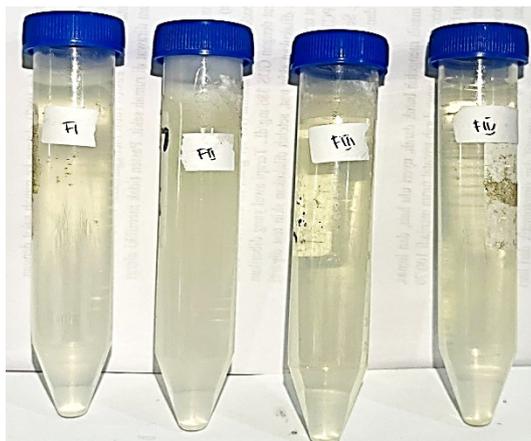


**Figure 1.** Centrifugation Test Results at 2000 rpm

The physical stability test conducted at a centrifugation speed of 2000 rpm revealed that all nanoemulsion formulations remained visually stable. At this relatively low speed, the centrifugal force generated was insufficient to cause separation between the oil and aqueous phases. Macroscopically, all formulations appeared homogeneous with no visible oil layer on the surface. Can be seen in figure 1

Formulations F1 and F2 exhibited a slightly turbid appearance with higher viscosity, indicating larger droplet sizes and less uniform distribution. Although no phase separation occurred, the interfacial protection in these two formulations was not fully optimal. At low rotational speeds, the mechanical force was unable to overcome Brownian motion and the steric barrier formed by nonionic surfactants. This observation is consistent with previous findings, which reported that centrifugal forces below 3000 rpm cannot penetrate the steric protection layer formed by nonionic surfactants (21).

In contrast, F3 and F4 displayed clearer and more uniform dispersions with no sign of flocculation or phase separation. The combination of Tween 80 and PEG 400 effectively reduced interfacial tension, leading to smaller and more stable droplets. Poloxamer 188 contributed to steric stabilization, while Transcutol-p enhanced oil solubility in the aqueous phase, resulting in a homogeneous system with improved resistance to mild mechanical stress. Therefore, at 2000 rpm, all formulations remained kinetically stable, with F3 and F4 showing the highest degree of stability compared to the others (22).



**Figure 2.** Centrifugation Test Results at 4000 rpm

Figure 2 presents the observation of nanoemulsion samples subjected to centrifugation at 4000 rpm. Increasing the centrifugation speed to 4000 rpm amplified the centrifugal force acting on the system by approximately fourfold compared to the previous stage. This condition initiated the onset of creaming or droplet accumulation in the upper layer, particularly in systems where droplet size distribution remained relatively broad. However, nanoemulsions with smaller droplet sizes (<300 nm) and low PDI values generally maintained good stability. This stability was attributed to the steric hindrance effect of Poloxamer 188 and the low interfacial tension generated through the synergistic action of Tween 80 and PEG 400, which together prevented phase separation (23). Furthermore, Transcutol-p contributed by maintaining solubility and viscosity, thereby minimizing the density difference between the oil and aqueous phases and slowing droplet migration during centrifugation. Poloxamer 188 also played a significant role in enhancing the viscosity and strengthening the interfacial film, which overall improved the formulation's resistance to moderate centrifugal stress (23). Can be seen in figure 2

Formulations F1 and F2 exhibited increased turbidity accompanied by a thin layer of separation at the top (indicating incipient creaming). This observation implies that larger droplet sizes create greater density contrasts between phases, facilitating droplet migration toward the surface. The relatively high interfacial tension resulting from the low surfactant ratio caused the protective film surrounding the droplets to lose elasticity and become incapable of resisting deformation from external forces. Droplets with diameters exceeding 400 nm tend to undergo rapid creaming under moderate centrifugal stress, as the density difference between phases increases exponentially with droplet size (24).

