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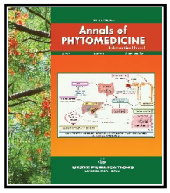

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Development and evaluation of nanogel containing genistein for topical applications

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Abstract

Genistein, a natural isoflavone found in soybeans, has gained attention for its diverse pharmacological benefits, including antioxidant, anti-inflammatory, and anticancer properties. However, the therapeutic efficacy of genistein is often limited by challenges such as poor solubility, stability, and absorption. This study aimed to explore the feasibility of nanogel for the topical delivery of genistein in prevention of skin damage. The solubility of genistein was determined in various oils, surfactants, and co-surfactants, and genistein nanoemulsion was prepared using an aqueous titration method. The optimized nanoemulsion consisted of Olive oil and Sefsol-218 (1:1 ratio) as the oil phase, Kolliphor RH40 as the surfactant, and polyethylene glycol (PEG-400) as the co-surfactant. The droplet size of the nanoemulsion was 89.76 nm, with a low polydispersity index (0.161 ± 0.022) and a negative zeta potential (-20.02 ± 0.234). Finally, a nanogel formulation was prepared using Carbopol 940 as the gelling agent. The triethanolamine (TEA) as the neutralizing agent, and glycerine was added as a humectant to provide a smooth and calming effect. The optimized nanogel gave sustained release with acceptable rheological and pharmaceutical properties. Further study is required to determine the potential benefits of utilizing genistein-loaded nanogel for prevention of skin damage when applied topically.

1. Introduction

In recent years, there has been growing interest in exploring natural compounds for their potential therapeutic benefits, particularly in the realm of pharmaceutical research. Genistein, a natural isoflavone abundantly found in soybeans and soy products, has garnered considerable attention due to its diverse pharmacological properties (Rasheed *et al.,* 2022). With documented anti-inflammatory, antioxidant, and anticancer effects, genistein holds promise as a therapeutic agent for various health conditions. However, the therapeutic potential has been hindered by challenges associated with poor solubility, stability, and absorption of genistein through conventional delivery methods. It is imperative to develop effective methods to mitigate UV-B-induced damage to keratin-forming cells. One promising approach is drug administration *via* the skin, which has garnered significant attention (Aqeel *et al.,* 2023). Topical applications offer some benefits included reduced systemic side effects and decreased first-pass metabolism. Due to the drug's potent sustained-release mechanism, a comparatively low dosage can enhance patient adherence and save treatment expenses. Topical drug delivery-induced skin irritation can be considerably decreased by new nanoemulsion carriers (Eqbal, *et al.,* 2021). Improved skin barrier function, prolonged medication release, and epidermal hydration to attain moisturization are some advantages of nanoemulsions (Barkat, *et al.,* 2017). As a result, by limiting drug accumulation in the skin, nanoemulsions can be utilized to increase the bioavailability and effectiveness of medications (Si *et al.*, 2010; Ullah *et al.,* 2011).

Genistein has a diphenol structure that is like human endogenous estrogen in terms of stereochemistry. Genistein may help to cure a variety of skin problems with minimal adverse effects when applied topically (Jahan *et al.,* 2022). The topical administration of drugs offers several advantages over systemic routes, including targeted delivery, reduced systemic side effects, and improved patient compliance. Nanogels hold significant promise for topical application due to their ability to encapsulate and deliver therapeutic agents precisely to target sites on the skin (Raina *et al.,* 2023). Their nanoscale size and tunable properties offer enhanced drug penetration, prolonged release, and improved therapeutic efficacy for various dermatological conditions. In this context, the development of nanogel-based delivery systems presents an attractive approach for enhancing the topical delivery of bioactive compounds such as genistein. Nanogels, composed of cross-linked polymer networks with a nanoscale size range, possess unique properties that make them well-suited for drug delivery applications. They offer high drug loading capacity, improved release kinetics, and the ability to protect encapsulated drugs from degradation, thereby overcoming some of the challenges associated with conventional formulations. Present study was aimed to address the limitations of genistein's therapeutic efficacy by developing a nanogel containing genistein for topical applications. By encapsulating genistein within a nanogel matrix, the solubility, stability, and targeted delivery of genistein can be improved, leading to enhanced therapeutic outcomes (Singh *et al.,* 2022) Furthermore, the use of nanogels for topical delivery can

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potentially offer controlled release of genistein, ensuring sustained drug release at the site of application while minimizing systemic exposure and associated side effects.

2. Materials and Methods

Genistein was sourced from Chengdu Herbpurify Co. (Ltd.), Chengdu, China. Olive oil was procured from SD Fine Chemicals, Mumbai, India. Sefsol 218, Kolliphor RH40 were graciously provided as gift samples by Nikko Chemicals (Tokyo, Japan) and BASF (Mumbai, India), respectively. Additionally, PEG 400 and Carbopol 940 were obtained from SD Fine Chem in Mumbai, India. All other chemical substances utilized in the study were of analytical grade and were under the GRAS (Generally Regarded as Safe) category.

