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FORMULATION AND EVALUATION OF COSMETIC CREAM CONTAINING PUNICA *GRANATUM* SEED OIL

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ABSTRACT

An advanced nanoemulsion-based drug delivery system was developed to overcome the limitations of conventional formulations, particularly for poorly soluble and poorly permeable compounds. In this study, a nano emulsion incorporating *Punica granatum* seed oil (PSO) was formulated and evaluated for transdermal application. Pomegranate seed oil is rich in bioactive constituents such as conjugated α-linolenic acids, especially punicic acid, along with fatty acids, flavonoids, alkaloids, saponins, and glycosides, which contribute to antioxidant, antimicrobial, antiaging, and potential anticancer properties. The Nano emulsion was prepared using Tween 80 and Span 80 as surfactants and optimized at a 1:1 oil-to-Smix ratio. The selected formulation (F9) demonstrated high drug content, excellent entrapment efficiency, suitable particle size, and a stable zeta potential value, confirming good stability. The optimized nanoemulsion was further converted into a nanoemulgel using Carbopol as a gelling agent, which exhibited enhanced drug release over 8 hours and maintained desirable physicochemical characteristics. Stability studies under ICH conditions indicated no significant changes in pH, viscosity, spread ability, or phase separation. The formulation showed promising antimicrobial activity against *E. coli* and demonstrated synergistic effects against the A375 melanoma cell line, suggesting potential antiaging and anticancer applications. Overall, the developed PSO-based nanoemulgel appears to be a stable and effective topical formulation with therapeutic and cosmetic benefits.

KEYWORDS: Punica granatum seed oil, nanoemulsion, nanoemulgel, punicic acid, transdermal delivery, antimicrobial activity, antiaging, melanoma.

INTRODUCTION

The advancement of pharmaceutical formulation science has significantly improved the delivery of bioactive compounds, particularly those with poor water solubility and limited permeability. Conventional topical and oral dosage forms often fail to provide optimal therapeutic outcomes when dealing with lipophilic natural oils due to poor absorption, instability, and inconsistent drug release. To overcome these challenges, nanotechnology-based delivery systems have gained considerable importance in recent years. Among them, nanoemulsions have emerged as an effective and versatile platform for enhancing the solubility, stability, and bioavailability of hydrophobic compounds[1].

Nanoemulsions are thermodynamically or kinetically stable dispersions consisting of oil and water phases stabilized by suitable surfactants. The droplet size of nanoemulsions typically ranges from 20 to 200 nanometers, which provides a large interfacial surface area. This nanoscale size plays a crucial role in improving drug dissolution, absorption, and penetration across biological membranes. Due to their transparent or translucent appearance, low viscosity, and high stability, nanoemulsions are widely used in pharmaceutical and cosmetic formulations. Their small droplet size reduces gravitational separation and enhances physical stability compared to conventional emulsions[2].

Punica granatum oil, obtained from pomegranate seeds, has attracted attention due to its rich composition of bioactive fatty acids, especially punicic acid. This conjugated fatty acid is known for its antioxidant, anti-inflammatory, antimicrobial, and potential anticancer properties. The presence of natural antioxidants in the oil helps neutralize free radicals and protect cells from oxidative stress. Oxidative stress is closely associated with premature skin aging, inflammation, and various dermatological disorders. Additionally, studies have suggested that pomegranate seed oil may exhibit cytotoxic effects against certain cancer cell lines, including melanoma cells. These biological properties make Punica granatum oil a promising candidate for topical therapeutic and cosmetic applications[3,4].

Despite its beneficial effects, direct application of pomegranate seed oil presents several limitations. The oil is highly lipophilic and poorly miscible with water, resulting in limited penetration through the skin barrier. It may also produce a greasy sensation, reducing patient compliance. Furthermore, exposure to environmental conditions such as light, heat, and oxygen can affect its stability. Therefore, incorporating Punica granatum oil into a

nanoemulsion system provides a logical and scientifically sound approach to enhance its delivery and effectiveness[5].

In the formulation of nanoemulsions, the selection of appropriate surfactants is critical for achieving stability and uniform droplet distribution. Tween 80 and Span 80 are commonly used non-ionic surfactants due to their safety, compatibility, and ability to reduce interfacial tension between oil and water phases. Tween 80 is hydrophilic in nature, while Span 80 is relatively lipophilic. The combination of these two surfactants allows adjustment of the hydrophilic–lipophilic balance (HLB), which is essential for forming a stable oil-in-water nanoemulsion. Proper optimization of the oil-to-surfactant ratio ensures formation of nanosized droplets with minimal aggregation[6].