Meanwhile, formulations F3 and F4 maintained visual stability with no signs of phase separation. This finding confirms that the interfacial film produced by combining Tween 80 and PEG 400 was sufficiently strong to preserve droplet integrity. Poloxamer 188 contributed to slowing droplet migration by increasing the viscosity of the dispersion medium, while Transcutol-P reduced interfacial tension, resulting in a system resistant to moderate mechanical stress. Overall, Poloxamer 188 enhanced the nanoemulsion's resistance to medium centrifugal force through both steric stabilization and viscosity enhancement effects (23).

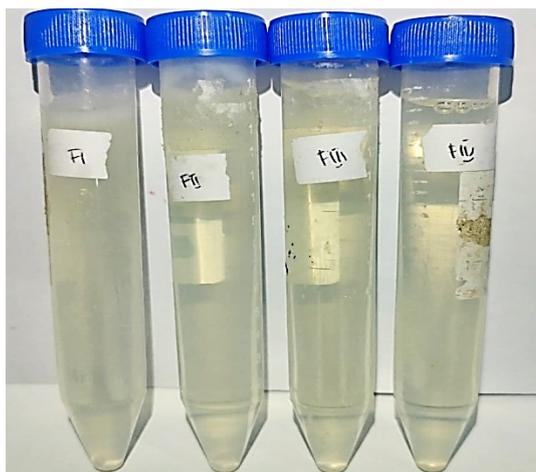


**Figure 3.** Centrifugation Test Results at 6000 rpm

Figure 3 depicts the nanoemulsion samples subjected to centrifugation at a speed of 6000 rpm. Under these conditions, the generated centrifugal force was strong enough to evaluate the system's stability threshold. When the interfacial film lacked sufficient strength or the droplet size remained relatively large, flocculation or coalescence could occur, manifested by an increase in turbidity and the formation of a thin oil layer on the surface after centrifugation. However, this effect was minimized in formulations containing Poloxamer 188, as the polyethylene oxide chains of the polymer created a thick steric barrier surrounding each droplet, effectively preventing direct collisions between them. Tween 80 and PEG 400 also maintained the balance between van der Waals attractive forces and steric repulsive forces at the interface. Additionally, well-distributed Transcutol-p enhanced the compatibility between the oil and aqueous phases, making droplets more resistant to separation under centrifugal stress. Nanoemulsion systems containing Poloxamer 188 exhibited superior resistance to mechanical stress, including centrifugation, due to steric stabilization and increased viscosity of the dispersion medium (25). Can be seen in figure 3

Formulations F1 and F2 exhibited higher turbidity, with a thin oil layer starting to form on the surface. This behavior indicated partial flocculation and coalescence. The instability of these formulations was attributed to an imbalance between the surfactant and co-surfactant ratios, which resulted in an interfacial film insufficiently strong to resist droplet collisions. A loosely packed surfactant film allowed droplet redistribution during centrifugal acceleration, thereby causing coalescence and reducing overall system stability (26).

Meanwhile, formulations F3 and F4 maintained excellent stability with no visible phase separation. Among them, F4 was identified as the most stable formulation due to its higher surfactant-co-surfactant ratio, which effectively reduced interfacial tension, supported by the steric protective role of Poloxamer 188. Under these conditions, the polyethylene oxide chains of Poloxamer 188 formed a physical barrier between droplets, preventing both flocculation and coalescence (25). Therefore, the incorporation of Poloxamer 188 enhanced the nanoemulsion's mechanical resistance, particularly against centrifugal stress, by reinforcing the interfacial film (25).