2.1 Solubility studies for screening of oil phase, surfactants and co-surfactants

The solubility study of genistein was evaluated in different oil, surfactants and co-surfactants. The procedure started by addition of drug in different oils, surfactants and co-surfactants to see how well they dissolved genistein. The capacity of oil to dissolve genistein was assessed by mixing an excess of the substance with two milliliters of specific oils, each stored in a five-millilitres stopper vial. To achieve equilibrium, these vials were thoroughly combined using a vortex mixer and then incubated on an isothermal shaker (IKAV R KS 400i, Staufen, Germany) for 72 h at a constant temperature of 25 ± 1°C. After equilibration, the vials were removed from the shaker and centrifuged using a REMI centrifuge (Mumbai, India) at 10,000 rpm for 15 min. A 0.45 µm membrane filter was used to filter the resultant supernatant. High Performance Liquid Chromatography (HPLC) was used to measure the amount of genistein in each oil at 261 nm (Jaiswal *et al.,* 2022; Ahmad, *et al.,* 2017).

2.2 Construction of pseudo ternary phase diagrams

The *in situ* emulsification method, commonly known as the aqueous titration approach, was used to carefully generate pseudo ternary phase diagrams in accordance with the methodology described by Talegaonkar *et al*. (2010). This required the exact blending of three necessary ingredients: purified water, S_{mix} (a blend of surfactants and co-surfactants), and oil. To optimize the experimental setting, the surfactant and co-surfactant were combined in different volume ratios inside a 50 ml stock solution. Using distinct glass vials, each phase diagram was meticulously created by combining the oil with a particular S_{mix} ratio in a range of volume combinations, from 1:9 to 9:1. Following the procedure, sixteen distinct ratios of oil to S_{mix} were analyzed, ranging from 1:1 to 9:1, and their transparency and flow properties were visually evaluated. Using the method described by Ubaidulla *et al.* (2007), sixteen distinct combinations of oil and S_{mix} were investigated, spanning ratios like 1:1, 1:2, 1:3, up to 9:1, and visually evaluated for transparency and flow characteristics.

2.3 Thermodynamic stability

The heating-cooling cycles, freezing-thawing cycles and centrifugation tests were performed on the chosen formulations during thermodynamic stability stress tests.

Heating cooling cycle: Formulations were evaluated thoroughly in both extreme situations by being exposed to 45°C and 0°C in succession for at least 48 h each cycle.

Freeze-thaw cycle: Over the course of at least 24 h, formulations were carefully exposed to two different temperatures (-20°C and 20°C). For every batch of formulation, three such cycles were carried out to imitate accelerated stability conditions, enabling a thorough assessment of their robustness and resilience.

Centrifugation tests: The formulations were subjected to centrifugal forces for 30 minutes at 5000 rpm using a REMI centrifuge located in Mumbai, India. All indications of cracking, creaming, or phase separation were carefully noted and monitored.

Based on the methods described by Akhtar *et al.* (2015), formulations exhibiting highest stability were selected for additional study after these stress testing.

2.4 Dispersibility tests

The study comprised introducing 1 milliliter of every formulation into separate containers holding 500 milliliters of 0.1N HCl and deionized water, which were kept at a precise 37 ± 0.5°C using a USP Dissolution Apparatus Type II. A conventional stainless steel dissolving paddle rotated at 75 rpm, gently stirring the solutions to encourage emulsification, in order to evaluate the effectiveness of self-emulsification (Singh *et al.,* 2022).

A specially designed grading system was used to evaluate the formulations, allowing for a comprehensive and distinct evaluation of their emulsification capability.

Grade A: A clear, instantly formed nanoemulsion that forms in less than a minute.

Grade B: An emulsion forms quickly, albeit a little less transparently.

Grade C: In less than two minutes, a fine, milky emulsion forms.

Grade D: A slightly greasy, dull white emulsion forms that needs to be carefully emulsified (taking more than two minutes).

Grade E: A formulation with big oil globules on the surface and poor or insufficient emulsification.

Formulations with acceptable dispersibility and stability, earning Grades A and B, were chosen to prepare batches loaded with genistein by using the least amount of S_{mix} for each oil fraction.

2.5 Preparation of genistein-loaded nanoemulsions by aqueous titration method

Ten milligrams of genistein were dissolved at different concentrations of 10%, 15%, 20%, and 25% v/v in the selected oil to start the formulation process. Then, using a vortex mixer, the prescribed S_{mix} ratio was added to the oil phase and thoroughly mixed. After that, the mixture was progressively mixed with the aqueous phase until the necessary nanoemulsion was achieved. This meticulous technique made sure that the emulsification process was precisely controlled, which made it easier to create stable and useful nanoemulsions (Singh *et al.,* 2022).

2.6 Characterization of genistein nanoemulsion

- **Visual examination**

It is performed to distinguish Genistein nanoemulsion and macroemulsion.