The process of nanoemulsion preparation typically involves controlled mixing of the oil phase and aqueous phase in the presence of surfactants. The resulting system should exhibit clarity or slight translucency, indicating formation of nanoscale droplets. Evaluation of parameters such as droplet size, polydispersity index, pH, viscosity, drug content, and zeta potential is necessary to confirm stability and performance. Zeta potential, in particular, provides information about surface charge and electrostatic repulsion between droplets. A sufficiently high positive or negative zeta potential value indicates reduced chances of droplet aggregation, thereby improving physical stability[7].

For topical application, liquid nanoemulsions may sometimes lack adequate viscosity and retention at the site of administration. To address this issue, nanoemulsions can be converted into nanoemulgel by incorporating a suitable gelling agent. Carbopol 940 is widely used as a polymeric gelling agent in topical formulations because of its high thickening efficiency and compatibility with various active ingredients. Upon neutralization with Triethanolamine, Carbopol forms a clear and stable gel network. The incorporation of nanoemulsion into this gel matrix enhances viscosity, improves spread ability, and prolongs residence time on the skin surface[8].

The resulting nanoemulgel system offers several advantages. It provides controlled and sustained release of Punica granatum oil, allowing prolonged therapeutic action. The nanosized droplets enhance interaction with the stratum corneum, the outermost protective layer of the skin, facilitating better penetration. Improved penetration can increase the availability of bioactive fatty acids in deeper skin layers, where they can exert antioxidant and protective effects. Additionally, the gel structure prevents rapid runoff from the application site and improves patient comfort due to its non-greasy and smooth texture[9].

Skin aging is a complex biological process influenced by intrinsic factors such as genetics and extrinsic factors such as ultraviolet radiation and pollution. Oxidative stress plays a central role in collagen degradation, reduced elasticity, and wrinkle formation. The antioxidant activity of Punica granatum oil may help counteract these effects by neutralizing reactive oxygen species. Therefore, a stable nanoemulgel containing pomegranate seed oil may serve as an effective antiaging formulation. The enhanced penetration achieved through nanoemulsion technology further supports its cosmetic value[10].

In addition to antiaging potential, the antimicrobial properties of pomegranate seed oil make it relevant for protecting the skin against microbial infections. The improved dispersion of the oil in nanosized droplets increases surface contact with microorganisms, which may enhance antimicrobial effectiveness. Furthermore, preliminary evidence of activity against melanoma cell lines suggests that bioactive components of the oil could contribute to supportive care in skin-related disorders. Although such effects require further investigation, the nanoemulsion platform provides an efficient means of delivering these compounds to target tissues[11].

Stability studies are essential to ensure the long-term performance of the formulation. Parameters such as pH and viscosity must remain within acceptable ranges to prevent skin irritation and maintain consistency. Evaluation under accelerated conditions helps predict shelf life and confirms resistance to phase separation or degradation. A stable nanoemulsion system with appropriate zeta potential and uniform droplet size distribution demonstrates good formulation robustness[12].

Overall, the development of a Punica granatum oil-based nanoemulsion and its subsequent conversion into a nanoemulgel represents a modern and rational approach in topical drug delivery. By combining natural bioactive compounds with nanotechnology-driven formulation strategies, it is possible to enhance solubility, stability, penetration, and therapeutic efficiency. The integration of Tween 80 and Span 80 as surfactants, along with Carbopol 940 and Triethanolamine for gel formation, enables the creation of a stable, effective, and cosmetically acceptable system[13].

In conclusion, nanoemulsion technology offers a promising platform for maximizing the benefits of pomegranate seed oil in dermatological and cosmetic applications. Through improved delivery and sustained release, the developed nanoemulgel has the potential to provide antioxidant protection, antimicrobial activity, antiaging benefits, and supportive effects in melanoma-related research. This approach highlights the importance of innovative formulation strategies in transforming traditional natural oils into scientifically validated and effective topical systems[14,15].