**Figure 4.** Centrifugation Test Results at 8000 rpm

Figure 4 presents the observation of nanoemulsion samples subjected to centrifugation at 8000 rpm. At this stage, the centrifugal force generated was approximately eight times greater than under initial conditions, causing systems with low stability to display clear phase separation. Large droplets or systems with high interfacial tension were more susceptible to coalescence and breaking. Under such conditions, the interfacial film could undergo deformation due to intense mechanical stress, particularly in formulations with an unbalanced surfactant-to-co-surfactant ratio. Weakly bound Tween 80 molecules tended to detach from the interface, leading to droplet coalescence. However, when sufficient concentrations of Poloxamer 188 and PEG 400 were present, the steric protective layer and the increased viscosity of the aqueous matrix helped slow down interfacial disruption (27). Nanoemulsion systems containing Transcutol® demonstrated higher sensitivity to mechanical stress because local polarity changes could trigger surfactant redistribution (28). Consequently, the stability of the system at 8000 rpm largely depended on the strength of molecular interactions and the thickness of the interfacial layer formed. Can be seen in figure 4

Formulations F1 and F2 exhibited complete phase breaking, in which the oil phase was visibly separated from the aqueous phase. The weak interfacial film failed to withstand extreme mechanical stress, allowing droplets to merge and form a distinct oil layer on the surface. This phenomenon indicated poor thermodynamic stability in formulations with low surfactant ratios. At centrifugal forces exceeding 7000 rpm, only systems possessing strong interfacial films were capable of maintaining droplet integrity without undergoing coalescence (29).

Formulation F3 displayed a slight formation of an oil layer on the surface, while F4 remained relatively stable, showing only minimal turbidity without total phase separation. This behavior confirmed that the interfacial film of F4 possessed high resistance to mechanical stress due to the synergistic effects of Tween 80, PEG 400, and Poloxamer 188 (28). The slight increase in turbidity in F4 was likely caused by the redistribution of Transcutol-p, which could alter local interfacial polarity under high centrifugal force. Excessive Transcutol content may reduce stability under extreme centrifugation conditions by inducing surfactant reorientation at the interface (28).

## Evaluation Results

### Organoleptic Test

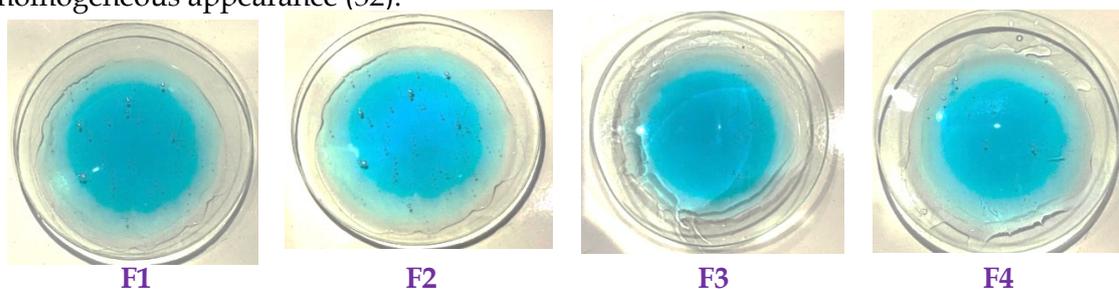
**Table 2.** Organoleptic and Emulsion Type Evaluation Results of the Nanoemulsion Base

Formula	Parameter		
	Color	Odor	Homogeneity
F1	Turbid	Characteristic odor of grapeseed oil	Homogeneous
F2	Turbid	Characteristic odor of grapeseed oil	Homogeneous
F3	Clear	Characteristic odor of grapeseed oil	Homogeneous
F4	Clear	Characteristic odor of grapeseed oil	Homogeneous

The organoleptic evaluation showed that the nanoemulsion base formulations F1-F4 were clear, homogeneous, and exhibited no phase separation during the observation period. The clarity of the formulations indicates the formation of nanosized droplets that minimize light scattering, which is a characteristic of physically stable nanoemulsions (30). Formulations based on nonionic surfactants have been reported to produce clear and homogeneous nanoemulsions without phase separation during initial observations. Moreover, good visual characteristics are considered an early indicator of the physical stability of topical cosmetic formulations (31). Can be seen in table 2

### Emulsion Type Test

The emulsion type test was conducted to determine whether the formulation was oil-in-water (o/w) or water-in-oil (w/o). The test was performed using a staining method by adding a water-soluble dye (methylene blue) to the formulation. In an oil-in-water (o/w) emulsion, oil droplets are dispersed in the aqueous phase. The external water phase interacts with the water-soluble dye, resulting in a uniformly colored and homogeneous appearance (32).