- **Dynamic light scattering (DLS) dimension**

The mean droplet size and polydispersity index (PDI) of formulated genistein nanoemulsion were measured by zetasizer ZS 90 (Malvern instruments, Worcestershire, UK). It measures the differences in light scattering produced by Brownian motion of the components. The formulation was diluted with distilled water and filtered through 0.45 µm membrane filter (Ahmad *et al*, 2017 and Akhtar, 2015).

- **Viscosity**

Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories Inc. Middleboro, MA) with spindle # CPE40 at 25 ± 0.5°C was used to determine the viscosity of formulated Genistein formulations.

- **Electrical conductivity**

The conductivity (s) of Genistein nanoformulations were analyzed by conductometer, CDM 230 (Radiometer, Copenhagen, Denmark). The interpretation was made at 94Hz, containing a cell constant of 0.11cm^{-1}. The dimensions were made at 25 ± 1°C.

- **Refractive index and percent transmittance**

The refractive index of the formulated Genistein was measured using Abbe refractometer (Bausch and Lomb optical Company, Garden City, NY). One drop of GLN was placed on the slide at 25°C. The percentage transmittance was determined by HPLC at 261 nm (Jaiswal *et al.,* 2022; Ahmad *et al*,. 2017; Bali *et al.*, 2011).

2.7 Preparation of genistein nanogel from nanoemulsion

The first step in the preparation of genistein-NEG was to dissolve 1.0% carbopol 940 in distilled water to allow for complete swelling. Following this, 5.0% glycerine was added to the mixture, and Triethanolamine (TEA) was carefully added in carefully measured amounts (1% to 1.5% w/w) to create a strong gel matrix (Singh *et al.,* 2022). In order to complete the formulation, the above hydrogel system was mixed with optimized Genistein nanoemulsion (Figure 1).

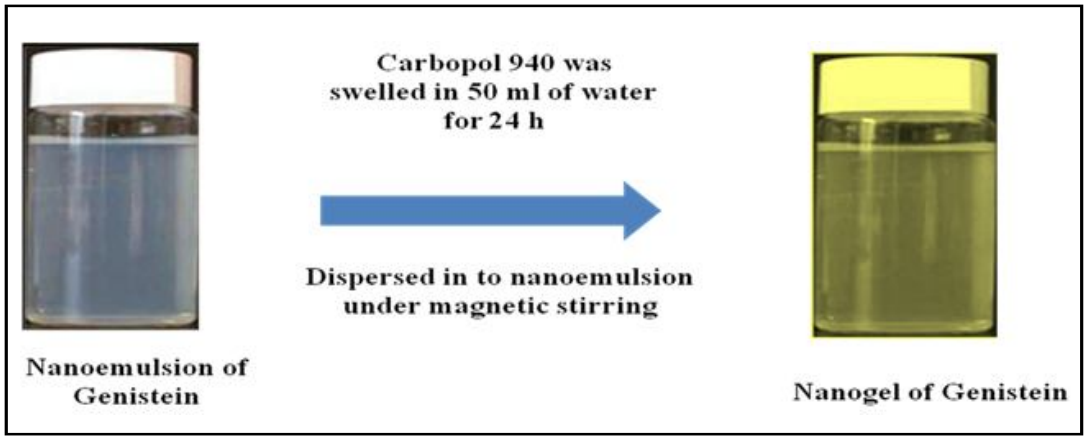


Figure 1: Genistein nanogel preparation.

2.8 Analysis of drug contents

For the analysis of drug content in nanogel, Genistein was extracted from pre-weighed 0.5 g of nanogel with 50 ml of phosphate buffer (pH 6.8) and mixture was filtered through membrane filter (0.45 µm). The absorbance of sample was determined using HPLC at 261 nm (Jaiswal *et al.,* 2022; Singh *et al.,* 2022).

2.9 Characterization of genistein nanogel

The ideal genistein-loaded nanogel was assessed for pH, viscosity, spread ability, texture analysis and drug content.

- **Determination of pH and viscosity**

The pH and viscosity of genistein nanogel was measured in triplicate by the digital pH meter and Brookfield DV III Ultra V6.0 RV viscometer respectively. Using spindle #CC14 rotated at 5 rpm. The measurements were carried out at a regulated temperature of 25 ± 0.5°C.

- **Spreadability**

Spreadability was determined by compressing 0.5 grams of the sample under glass plates with a given weight. As a function of applied mass, the spreading area was calculated (Singh *et al.,* 2022).

- **Texture analysis**

The texture properties of the nanogel were determined using a Texture Analyzer TA XTPlus (Stable Micro Systems Ltd., Surrey, UK). A standard beaker (100 ml) was filled with approximately 50 ml of the nanogel formulation, avoiding the introduction of air into the sample and ensuring the generation of a smooth upper surface. A disc 40 mm in diameter was compressed into the nanogel and redrawn. The method settings, involving speed rate and distance (depth of insertion), were chosen based on the type of nanogel. The force-time plot was used to calculate nanogel parameters such as firmness, consistency, cohesiveness, and work of cohesion. The maximum force represents the hardness/firmness of the nanogel formulation (the maximum positive force required to achieve a given deformation, F_{max}). However, cohesiveness is described as the amount of work required to deform the nanogel during the probe's downward movement (the negative area under the force-time curve: characterizes the work required to pull the probe away from the sample).