Materials and Methods Materials:

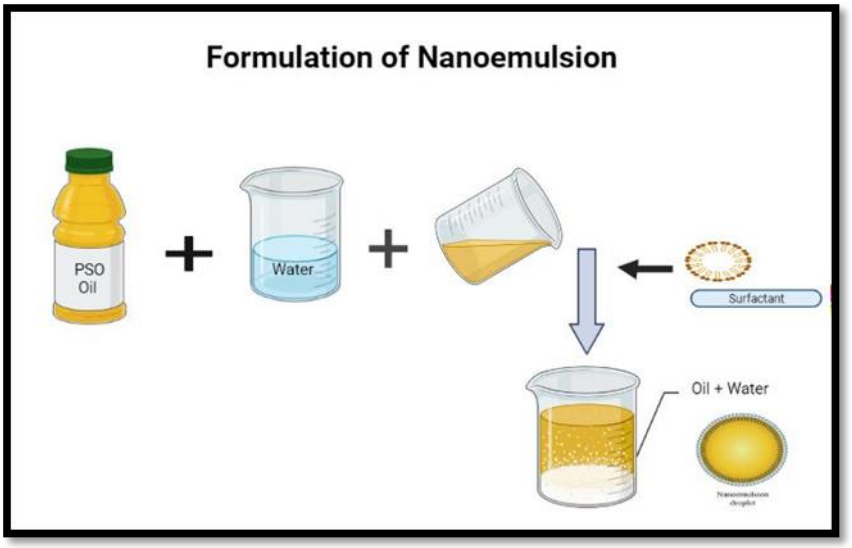
The following drugs, excipients and chemicals were used for the formulation of nanoemulsion.

List of Materials

S. No.	Drug / Excipients/ Solvents	Manufacture
1.	*Punica granutum* oil	National Research center for Pomegranate Solapur
2.	Tween 80	SD Chemicals, Mumbai
3.	Span 80	Rajesh Chemicals, Mumbai
4.	Carbopol 940	SD Fine Chemicals, Mumbai
5.	Triethanolamine	SD Fine Chemicals, Mumbai
6.	n- Hexane	SD Fine Chemicals, Mumbai
7	Hydrochloric Acid	SD Fine chemicals Mumbai
8	Sodium Orthohydrogen diphosphate	SD Fine chemicals Mumbai
9	Stearic acid	SD Fine chemicals Mumbai
10	Cetyl Alcohol	SD Fine chemicals Mumbai
11	Glyceryl Monostearate	SD Fine chemicals Mumbai
12	Hydroxypropyl Methyl Cellulose	SD Fine chemicals Mumbai
13	Glycerin	SD Fine chemicals Mumbai
14	Methyl Paraben	SD Fine chemicals Mumbai
15	Propyl Paraben	Rajesh Chemicals, Mumbai
16	Propylene Glycol	Rajesh Chemicals, Mumbai
17	Triethanolamine	Rajesh Chemicals, Mumbai

FORMULATION OF NANOEMULSION[16]:

The quantitative composition of Nanoemulsions was selected based on the phase diagram. Nano-emulsions were prepared using the spontaneous emulsification mechanism, which is taking place when an organic phase and an aqueous phase are mixed. The organic phase contains a homogeneous solution of oil, lipophilic surfactant, and co-surfactant; the aqueous phase consists of distilled water. The selected oil phase was taken. Surfactant and co-surfactant (Smix) were mixed in different volume ratios (1:1, 1:2, 1:3). These Smix ratios were chosen to reflect the increasing concentration of co-surfactant with respect to surfactant, increasing concentration of surfactant with respect to co-surfactant, for the detailed study of the phase diagrams for the formulation of NE. A mixture of oil and Smix was prepared in different volume ratios (1:1, 1:2, 1:3, 1:4) add required amount of water drop wise in the oil phase with a continuous stirrer using a homogenizer at 4000 rpm for 1 hour.



Formulation of nanoemulsion

Formulation table F1 to F12 containing PSO

S. No.	Oil	Surfactant(gm)	Water(ml)
F1	1.5	0.5	20
F2	2	1	20
F3	2.5	1.5	20
F4	3	2	20
F5	1.5	1.5	20
F6	2	2	20
F7	2.5	2.5	20
F8	3	3	20
F9	1.5	S mix (1:1)	20
F10	2	S mix (1:2)	20
F11	2.5	S mix (1:3)	20
F12	3	S mix (1:4)	20

Methods of Evaluation of Nanoemulsion

Physical examination and homogeneity[17]:

The final prepared Nanoemulsion formulations were inspected visually for their color intensity variation. All developed nanoemulsion was tested for homogeneity by visual inspection after the Nanoemulsions have been placed in the container. They were also examined for their appearance and presence of any aggregates.

