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Evaluation of the Physical Stability of a Grape Seed Oil Nanoemulsion Base Using a Centrifugation Test

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ABSTRAK

Nanoemulsi adalah sistem dispersi berskala nanometer yang digunakan sebagai basis sediaan farmasi dan kosmetik karena kemampuannya meningkatkan solubilisasi dan stabilitas fisik bahan-bahan lipofilik. Penelitian ini mengevaluasi stabilitas fisik basis nanoemulsi minyak biji anggur yang mengandung Tween 80, PEG 400, Poloxamer 407, dan Transcutol HP menggunakan uji sentrifugasi dipercepat. Empat formulasi dengan konsentrasi surfaktan dan ko-surfaktan yang berbeda disiapkan dan disentrifugasi pada kecepatan 2.000, 4.000, 6.000, dan 8.000 rpm. Homogenitas, kejernihan, kekeruhan, dan pemisahan fase diamati secara visual setelah setiap perlakuan. Semua formulasi tetap homogen tanpa pemisahan fase yang terlihat pada kecepatan 2.000 dan 4.000 rpm. Pada kecepatan 6.000 rpm, beberapa formulasi menunjukkan peningkatan kekeruhan namun tidak terjadi pemisahan fase. Pada kecepatan 8.000 rpm, formulasi tetap homogen dan tidak menunjukkan pemisahan fase yang terlihat. Stabilitas sistem ini kemungkinan berkaitan dengan peran Tween 80 dalam menurunkan tegangan antarmuka, PEG 400 dan Transcutol HP dalam mendukung solubilisasi fase minyak, serta Poloxamer 407 dalam memberikan stabilisasi sterik. Hasil ini menunjukkan stabilitas fisik yang memuaskan pada kondisi sentrifugasi yang diterapkan dan mendukung evaluasi lebih lanjut melalui analisis ukuran droplet, indeks polidispersitas, potensial zeta, reologi, dan studi penyimpanan jangka panjang.

Kata Kunci: Nanoemulsi; minyak biji anggur; uji sentrifugasi; stabilitas fisik; Poloxamer 407;

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ABSTRACT

Nanoemulsions are nanometer-scale dispersion systems used as bases for pharmaceutical and cosmetic preparations because they can improve the solubilization and physical stability of lipophilic ingredients. This study evaluated the physical stability of a grape seed oil nanoemulsion base containing Tween 80, PEG 400, Poloxamer 407, and Transcutol HP using an accelerated centrifugation test. Four formulations with different surfactant and co-surfactant concentrations were prepared and centrifuged at 2,000, 4,000, 6,000, and 8,000 rpm. Homogeneity, clarity, turbidity, and phase separation were assessed visually after each treatment. All formulations remained homogeneous without visible phase separation at 2,000 and 4,000 rpm. At 6,000 rpm, several formulations showed increased turbidity but no phase separation. At 8,000 rpm, the formulations remained homogeneous and showed no visible phase separation. The stability of the system may be associated with Tween 80 reducing interfacial tension, PEG 400 and Transcutol HP supporting solubilization of the oil phase, and Poloxamer 407 providing steric stabilization. These results demonstrate satisfactory physical stability under the applied centrifugation conditions and support further evaluation through droplet-size analysis, polydispersity index, zeta potential, rheology, and long-term storage studies.

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Keywords: *Grape seed oil; centrifugation test; physical stability; Poloxamer 407*

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1. Introduction

Nanoemulsions are biphasic colloidal dispersion systems in which one immiscible liquid is dispersed as nanometer-scale droplets within another liquid and stabilized by an appropriate interfacial film. In pharmaceutical and cosmetic development, these systems are attractive because their small droplet size provides a large interfacial area, facilitates the incorporation of lipophilic compounds into aqueous preparations, and may improve dissolution, absorption, and product performance [1,2]. Nanoemulsions can also provide a transparent or translucent appearance and favorable sensory properties, making them suitable as versatile bases for oral, topical, and transdermal delivery systems.

Unlike microemulsions, nanoemulsions are not thermodynamically stable and therefore require sufficient kinetic stabilization to maintain their physical characteristics during processing and storage [3,4]. Their stability is governed by the balance between droplet-disrupting forces and the protective interfacial layer formed by surfactants, co-surfactants, and other stabilizers. Inadequate interfacial coverage or changes in the continuous phase may promote creaming, sedimentation, flocculation, coalescence, or Ostwald ripening. These instability mechanisms can increase droplet size, alter clarity and viscosity, and ultimately cause phase separation, thereby reducing the consistency, quality, and acceptability of the preparation [3-5].