2.10 *In vitro* drug release study

The drug release from suspension, nanoemulsion and nanogel formulation was determined by using a common apparatus known as Franz Diffusion cell. This apparatus consists of a cylindrical glass tube which was opened at both the ends. Dialysis membrane

previously soaked overnight in the dissolution medium at room temperature was mounted carefully between the donor and receptor compartments of diffusion cell. The receptor compartment was filled with freshly prepared 50 ml of phosphate buffer (pH 6.8). Fixed amount of nanoemulsion and nanogel were placed in the donor compartment in two separate set of experiments. The fixed assembly was placed on a magnetic stirrer with continuous stirring of 100 rpm at 37ºC with a thermostatic control. 1 ml aliquots of the receptor medium were withdrawn at predefined intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 12 h) in order to compare the release kinetics of genistein nanoemulsion, nanogel with genistein suspension. Equal volumes of fresh receptor medium solution were replaced immediately. The samples were analyzed for genistein concentration using HPLC at 261 nm (Ahmad *et al.,* 2017).

2.11 Stability studies

Accelerated stability studies were performed as per the International Conference on Harmonization (ICH) guidelines (ICH, 1993). By keeping the samples of prepared nanogel in ambient colour vials sealed with aluminium foil. The pH, clarity, rheological evaluation, gelling capacity and drug content were determined at 0, 30, 60, and 90 days (Akhtar, *et al.,* 2014; Choudhury *et al.,* 2014).

2.12 Statistical analysis

The data was presented as mean values plus or minus standard deviation (SD). Analysis of variance (ANOVA) was used to determine statistical significance through GraphPad Prism (version 9.01).

3. Results and Discussion

3.1 Solubility studies

The important criterion to choose the components is their pharmaceutical acceptability. It has been verified that only very precise pharmaceutical excipient mixtures lead to efficient nanoemulsion formulations. All excipients under the GRAS (generally regarded as safe) category were preferred for formulation of genistein nanoemulsion. The solubility of the drug in oils is essential, as the ability of the nanoemulsion to hold the drug in solubilized form is significantly influenced by the solubility of the drug in the oil phase. If, the surfactant or cosurfactant is contributing to drug solubilization, there could be a risk of precipitation, particularly when oral or parenteral nanoemulsion is the goal (Gursoy and Benita, 2004). To fulfill the requirement of delivery system, combination of oils was tested. This confirms the marked increase in the solubility of drug in comparison to the individual oils. Among the oils and their combinations tested, the solubility of genistein was found to be highest (129.12 ± 1.02 mg/ml) in Olive oil + Sefsol-218 (1:1) Thus, Olive oil + Sefsol-218 (1:1) was selected as the oil phase for the origination of the formulation (Figure 2) (Jaiswal *et al.,* 2022; Ubaidulla *et al*, 2007; Talegaonkar *et al*, 2010).

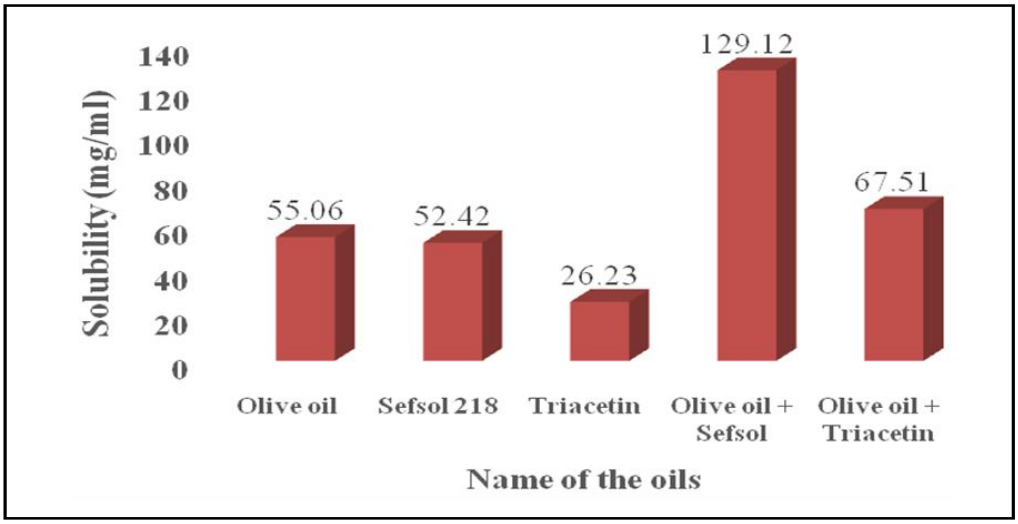


Figure 2: Solubility of genistein in selected oils.