**Figure 5.** Emulsion Type Test Results

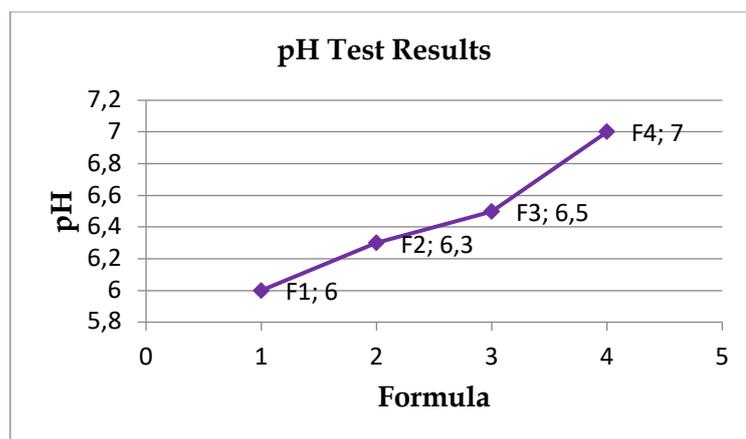
The results of the emulsion type test using the methylene blue staining method demonstrated that all nanoemulsion formulations were classified as oil-in-water (O/W) systems. Methylene blue, a hydrophilic dye, disperses uniformly when the continuous phase of the system is aqueous. The homogeneous distribution of color throughout the formulation indicates that water functions as the external phase, while oil exists as dispersed globules. In O/W systems, oil droplets are suspended and surrounded by the aqueous phase, allowing the water-soluble dye to interact with the continuous medium and produce a uniform coloration. This principle has been widely applied as a standard method for emulsion type identification, where the rapid diffusion of a water-soluble dye serves as an indicator of water being the dominant continuous phase (33,34). Can be seen in figure 5

### pH Test

**Table 3.** The pH test results of the nanoemulsion base

Formulations	pH
F1	6,0
F2	6,3
F3	6,5
F4	7,0
Mean $\pm$ SD	6.45 $\pm$ 0,42

The pH values of the nanoemulsion bases F1-F4 were within an appropriate range for topical application, indicating that the formulations are safe and comfortable for use on the skin. A pH close to the physiological pH of the skin is important to prevent irritation and to maintain the chemical stability of the formulation. pH values near 6.8-7.0 have been reported to be acceptable for nanoemulsion stability and compatibility (35). In addition, stable liquid topical formulations typically exhibit pH values within the physiological range of the skin and do not show significant changes during physical evaluation (36). The stability of pH also contributes to the physical stability of water-based cosmetic formulations, as pH variations may affect the integrity of the dispersed system (37). Can be seen in table 3



**Figure 6.** pH Test Results

Based on the graph, the pH values of the nanoemulsion bases F1-F4 showed a gradual increase from 6.0 to 7.0. This increase may be attributed to variations in the formulation composition, particularly differences in the concentration of surfactants, co-surfactants, and the oil-to-water phase ratio, which influence the ionic balance within the dispersed system. Changes in nanoemulsion composition are known to modify the interfacial environment of the droplets, thereby affecting physicochemical characteristics, including pH and system stability (38). Can be seen in figure 6