Average particle size and Particle size distribution[18]:

Average particle size and size distribution of selected batches of Nanoemulsion was determined by using SAGLOSOFT Micro-Imaging Software version-2. The optimized batches F9 were evaluated by using Malvern Particle size Analyzer.

Determination of viscosity:

The viscosity of the formulations was determined using Brookfield DVE viscometer. The sample was taken for analysis without diluting the sample by using Spindle No. 63 at 100rpm and viscosity measured in centipoise.

Determination of pH:

The prepared neutral, positive, negative nanoemulsion formulations were measured using (Systronics, 361-micro pH meter) pH determination. The pH value determination monitoring the pH value is important for determining the Nanoemulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. The Nanoemulsions had stable pH values between (5.22-5.52) for almost all formulations tested.

Entrapment efficiency:

Percentage drug entrapment efficiency was determined for drug content in formulation. The drug content of nanoemulsion formulation was determined by UV visible spectrophotometric method. Each 1 ml sample was cooling centrifuged at 3500 rpm for 1 hr. after centrifuge; supernatant transparent layer was taken and diluted with 10ml distilled water. The samples were measured at 272 nm using UV- VIS spectrophotometric method. Results were taken in triplicate and the average was taken into consideration.

Drug content:

The dose of drug was well below the saturation point. The amount of drug release after incorporation should be checked. Hence, the drug content was calculated by UV Visible spectrophotometer. 0.1ml of nanoemulsion was dissolved in 10ml of distilled water with constant stirring. The solution was filtered through Whatman filter paper. Further dilutions are made to get required concentration and the absorbance solution was measured at 273nm by using systronics double bead spectrophotometer 2202 against blank reagent distilled water.

The drug content was calculated using formula:

Drug content= Practical value / Theoretical value × 100

Globule size and zeta potential:

Zetasizer ZS 90 (Microtrac Zetasizer) have been used to determine the mean Globule size (GS) and Zeta potential (ZP) of NEs. Based on photon correlation spectroscopy technique the mean globule size was measured. This analyzes the fluctuations in dynamic light scattering due to the Brownian motion of the particles. The mean diameter was obtained at an angle of 90° in 10mm diameter cells at 25°C. The zeta potential, reflecting the electric charge on the

particle surface and it was very useful way of evaluating the physical stability of any colloidal system. It was determined based on an electrophoretic light scattering technique. All ZP measurements were conceded at 25^{o}C using disposable polystyrene cells, disposable 5plain folded capillary zeta cells and after suitable dilution of all samples with the original dispersion medium.

Identification test of emulsion[19]:

The type of emulsion was determined by dilution test and dye test.

Dye Test:

In a dye test a nanoemulsion was mixed in water soluble dye (amaranth) and observed under microscope. If continuous phase was appearing red it was considered as emulsion is o/w type and if the continuous phase was colorless it was considered as emulsions were considered as w/o type.

Dilution Test[20]:

In the dilution test, the nanoemulsion was done to find out the oil in water emulsion. Take a small amount of nanoemulsion, dissolve in small amount of water if the nanoemulsion was completely dissolved in water then the emulsion is o/w type and if the nanoemulsion is not dissolved in water the emulsion is w/o type.

Thermodynamic stability tests[21,22]:

All selected formulations were subjected to keep on different thermodynamic stability tests.

a) **Heating cooling cycle**: In this test the all the prepared formulations F1 to F12 were kept in a refrigerated up to 4°c for 24 hr. After 24hr the formulations removes from refrigerator and heat it up to 45°c on a heating metal. The six cycles are done for this study. The stable formulations, at these temperatures, were subjected to centrifugation.

b) **Centrifugation:** All the formulations those passes the heating cooling cycle are taken for centrifugation test. All formulation taken and centrifuged at 3500 rpm for 30min in a centrifugation machine. The formulations which have not shown any phase separation was taken for freeze-thaw cycle test.

c) **Freeze-thaw cycle:** The formulation those passes the centrifugation test is taken for freeze thaw study. Take all 12 batches all were kept at the storage temperature of freeze-thaw cycles between – 21°C and +25°C for 48 hr. after 48 hr. observed all the formulation shows any phase separation or not.