3.2 Construction of pseudoternary phase diagrams

Based on the concepts of pseudo-ternary phase diagrams, the nanoemulsion was prepared by utilizing a low-energy emulsification process called aqueous titration (Ubaidulla *et al*., 2007; Talegaonkar *et al*., 2010). These graphs show how a mixture's composition and phase behaviour relate to one another, making it possible to identify metastable systems and optimize oil concentration. For every set of S_{mix} ratios, distinct pseudo-ternary phase diagrams were created in order to pinpoint the areas that are best suited for the development of oil-in-water nanoemulsions and metastable systems. The oil-in-water nanoemulsion region is shown in these diagrams, with turbid and standard emulsions represented in other sections. In general, surfactant alone cannot reduce the oil interfacial tension sufficiently to yield a nanoemulsion; this demands the addition of an amphiphilic molecule or co-surfactant to reduce the surface tension. Co-surfactants penetrate into the surfactant monolayer, contributing additional fluidity to the interfacial film and thus disrupting the liquid crystalline phases which are formed when the surfactant film is too rigid. This credited to the fact that transient negative interfacial tension and fluid interfacial film is hardly ever achieved by the utilization of single surfactant, typically necessitating the addition of a cosurfactant. Different regions of nanoemulsion production were shown by the phase diagram investigation of the Genistein formulation, which included olive oil and Sefsol 218 (1:1) as the oil phase, Kolliphor RH 40 as the surfactant, and PEG 400 as the

cosurfactant. Specifically, using an equal S_{mix} ratio (1:1) led to the creation of a wider region of nanoemulsions that were transparent and had a blue tinge (N3). This area's low interfacial tension between the water and oil phases allowed for the solubilization of a maximum amount of oil (16.67% v/v). PEG 400's role as a cosurfactant improved the interfacial film's pliability and encouraged the oil phase to become extensively soluble. Additionally, the investigation demonstrated that the degree of nanoemulsion generation was dependent on the concentrations of S_{mix} ratios. A S_{mix} ratio of 1:1 produced the best results, offering larger nanoemulsion zones than other ratios. On the other hand, increased surfactant concentrations resulted in smaller nanoemulsion areas and the formation of liquid crystalline phases, underscoring the significance of meticulously adjusting surfactant and cosurfactant concentrations (Jaiswal *et al.,* 2022). Overall, the phase diagram analysis sheds light on the functions and ratios of water, surfactants, and cosurfactants in the creation of nanoemulsions, emphasizing how crucial it is to choose the right S_{mix} ratio to produce the intended formulation (Figure 3).

