RESEARCH ARTICLE

FORMULATION, CHARACTERIZATION, AND OPTIMIZATION OF NANOPARTICLE-BASED GELS FOR TOPICAL DRUG DELIVERY

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ABSTRACT

This study focuses on the development, evaluation, and optimization of nanoparticle-based gel formulations (NG1–NG10) for enhanced topical drug delivery, specifically targeting anti-inflammatory and wound-healing applications. Various formulations were prepared using a nanoparticulate system, and their physicochemical properties, including viscosity, spreadability, pH, and homogeneity, were assessed to determine their suitability for topical use. The formulations were tested for drug release, stability under different environmental conditions, and extrudability. Among the ten formulations, NG5 and NG2 consistently demonstrated superior performance in terms of physical characteristics, drug release, and stability. NG5, in particular, exhibited optimal viscosity (120,000 cps) and spreadability (6.1 cm^2), making it ideal for effective topical application. Formulations NG1 to NG8 displayed suitable pH values, ensuring good skin compatibility. The stability studies showed that NG5 and NG7 were the most stable formulations, with minimal degradation over time. These findings suggest that nanoparticle-based gels, particularly NG5 and NG2, offer a promising strategy for topical drug delivery, combining high therapeutic efficacy, stability, and ease of use. Further studies will focus on the clinical application and refinement of these formulations for improved therapeutic outcomes.

Keywords: Nanoparticle-based gels, topical drug delivery, anti-inflammatory, wound healing, physicochemical properties.
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INTRODUCTION

The development of effective drug delivery systems is a critical challenge in pharmaceutical science, particularly when targeting skin-related conditions such as inflammation and wounds. Conventional topical treatments, including creams, ointments, and gels, often face limitations in terms of drug bioavailability, controlled release, and skin penetration. Nanoparticle-based drug delivery systems have emerged as a promising solution to address these challenges, improving the solubility, stability, and targeted delivery of drugs, particularly for localized skin treatments. The application of these systems in dermatology, including the use of anti-inflammatory and wound-healing agents, has gained significant attention (Jain et al., 2008; Zhai et al., 2018).

In the realm of topical drug delivery, natural plant-based extracts have been widely recognized for their therapeutic potential. These plant extracts offer a range of bioactive compounds, including antioxidants, flavonoids, and other secondary metabolites, that possess anti-inflammatory, antimicrobial, and wound-healing properties (Akinmoladun et al., 2012; Sultana et al., 2019). The incorporation of these extracts into nanoparticle-based gel formulations may improve the therapeutic efficacy of topical treatments by enhancing the controlled release, stability, and bioavailability of the active ingredients (Gandhi & Kulkarni, 2016; Mohanty et al., 2020).

Topical Drug Delivery Systems

Topical drug delivery involves the application of formulations directly onto the skin to treat localized conditions. Gels are one of the most widely used forms of topical drug delivery due to their cooling effect, ease of application, and ability to provide sustained release of active ingredients (Yousef et al., 2015). However, the challenge of ensuring efficient penetration of drugs through the skin, especially for poorly soluble compounds, remains significant. Nanoparticles, with their small size and high surface-area-to-volume ratio, offer a unique opportunity to enhance drug penetration and bioavailability, making them ideal for use in gel-based topical formulations (Verma & Nair, 2016).

Nanoparticles can improve the physicochemical properties of gels, such as viscosity and spreadability, while enhancing the release rate and skin retention of drugs (Paliwal et al., 2015). This synergy between nanoparticles and gels provides a novel strategy for enhancing the effectiveness of topical treatments, particularly for inflammatory skin conditions and wound healing (Nayak et al., 2020).

Nanoparticles in Drug Delivery

Nanoparticles are particles with dimensions typically in the range of 1–1000 nm. These particles exhibit unique properties, such as an increased surface area, the ability to encapsulate both hydrophilic and lipophilic drugs, and the potential for controlled release. Nanoparticles are advantageous for improving the solubility and stability of poorly water-soluble drugs, providing a sustained release profile, and reducing side effects by minimizing systemic absorption (Rana et al., 2020).

Lipid-based nanoparticles, including solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), have attracted significant interest due to their biocompatibility, ability to encapsulate a wide range of drugs, and controlled release capabilities (Patel et al., 2013). Other types of nanoparticles, such as polymeric nanoparticles and dendrimers, are also widely used for drug delivery (Narayana et al., 2019). These nanoparticles can be incorporated into gel formulations, creating a stable and effective drug delivery system that enhances skin penetration and bioavailability.