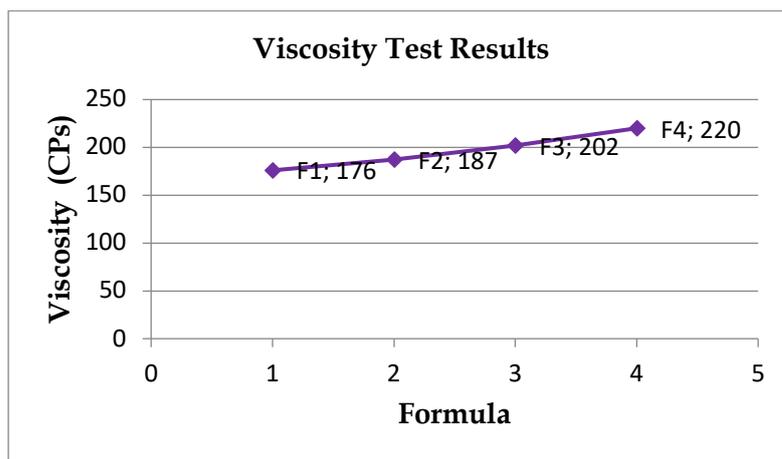
Surfactants play a crucial role in reducing interfacial tension and stabilizing oil droplets within the aqueous phase. Variations in the type and concentration of surfactants can alter the interfacial structure and the distribution of surface charge on the droplets, which may ultimately lead to differences in the measured pH values among formulations (39). Can be seen in figure 6.

**Viscosity Test**

**Table 4.** Organoleptic and Emulsion Type Evaluation Results of the Nanoemulsion Base

Formulations	Viscosity
F1	176
F2	187
F3	202
F4	220
Mean ± SD	196,25 ± 18,89

The viscosity measurements of the nanoemulsion bases F1-F4 indicated that the viscosity values were within an appropriate range for liquid topical bases. The obtained viscosity values were comparable to those reported for other nanoemulsion systems, which typically range from approximately 50 to 5,000 cPs depending on formulation composition (40). The increase in viscosity reflects the role of surfactants, cosurfactants, and stabilizing agents in enhancing system consistency, which contributes to physical stability by slowing droplet movement (30). The addition of stabilizers to nanoemulsion systems has been reported to increase viscosity and improve physical stability. Furthermore, increased viscosity in topical formulations contributes to greater resistance to phase separation during storage (41). Can be seen in table 4



**Figure 7.** Viscosity Test Results

Based on the graph, the viscosity values of the nanoemulsion bases F1-F4 showed a gradual increase from 176 to 220. This increase in viscosity is generally associated with changes in the formulation composition, particularly an increase in the volume fraction of the dispersed phase (oil) and variations in surfactant concentration, which can enhance interdroplet interactions. As a result, the system becomes more viscous and tends to exhibit a more structured behavior at certain volume fractions. The increase in viscosity observed from F1 to F4 may also be attributed to variations in the concentration of surfactants such as Tween (polysorbate) and Span (sorbitan ester). The combination of Tween and Span is commonly used to achieve an appropriate Hydrophilic-Lipophilic Balance (HLB) value in oil-in-water (O/W) nanoemulsion systems. An increase in the total surfactant concentration can enhance the density of the interfacial layer surrounding the oil droplets, thereby strengthening steric stabilization and increasing the flow resistance of the dispersed system (38). Can be seen in figure 7.

#### 4. Conclusion

This study demonstrates that increasing centrifugation speed is an effective accelerated approach to discriminate the mechanical robustness and physical stability of nanoemulsion bases. All formulations remained visually stable at 2000 rpm; however, clear differences emerged at 4000–6000 rpm, where F1–F2 showed increased turbidity and early signs of creaming, indicating weaker resistance to centrifugal stress. At 8000 rpm, F1–F2 underwent complete phase separation, F3 exhibited a slight surface oil layer, while F4 was the most stable, showing only minimal turbidity without total phase breaking. All formulations were confirmed as oil-in-water (O/W) systems with pH and viscosity values remaining within acceptable ranges for liquid topical bases. Notably, comprehensive nanoemulsion characterization parameters (e.g., droplet size distribution, polydispersity index, and zeta potential) have been reported in a separate published article; therefore, the present work specifically strengthens the evidence for physical stability under mechanical stress as a key screening step in nanoemulsion base development.

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