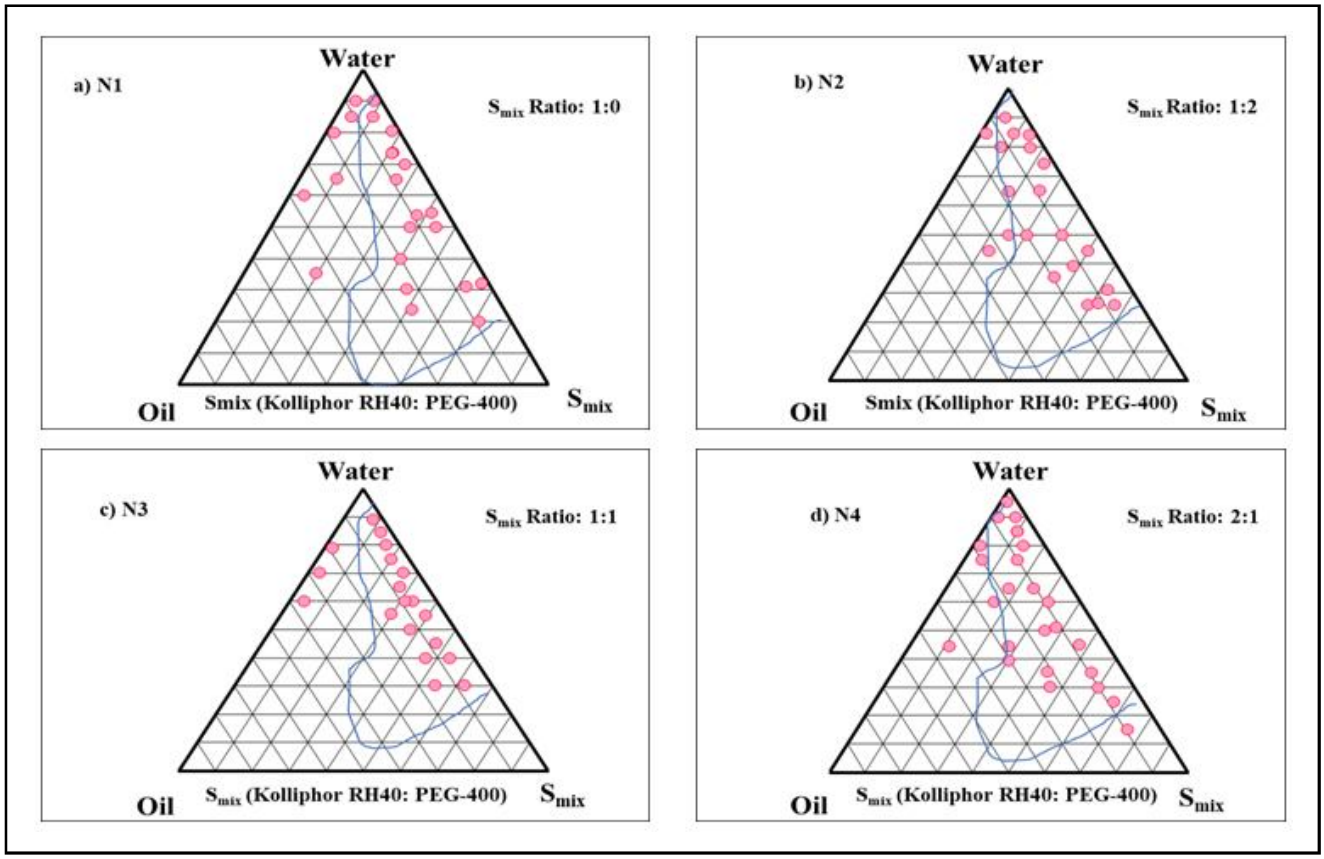


Figure 3: Ternary phase diagrams indicating o/w nanoemulsion region at the different S_{mix} ratio: N1(S_{mix} 1:0), N2 (S_{mix} 1:2), N3 (S_{mix} 1:1), N4 (S_{mix} 2:1).

Table 1: Thermodynamic stability studies

F. code	Heating cooling cycle (4°C-40°C)	Centrifugation test (5000 rpm)	Freeze thaw cycle(25°C/+25°C)	Inferences
F2B	✔	✔	✔	PASS
F2M	✔	✔	✔	PASS
F3K	✔	✔	✔	PASS
F3C	✔	×	×	FAIL
F4D	✔	✔	✔	PASS
F4N	✔	✔	×	FAIL
F2P	✔	✔	✔	PASS
F4O	✔	✔	✔	PASS
F4P	✔	✔	✔	PASS
F5K	✔	✔	✔	PASS
F5B	×	×	×	FAIL
F5M	✔	✔	✔	PASS
F6L	✔	×	✔	FAIL

As the concentration of surfactant in S_{mix} is further increased to 2:1 (N4), the nanoemulsion region reduced as compared to 1:1. Liquid crystalline area started to appear in the phase diagram, this may be due to higher surfactant concentration as in 2:1 (N4). Phase diagram obtained from N4 signify nanoemulsion formation in the aqueous region. S_{mix} ratio of 1:2 was used for this system (N2) and due to higher concentration of PEG 400 in the system interfacial tension seems to have been decreased much lower as compared with other systems. Therefore, the study of phase diagram (Figure 3) imparts helpful information on the role and concentration of surfactants, cosurfactants, and water in development of nanoemulsion. It is scrutinized from the diagram that an optimal concentration of S_{mix} ratio (1:1) provided better results.

3.3 Thermodynamic stability tests

The creation of nanoemulsions at particular concentrations of water, surfactant, and oil inhibits phase separation, creaming, and cracking, and provides thermodynamic and physical stability (Baboota *et al*., 2007). For example, nanoemulsions have intrinsic thermostability, but emulsions with kinetic stability are more likely to phase separate with time (Lawrence and Rees, 2000). Utilizing centrifugation, heating-cooling cycles, and freeze-thaw cycles, the physical (dispersion) stability of the formulations was evaluated. Nine formulations from the N3 of S_{mix} ratio (1:1) *i.e.,* F2B, F2M, F3K, F4D, F2P, F4O, F4P, F5K, and F5M were chosen for additional research after passing these tests of thermodynamic stability (Table 1).

3.4 Preparation of genistein nanoemulsion

Genistein was incorporated using the optimized formulas that passed all testing and focused on the tiniest surfactant concentrations.

- **Visual appearance**

Genistein nanoemulsion was transparent, clear, and devoid of any turbidity. The purpose of this test was to differentiate opaque-in-emergence macroemulsions from nanoemulsions.

- **Dynamic light scattering (DLS) analysis**

The DLS method was used to measure the droplet size distribution, polydispersity index, and zeta potential. The formulations F2B, F2M, F3K, F4D, F2P, F4O, F4P, F5K and F5M that passed the thermodynamic stability tests subjected to DLS analysis were having globule sizes less than 100 nm. The polydispersity index values for formulations F2B, F2M, F3K, F4D, F2P, and F5M were 0.512 ± 0.023, 0.612 ± 0.008, 0.520 ± 0.175, 0.550 ± 0.995, 0.161 ± 0.022 and 0.201 ± 0.175, respectively. A lower polydispersity index result indicated that the globule size of the nano-formulations was homogeneous. The chosen formulation composition gave much better results in terms of droplet size and polydispersity index, and the combination of oil, S_{mix}, and water was able to create small and stable globules on a nanoscale. As a result, an array of appropriate oil, surfactant, and co-surfactant ratios was identified in the data. In order to create a stable nanoemulsion and produce fewer globules, the above selection is crucial. Based on the entirety of the data, it was determined that the oil droplet size distribution in N3 formulations was suitable. The PDI was greater in the F2M (0.612 ± 0.008). However, F2P was chosen for additional research because, in comparison to other genistein nanoemulsions that were chosen, it had a more uniform droplet size, the lowest PDI (0.161 ± 0.022), the lowest viscosity (11.30 ± 0.517), the lowest refractive index (1.30 ± 0.011), and the highest electrical conductivity (399.056 ± 1.091) (Table 2).