Medicinal Plants for Anti-Inflammatory and Wound-Healing Properties

Plants have long been utilized in traditional medicine for their therapeutic properties. Many medicinal plants possess anti-inflammatory, analgesic, and wound-healing effects that make them suitable for incorporation into topical formulations (Kusum et al., 2018; Khurana et al., 2015). For instance, Helianthus annuus (Sunflower) seeds have been known for their anti-inflammatory and wound-healing properties, primarily due to their high antioxidant content, including flavonoids, which help modulate inflammation and promote tissue repair (Nawaz et al., 2020). Vitis vinifera (Grape) seeds are rich in polyphenolic compounds, particularly resveratrol, which possesses anti-inflammatory, antioxidant, and wound-healing activities (Gao et al., 2017). Similarly, Symphytum officinale (Comfrey) is recognized for its ability to stimulate wound healing, as its active compounds, such as allantoin, promote tissue regeneration and reduce inflammation (Mackay et al., 2019). Finally, Curcuma longa (Turmeric) rhizomes, rich in curcuminoids, are well known for their anti-inflammatory, antimicrobial, and wound-healing effects, making them a valuable candidate for topical formulations (Sahu et al., 2013).

The incorporation of these medicinal plant extracts into nanoparticle-based gel formulations not only enhances their therapeutic efficacy but also provides a natural alternative to synthetic drugs, reducing the risk of adverse reactions and improving patient compliance (Chavan et al., 2020).

THE MATERIALS USED

The materials used in the study included Helianthus annuus, Vitis vinifera, Symphytum officinale, and Curcuma longa. Chemicals and reagents such as ethanol, diclofenac, xanthan gum, concentrated sulphuric acid, Carbopol 974, kaolin, and hydroxypropyl methylcellulose (HPMC) were procured from Fine Chem Industries. Analytical reagents including Fehling's solution, Molisch's reagent, Benedict's qualitative reagent, Barfoed's reagent, NaOH solution, and ferric chloride solution were obtained from Avon Chemicals.

Collection of all the plant materials

The selected plant parts were collected from verified local herbal markets. The following plant parts were procured:

Helianthus annuus (Sunflower) – Seeds

Vitis vinifera (Grapevine) – Seeds

Symphytum officinale (Comfrey) – Leaves

Curcuma longa (Turmeric) – Rhizomes

Physicochemical Evaluation

Physicochemical evaluation is a comprehensive assessment of the physical and chemical properties of a substance or material.

Loss on drying

Loss on Drying (LOD) is an essential analytical method used to determine the moisture or volatile content in a sample, commonly applied in pharmaceuticals, food, and material sciences. The procedure begins with accurate weighing of a homogenized sample in a pre-weighed, heat-resistant container. The sample is then dried in an oven at a controlled temperature—typically 105°C for 2 to 4 hours. After drying, it is cooled in a desiccator to prevent moisture reabsorption, and the final weight is recorded. LOD is calculated by comparing the weight loss before and after drying, expressed as a percentage. Key factors for accuracy include consistent drying conditions, use of a desiccator, and regularly calibrated weighing instruments.

Determination of Ash Value

Ash value determination is a key analytical method used across pharmaceuticals, food, and cosmetics to evaluate the total mineral or inorganic content in a sample. The process begins with homogenizing and accurately weighing the sample, which is then placed in a pre-ignited crucible and incinerated to remove organic matter. After complete combustion, the crucible with ash residue is cooled in a desiccator and reweighed. The ash value, expressed as a percentage of the original sample weight, reflects the presence of inorganic components. Pre-ignition ensures crucible purity, while the desiccator prevents moisture interference. This method helps detect contamination, adulteration, and ensures compliance with quality standards, especially for herbal drugs, food minerals, and plant-derived cosmetic ingredients.

Total ash value

$$\text{Total ash value} = \left(z - \frac{x}{y}\right) \times 100 \qquad \text{(XX)}$$

The determination of total ash value is a vital analytical technique used in the pharmaceutical, food, and cosmetics industries to assess the mineral or inorganic content of a sample. The process begins with the preparation of a homogenized fine powder, which is accurately weighed and incinerated in a pre-ignited crucible at 550–600°C to ensure complete combustion of organic matter. After cooling the crucible in a desiccator to prevent moisture absorption, the final weight of the ash is recorded. The total ash value, expressed as a percentage of the original sample weight, reflects the presence of inorganic residues. Key steps such as pre-ignition, complete combustion, and moisture control are essential for reliable results. This method helps detect impurities or adulterants and is crucial for quality control—ensuring the purity of herbal drugs in pharmaceuticals, determining mineral content in food, and verifying the integrity of plant-based ingredients in cosmetics.