The selection of the oil phase is a critical formulation step because it influences solubilization capacity, interfacial behavior, droplet formation, and the intended application of the nanoemulsion. Grape seed oil has been investigated as a plant-derived carrier oil in several nanoemulsion-based systems. It has been used in lutein loaded nanoemulsions, where the oil phase contributed to the chemical stability of the incorporated lipophilic compound [6]. Grape seed oil has also been developed into a dermocosmetic nanoemulgel and evaluated on human facial skin [7], as well as formulated into nanoemulsions and nanoemulgels for the transdermal delivery of statins [8]. These studies demonstrate the potential versatility of grape seed oil as a lipid phase, while also emphasizing that a stable nanoemulsion base must be established before an active pharmaceutical or cosmetic ingredient is incorporated.

In the present formulation, Tween 80 was selected as the principal nonionic surfactant, while PEG 400 and Transcutol HP were used to support solubilization and interfacial-film formation. Nonionic surfactants are frequently selected for nanoemulsion development because they can reduce oil-water interfacial tension and provide an interfacial barrier around dispersed droplets [9]. Co-surfactants can further improve the flexibility and packing of the interfacial film, which facilitates droplet disruption during mixing and sonication. Poloxamer 407 was included as a polymeric stabilizer because its amphiphilic structure can contribute to steric stabilization and improve the performance of self-nanoemulsifying systems [10]. The combined concentrations of these components are therefore expected to determine whether the grape seed oil phase remains uniformly dispersed under physical stress.

Physical-stability evaluation is essential during the early development of nanoemulsion bases. Although real-time and accelerated storage studies provide the most comprehensive information, they require longer observation periods and several complementary measurements. Centrifugation is commonly used as a rapid preliminary screening method because it applies an increased gravitational field that accelerates droplet migration and may reveal a tendency toward creaming, sedimentation, coalescence, or visible phase separation. Direct and accelerated centrifugation approaches have been used to differentiate nanoemulsion formulations according to their resistance to destabilization [11]. However, the absence of phase separation after centrifugation should be interpreted as evidence of resistance under the applied test conditions rather than proof of long-term thermodynamic stability.

Accordingly, this study aimed to evaluate the physical stability of grape seed oil nanoemulsion bases containing different concentrations of Tween 80, PEG 400, and Transcutol HP, with Poloxamer 407 maintained as a stabilizing component. The formulations were subjected sequentially to centrifugation at 2,000, 4,000, 6,000, and 8,000 rpm, and changes in homogeneity, clarity, turbidity, and phase separation were visually assessed. The study was designed to identify formulations capable of maintaining physical uniformity under progressively increasing centrifugal stress and to provide a rational basis for subsequent instrumental characterization and long-term stability testing.

2. Methods

Centrifugation tests were performed to evaluate the resistance of the nanoemulsion bases to increased gravitational force. Equal volumes of each formulation were transferred into centrifuge tubes and centrifuged at 2,000, 4,000, 6,000, and 8,000 rpm for 30 minutes at each test speed. After each treatment, the samples were visually examined for homogeneity, clarity, turbidity, phase separation, and the formation of distinct oil or aqueous layers.

A nanoemulsion was considered physically stable when no visible layer separation was observed after centrifugation. This accelerated method simulates the effect of increased gravitational stress and is useful for the preliminary estimation of dispersion stability during storage [11].

Materials

The materials used were purified water, grape seed oil (Sigma-Aldrich), polyethylene glycol 400 (PEG 400; Sigma-Aldrich), Poloxamer 407 (Sigma-Aldrich), polysorbate 80 (Tween 80; Sigma-Aldrich), Transcutol HP (Chemical Bull), 70% ethanol for equipment disinfection, and 95% ethanol as the solvent.

Nanoemulsion Base Formulation

Table 1. Composition of the grape seed oil nanoemulsion base

Materials	Concentration (%)			
	F1	**F2**	**F3**	**F4**
Grape seed oil	5	5	5	5
Tween 80	27.5	27.5	30	30
PEG 400	30	30	32.5	32.5
Poloxamer 407	2	2	2	2
Transcutol HP	0.5	1	1	2
Purified water	q.s. to 50 mL			

All equipment was cleaned and disinfected with 70% ethanol, and the ingredients were weighed according to the composition shown in Table 1. Poloxamer 407 was first dissolved in 95% ethanol. Tween 80 and PEG 400 were then mixed in a beaker using a magnetic stirrer until homogeneous, after which grape seed oil was added gradually as the oil phase.

Purified water was added slowly under continuous stirring, followed by the gradual addition of the Poloxamer 407 solution and Transcutol HP. Each formulation was sonicated for 1 hour to reduce droplet size and was then subjected to centrifugation at 2,000, 4,000, 6,000, and 8,000 rpm for 30 minutes at each speed. Physical stability was assessed from the clarity, homogeneity, turbidity, and absence of phase separation.

3. Results and Discussion

The centrifugation test is an accelerated method for evaluating the physical stability of nanoemulsions by applying a gravitational force greater than normal gravity. It can accelerate instability phenomena such as creaming, flocculation, and coalescence, thereby enabling rapid visual screening. Nanoemulsions are generally characterized by droplets in the nanometer range and are stabilized by surfactants and co-surfactants; consequently, their physical stability is strongly influenced by droplet size and interfacial properties [4].