RESULTS & DISCUSSION:

Characterization of Nanoemulsion:

Physical Examination and Homogeneity:

The formed Nanoemulsion observed visually it is milky, translucent and easily flowable emulsion is formed. All the emulsions are show good homogeneity.

Determination of pH:

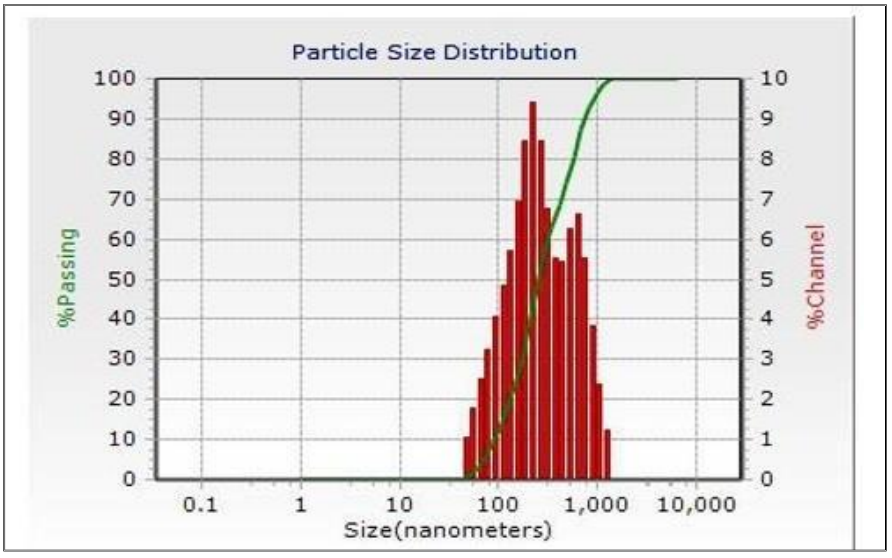
The pH of all the prepared batches F1 to F12 were evaluated with digital pH meter, it was observed in between within rang 6 to 6.5 it is slightly acidic. The results pH of all the batches are shown in table.

Viscosity:

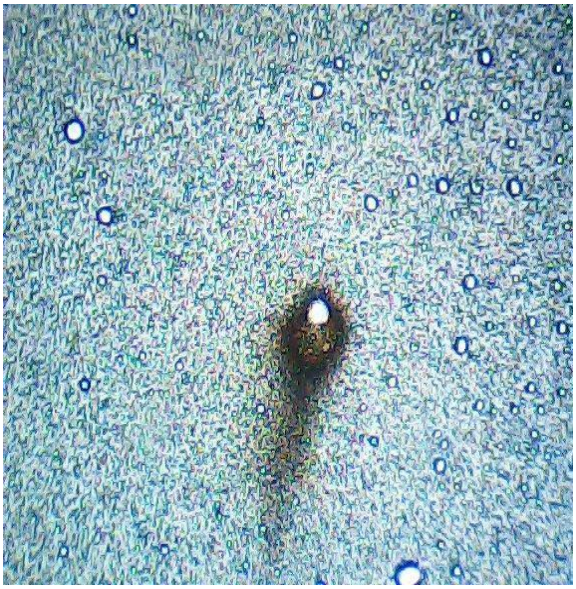
The viscosity of prepared nanoemulsion batches F1 to F12 were determined by using Brookfield viscometer. The viscosity of F1 to F12 in between 52.7 cps to 80 cps. All the results are shown in table.

Average particle size and particle size distribution:

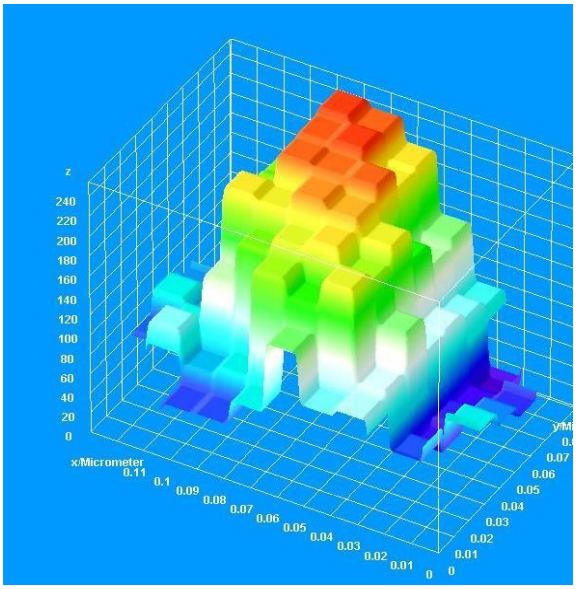
The average globule size of emulsion was in the range of µm given in Table no. 10. The globule size analyzed by the SAGLO soft imaging Analysis software version-2. The optimized batch No. F9 shows average particle size of 91.9 nm and the graph of particle size distribution of nanoemulsion of batch F9 was given in Fig.