Table 2: Characterization of optimized genistein nanoemulsion

Formulation Code	Droplet size (nm)	PDI	Zeta potential (mV)	Refractive Index	Conductivity (mS/cm)	Viscosity (cP)	% Transmittance
F2B	103.4	0.512 ± 0.023	-14.40 ± 0.501	1.32 ± 0.025	398.056 ± 1.091	11.35 ± 0.166	99.88 ± 0.55
F2M	108.6	0.612 ± 0.008	-18.40 ± 0.161	1.36 ± 0.029	354.212 ± 1.213	19.10 ± 0.720	99.38 ± 0.54
F3K	123.6	0.520 ± 0.175	-15.50 ± 0.171	1.35 ± 0.012	351.258 ± 1.051	21.71 ± 0.831	98.85 ± 0.34
F4D	109.7	0.550 ± 0.995	-9.70 ± 0.341	1.34 ± 0.021	378.133 ± 1.201	15.57 ± 0.961	98.20 ± 0.93
F2P	**89.76**	**0.161 ± 0.022**	**-20.02 ± 0.234**	**1.30 ± 0.011**	**399.056 ± 1.091**	**11.30 ± 0.517**	**99.84 ± 0.84**
F4O	237.5	0.450 ± 0.025	-20.50 ± 0.151	1.32 ± 0.071	353.159 ± 1.251	12.35 ± 0.775	99.55 ± 0.33
F4P	66.07	0.292±0.022	-22.03 ± 0.231	1.32±0.025	370.159 ± 1.362	11.35±0.166	99.54 ± 0.93
F5K	112.7	0.566 ± 0.022	-10.40 ± 0.161	1.35 ± 0.024	397.056 ± 1.091	13.35 ± 0.164	97.35 ± 0.86
F5M	108.5	0.201 ± 0.175	-22.5± 0.351	1.31 ± 0.016	369.056 ± 1.091	11.35 ± 0.165	99.84 ± 0.66

All the values were expressed as Mean±SD, (n=3).

3.5 Characterization of genistein nanogel

Following parameters were used to characterize the Genistein nanogel (F2P):

- **Visual appearance and clarity**

The prepared genistein nanogel was visually inspected for clarity and color. The genistein nanogel system appeared clear, with no presence of particles.

- **pH, viscosity and drug contents of nanogel**

In order to minimize the risk of skin irritation during topical administration, the pH evaluation of the genistein nanogel (G-NEG) formulation was crucial in ensuring its suitability. Preserving the skin's natural acid mantle requires keeping the pH within a desired range. The optimized G-NEG formulation's measured pH was found to be 5.51 ± 0.05, which is quite similar to the pH of human skin. Using a parallel plate viscometer, the rheological characteristics of the manufactured placebo gel and the Genistein nanogel were thoroughly examined. The results showed that the rheological behaviour of the nanogel and the placebo gel was similar, indicating

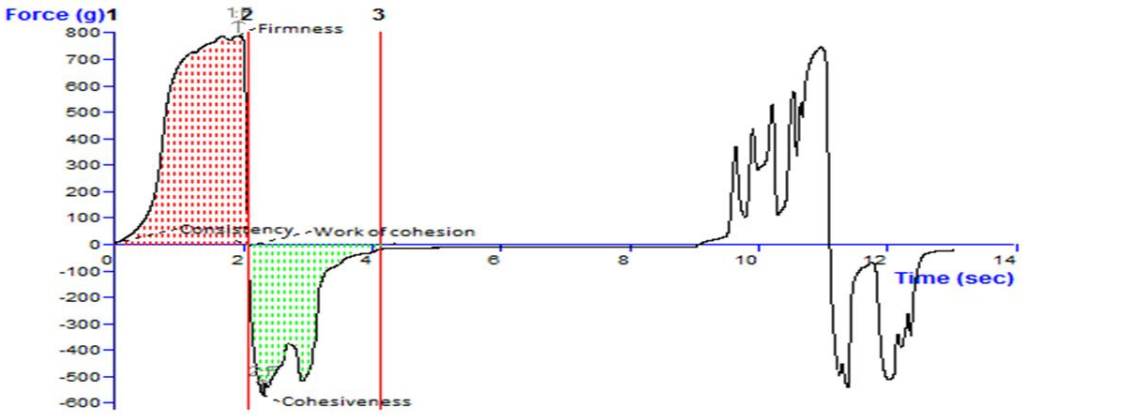
that the addition of nanoemulsion to the carbopol 940 hydrogel system did not substantially alter its rheological properties. The created hydrogel system based on nanoemulsion demonstrated thixotropic and pseudoplastic characteristics, proving its effectiveness as a vehicle for topical applications. The G-NEG spreading characteristics, which are an important criterion for evaluating topical semisolid formulations, showed that an increase in weight, or applied force, led to an extension of the spreading region. The improved G-NEG showed positive spreadability characteristics, confirming that it is appropriate for topical administration. The drug content analysis involved quantifying the concentration of genistein in the nanogel formulation, which was found to be over 90%.