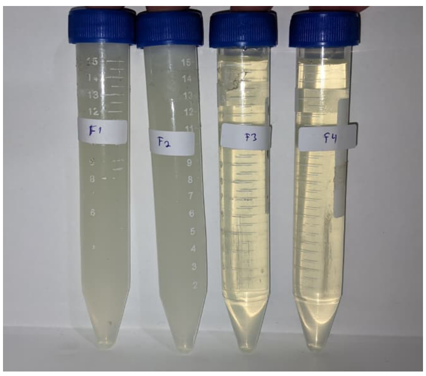

Figure 1. Centrifugation test results at 2,000 rpm

At 2,000 rpm, all nanoemulsion formulations remained homogeneous and showed no visible phase separation. The applied centrifugal force was therefore insufficient to disrupt the dispersion system. Smaller droplets generally exhibit greater resistance to gravitational separation because they are more uniformly dispersed throughout the continuous phase [12].

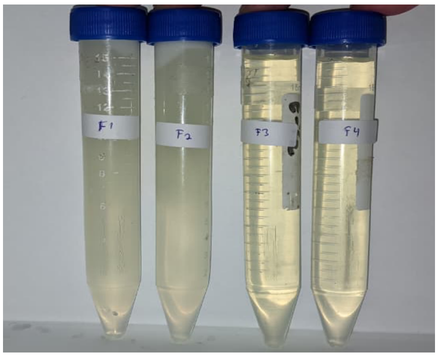

Figure 2. Centrifugation test results at 4,000 rpm

At 4,000 rpm, all formulations remained homogeneous, although differences in clarity became more apparent. This finding indicates that Tween 80 effectively reduced interfacial tension, while PEG 400 increased the flexibility of the interfacial layer and helped maintain dispersion stability [13].

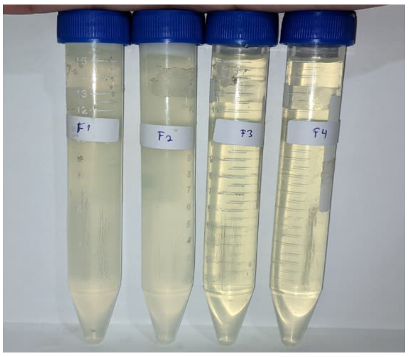

Figure 3. Centrifugation test results at 6,000 rpm

At 6,000 rpm, several formulations showed increased turbidity, suggesting a change in droplet distribution or a temporary increase in droplet association. The higher centrifugal force may have increased the frequency of droplet collisions and promoted reversible flocculation [14]. Nevertheless, no visible phase separation occurred, indicating that the interfacial layer continued to prevent permanent coalescence.

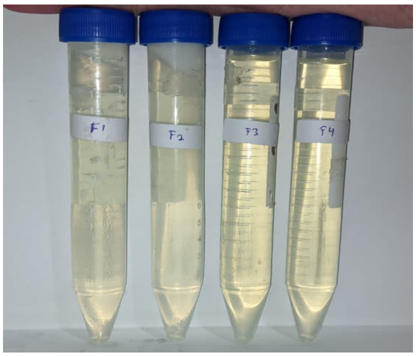

Figure 4. Centrifugation test results at 8,000 rpm

At 8,000 rpm, the nanoemulsion formulations remained homogeneous and showed no visible phase separation. This observation indicates satisfactory kinetic stability under the highest centrifugal stress applied in this study. Nanoemulsions that remain physically uniform after high-speed centrifugation generally show greater resistance to accelerated phase separation [15].

The observed stability was supported by the combined functions of the formulation components. Tween 80 reduced interfacial tension, PEG 400 and Transcutol HP promoted solubilization of the oil phase, and Poloxamer 407 provided steric stabilization that limited droplet coalescence. This combination can support a more uniform droplet distribution and improve the kinetic stability of the nanoemulsion system [12].

Overall, increasing the centrifugation speed provided a progressively more stringent assessment of the nanoemulsion bases. The absence of visible phase separation at 8,000 rpm indicates satisfactory physical stability under the applied accelerated conditions [15]. However, centrifugation is a screening test and should be complemented by long-term storage and instrumental characterization.

4. Conclusion

The grape seed oil nanoemulsion bases containing Tween 80, PEG 400, Poloxamer 407, and Transcutol HP maintained homogeneity and showed no visible phase separation after centrifugation at speeds up to 8,000 rpm. These findings demonstrate satisfactory physical stability under the applied accelerated conditions and indicate that the surfactant, co-surfactant, and polymeric stabilizer contributed to maintaining the dispersion system. The formulations therefore have potential for further development as bases for pharmaceutical and cosmetic preparations containing lipophilic active ingredients. Further studies should evaluate droplet size, polydispersity index, zeta potential, viscosity, pH, thermal cycling, and long-term storage stability to establish a more comprehensive stability profile.

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