Particle size distribution of batch F9



Optimized batch F9 observed under Saglosoft Image Analyzer



3D Surface plot for optimized batch F9

Evaluations parameters of PSO-based Nanoemulsion F1 to F12

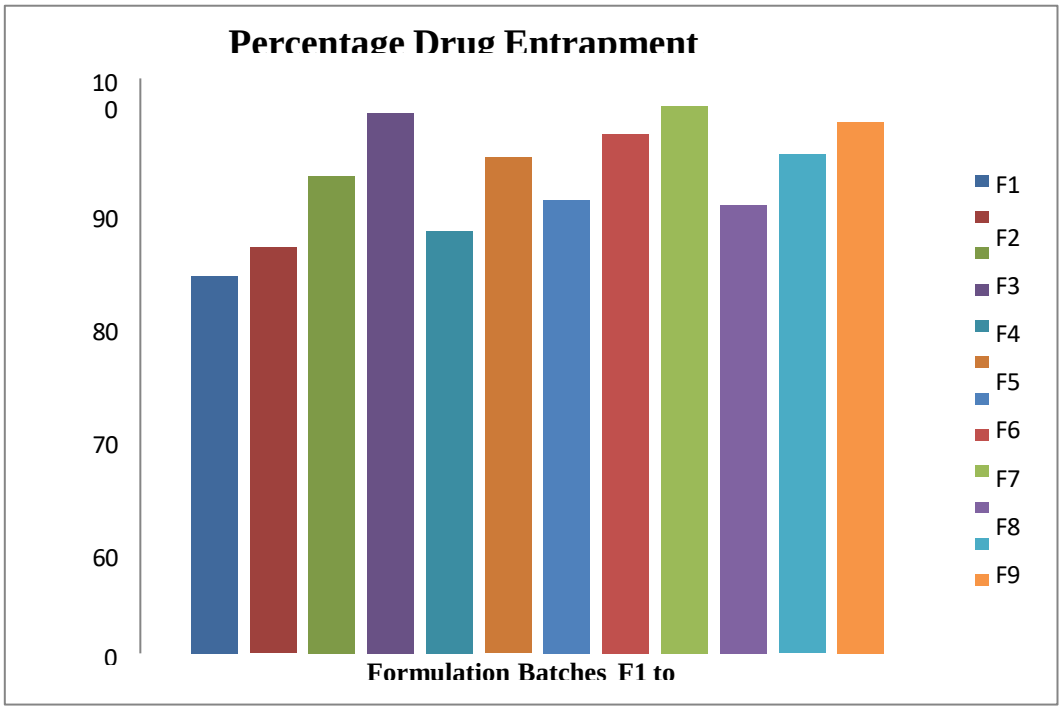
Formulation	Viscosity (cps)	pH	Drug Content (%)	Particle size (nm)
F1	70.7	6	76.93	7800
F2	59	6.5	84.39	6370
F3	67	6.2	86	3190
F4	52.7	6	94.6	2610
F5	54	6.4	89.6	4310
F6	58	6	85.48	6850
F7	64	6.1	84	4950
F8	73	6	92.65	4720
F9	68	6.2	95.67	6580
F10	80	6	90	3780
F11	82	6.4	88.5	6150
F12	80	6.3	93.25	5090

Drug Entrapment Efficiency:

The drug entrapment efficiency of all prepared batches F1 to F12 was found in range of 65.61 to 95.16%. All the batches showed good entrapment efficiency is given in Table and Fig.**Percent (%) Drug Entrapment Efficiency of PSO-based Nanoemulsion F1 to F12**

Formulation	Drug Entrapment Efficiency (%)
F1	65.61
F2	70.6
F3	83
F4	94
F5	73.5

F6	86.26
F7	78.9
F8	90.4
F9	95.16
F10	78
F11	86.8
F12	92.5



Percent (%) Drug Entrapment Efficiency Formulation F1 to F12.