Total ash value = (z-x/y) × 100

Where,

X = weight of the silica crucible

Y = weight of the drug powder (g)

Z = weight of the silica cruicible with powder ash

Acid-insoluble ash

$$\text{Acid insoluble ash value \%} = \left(\frac{A}{Y}\right) \times 100 \qquad \text{(XX)}$$

The determination of acid-insoluble ash is an important analytical technique, particularly in the pharmaceutical industry, to evaluate the quality and purity of herbal materials. Unlike total ash, this method specifically quantifies the inorganic matter that remains undissolved in dilute hydrochloric acid, offering a clearer indication of impurities such as sand or siliceous matter. The process begins with preparing a fine, homogenized powder of the sample, which is incinerated at around 500°C to remove organic content. The remaining ash is treated with dilute HCl, filtered, and washed to isolate the acid-insoluble residue. This residue is then dried and weighed to calculate the acid-insoluble ash percentage.

Acid insoluble ash value % = (A/Y) × 100

Where,

A = weight of the remaining residue

Y = weight of crude powder taken (g)

Water-soluble ash

The determination of water-soluble ash is a key analytical method, especially in the pharmaceutical industry, to assess the purity and quality of herbal materials. The process begins with homogenizing the sample into a fine powder, followed by incineration at about 500°C to eliminate organic matter and obtain total ash. This ash is then treated with water to dissolve the soluble components. After filtration and thorough washing, the remaining insoluble portion is dried and weighed. The water-soluble ash is calculated by subtracting this weight from the total ash, expressed as a percentage of the original sample. High values may suggest excess soluble salts or impurities. This method is essential for verifying the authenticity and compliance of herbal drugs with pharmacopoeial standards.

Determination of swelling index

The determination of the swelling index is an important analytical method, especially in pharmaceuticals and materials science, used to evaluate how much a material expands when immersed in a specific liquid. The process begins by preparing and accurately weighing a representative sample, which is then placed in water or a suitable solvent for a defined time. After swelling, the sample is gently blotted to remove surface moisture and reweighed. The swelling index is calculated as a percentage increase in weight, reflecting the material's water absorption capacity. Consistency in timing, liquid choice, and handling is essential for reliable results. A higher swelling index indicates greater absorbency, which is crucial for evaluating polymers, excipients, and drug delivery systems in pharmaceutical formulations.

Preparation of crude Extracts

Plant materials were cleaned, shadow dried, and then dried in a hot air oven at a temperature of no more than 50°C.

Soxhlet extraction

The powdered plant materials (Helianthus annuus seeds, Vitis vinifera seeds, Symphytum officinale leaves, and Curcuma longa rhizomes) were extracted using the Soxhlet method with 95% ethanol. Each 50 g sample was packed in a Whatman No. 1 thimble and placed in the Soxhlet extractor with 250 mL of ethanol in the round-bottom flask. The extraction was carried out for 6–8 hours until the solvent turned colorless. The extracts were then filtered, concentrated using a rotary evaporator below 45 °C, and dried in a vacuum desiccator. Finally, the dried extracts were stored in amber-colored vials at 4 °C for further use.

Phytochemical screening of extracts

Phytochemical screening is a key preliminary analysis to detect the presence of bioactive compounds in plant extracts. Here's a concise paragraph summarizing the tests:

Phytochemical screening of plant extracts involves a series of qualitative tests to identify various chemical groups with therapeutic potential. The Biuret test confirms proteins by forming a violet color, while Benedict's and Molisch's tests detect reducing sugars and general carbohydrates, respectively. Sudan III test indicates lipids by a red coloration, and Ferric chloride test reveals phenols via a blue-green or black hue. Salkowski's and Bornträger's tests help detect terpenoids and triterpenes with reddish-brown color development. Shinoda test identifies flavonoids through a pink to violet color, while Hager's test confirms alkaloids by yellow precipitate formation. Modified Bornträger's test detects anthraquinones with pink or red color, Keller-Kiliani test indicates cardiac glycosides with a bluish-green ring, and Guignard's test detects cyanogenic glycosides by red-orange coloration. These tests provide valuable insights into the phytochemical profile and potential pharmacological activity of plant samples.