- **Texture analysis of optimized nanogel**

Texture analysis of G-NEG demonstrated firmness, suggesting robust cohesiveness within the gel structure. These findings emphasized the gel's potential suitability for applications requiring stable and cohesive formulations (Figure 4).

3.6 *In vitro* drug release profile

In vitro drug release study over a period of 12 hours showed improved genistein release from G-NEG (87.11%) as compared to Genistein nanoemulsion (70.81%) and Genistein suspension (21.20%). G-NEG showed prolonged drug release characteristics as depicted in Figure 5. Drug release pattern observed suggest a steady increase in the release of genistein over a period from 4 to 12 h.



Test ID	Batch		Firmness g Force 1	Consistency g.sec Area F-T 1:2	Cohesiveness g Force 2	Work of Cohesion g.sec Area F-T 2:3
Start Batch TEST	TEST					
TEST1	TEST		790.85	1012.44	-573.38	-546.39
End Batch TEST	TEST					
Average:	TEST (F)	AVERAGE("BATCH")	790.85	1012.44	-573.38	-546.39
S.D.	TEST (F)	STDEVP("BATCH")	0.00	0.00	0.00	0.00
C.V.	TEST (F)	STDEVP("BATCH")/AVERAGE("BATCH")*100	0.00	0.00	0.00	0.00
End of Test Data						

Figure 4: Texture analysis report.

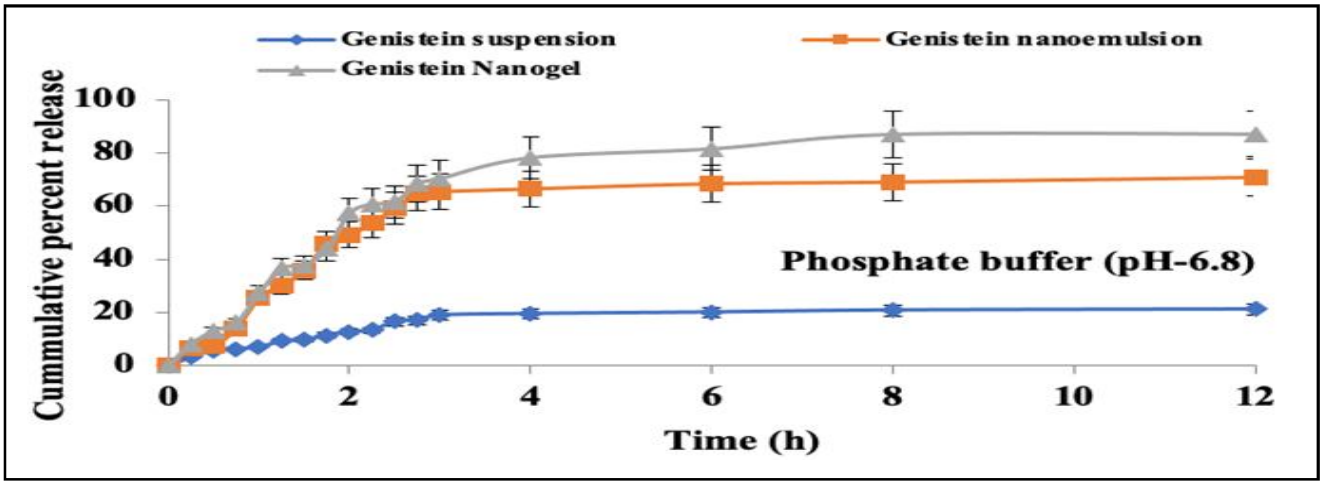


Figure 5: Cumulative *in-vitro* drug release of genistein loaded nanogel and their comparison with genistein nanoemulsion and genistein suspension.

3.7 Accelerated stability studies

The stability study of optimized nanogel formulation (G-NEG) formulation showed increased droplet size. However, there was reduced percent transmittance in comparison to formulations at day zero. No phase separation was noticed and G-NEG was found to be most stable ($p>0.05$) at 25±2°C/60±5% RH than at higher temperatures 40±2°C/65±5% RH, and 60±2°C/75±5% RH, respectively.

4. Conclusion

Genistein-loaded nanogel was successfully developed and characterized. The optimized nanogel formulation exhibited desired characteristics such as improved drug release profile and good stability. This finding suggests the potential of Genistein nanogel as a topical delivery system in skin care. However, further research is warranted to fully explore the safety and efficacy of Genistein nanogel in preventing skin damage.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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