Drug content:

The drug content of all prepared formulation batches F1 to F12 were determined and ranged between 76.96 % to 95.67 %. From all the batches, F4= 94.6 % and F9= 95.67% batch shows the highest drug content. The drug content of all the batches is shown in table.

Zeta potential:

Zeta potential of optimized batch F9 was observed. The Zeta potential value of F9 batch was found to be −32.48 mv. The values are shown to threshold agglomeration.

Zeta potential of Optimized Nanoemulsion Formulation F9

Zeta Potential	
Mobility	-0.54u/ s/ V/ c m
Zeta Potential	-32.48mv
Charge	-0.01016 Fc
Polarity	Negative

Conductivity	278 Us/c m
Field Strength	5.0 k V/ m
Sample Information	
Fluid	
Viscosity	0. 751
Temperature	32. 88 C
Dielectric Const	79
Dispersant	
p H	7

Identification test for Emulsion:

a) Dye Test: Take 1ml of nanoemulsion mixed in water soluble dye (amaranth) take small amount of sample on lass slide and observed under optical microscope the continuous red colour is observed which confirm that emulsion in o/w type.

b) Dilution Test: Take 1ml of sample dilute in distilled water the emulsion is dissolved completely in distilled water does not show any phase separation which confirmed that the emulsion is o/w type.

Thermodynamic studies:

The results thermodynamic stability of Prepared nanoemulsion batches F1 to F12 containing the PSO oil, passes the all Heating cooling cycle, centrifugation cycle, freeze thaw cycle the results are given.

Thermodynamic Stability Study of PSO based Nanoemulsion batches F1 to F12

Formulation	Heating Cooling Cycle	Centrifugation Cycle	Freeze-thaw Cycle
F1	Pass	Pass	Pass
F2	Pass	Pass	Pass
F3	Pass	Pass	Pass
F4	Pass	Pass	Pass
F5	Pass	Pass	Pass
F6	Pass	Pass	Pass
F7	Pass	Pass	Pass
F8	Pass	Pass	Pass
F9	Pass	Pass	Pass
F10	Pass	Pass	Pass
F11	Pass	Pass	Pass
F12	Pass	Pass	Pass

Thermodynamic Stability Study of Formulation F1 to F12

SUMMARY AND CONCLUSION:

Nanoemulsions are advanced emulsion systems characterized by very small droplet sizes, improved stability, and enhanced drug delivery performance compared to conventional emulsions. In the present study, an emulsion system was developed using *Punica granatum* seed oil (PSO) as the oil phase. PSO was selected because of its rich fatty acid composition and bioactive polyphenolic compounds, which contribute to antioxidant and anti-inflammatory properties.

The nanoemulsion was formulated using Tween 80 as a surfactant and Span 80 as a co-surfactant/emulsifying agent to stabilize the oil and aqueous phases. Different concentrations of oil and surfactant were employed to prepare twelve formulation batches (F1–F12). The phase behaviour study helped in selecting appropriate ratios to obtain stable, white, and easily flowable emulsions. The optimized emulsion system was further incorporated into a 2% Carbopol gel base for improved consistency and topical applicability; however, the primary focus remained on the characteristics of the emulsion system itself.

All prepared emulsion batches were evaluated for key physicochemical parameters including pH, spread ability, viscosity, drug content, particle size, zeta potential, and in vitro diffusion. The results indicated that formulation variables significantly influenced the stability and performance of the emulsion. Among all batches, F9 demonstrated superior characteristics, including high drug content (approximately 95%), satisfactory viscosity, good spread ability, and enhanced in vitro drug release profile. The optimized emulsion exhibited a uniform droplet size distribution within the micro/nano range and showed acceptable zeta potential values, indicating good physical stability. Overall, the study confirms that a properly optimized nanoemulsion system containing *Punica granatum* seed oil can be successfully

formulated using suitable surfactant combinations. The optimized emulsion (F9) demonstrated desirable physicochemical properties, stability, and effective drug release behaviour. These findings suggest that the developed nanoemulsion system is a promising carrier for topical drug delivery applications.

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