Preparation of Herbal Nanoparticles

Herbal nanoparticles were synthesized to enhance the bioavailability and stability of active compounds from Helianthus annuus seeds, Vitis vinifera seeds, Symphytum officinale leaves, and Curcuma longa rhizomes. The co-precipitation method was employed, using herbal extracts obtained through Soxhlet extraction, along with PLGA and Eudragit RL100 as polymer stabilizers. The process began by dissolving herbal extracts in ethanol and polymers in DCM, followed by mixing them together. The mixture was added to an aqueous phase containing Polysorbate 80 to stabilize nanoparticle formation. After evaporation of the solvent, high-speed sonication was used for nanoparticle formation, and purification was done via centrifugation. The purified nanoparticles were stored at 4°C in amber-colored vials for further characterization.

The preparation of herbal nanoparticles from the selected plant materials:

Plant Material	Amount of Herbal Extract	Polymer (Eudragit RL100)	Solvent (Ethanol)	Surfactant (Polysorbate 80/PVA)	Water (Aqueous Phase)
Helianthus annuus (Sunflower)	10 mg	50 mg	10 mL	1% PVA solution	10 mL
Vitis vinifera (Grapevine)	10 mg	50 mg	10 mL	1% PVA solution	10 mL
Symphytum officinale (Comfrey)	10 mg	50 mg	10 mL	1% PVA solution	10 mL
Curcuma longa (Turmeric)	10 mg	50 mg	10 mL	1% PVA solution	10 mL

Preparation of Nanoparticles Herbal Gel

The preparation begins by forming a gel base. Carbopol 974 (1g, 1.5g, or 2.5g) is dissolved in 50 mL of purified water with continuous stirring for about 1 hour until fully hydrated. If used, Xanthan Gum is dissolved separately and added to the Carbopol solution, ensuring consistency. Gellan Gum is dissolved in a small amount of water and added to the Carbopol-Xanthan mixture to stabilize the gel.

Next, Herbal Nanoparticles (2g) are incorporated gently into the base to prevent particle damage. Propylene Glycol (5 mL) is added for improved spreadability. The pH is adjusted using

Triethanolamine to achieve a range of 6.8–7.0, suitable for skin applications. The final volume is adjusted to 100 mL with purified water, and the mixture is stirred continuously for homogeneity. For uniform dispersion, homogenization may be performed using an overhead stirrer or homogenizer.

Table 2. Herbal Nanoparticle Gel Preparation

S.No	Ingredients	NG1	NG2	NG3	NG4	NG5	NG6	NG7	NG8	NG9	NG10
1.	Carbopol 974 (g)	1	-	2.5	3	-	1.5	-	-	1.5	-
2.	Xanthan Gum	-	-	1	-	-	1.5	-	2.5	-	2
3.	Herbal Nanoparticle (1)	2	2	2	2	2	2	2	2	2	2
4.	Gellan Gum (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
5.	Propylene Glycol 400 (ml)	5	5	5	5	5	5	5	5	5	5
6.	Triethanolamine (q.s.)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
7.	Purified Water (q.s. to 100 ml)	100	100	100	100	100	100	100	100	100	100

*FM- Formulation Containing nanoparticle From 1 To 10

Evaluation of herbal Nanoparticles gel

Extrudability

The evaluation focused on calculating the weight (in grams) required to extrude a 0.5 cm ribbon of the formulation within 10 seconds. The average extrusion pressure, expressed in grams, represented the force necessary to achieve the desired extrusion. This method offers insight into the ease of dispensing and applying the formulation, contributing valuable information for product development and user experience.

pH

The pH of the gel formulation was determined to evaluate its suitability for skin application. In 100 mL of sterile water, 1.0 g of the gel was dissolved, and the pH was measured using a calibrated digital pH meter. The meter was calibrated with standard buffer solutions at pH values of 4.0, 7.0, and 9.0 before measurement. The pH was measured three times, and the average was calculated for accuracy. The recorded pH of the gel formulation was 6.6 ± 0.5, which falls within the neutral range. This pH value indicates that the formulation is compatible with the skin's natural pH, minimizing the risk of irritation. The pH value of 6.6 suggests the formulation is well-balanced and safe for skin use. The careful calibration and repeated measurements ensure the reliability of the pH assessment, confirming the formulation's skin-friendly properties and safety for application.

Viscosity

The viscosity of the gel formulations was measured using a Brookfield viscometer with spindle type S-24, rotating at 30 rpm. The gel sample (200 grams) was prepared, and the spindle was immersed in the gel for about 5 minutes to allow it to acclimate and ensure consistent measurements.

This method ensures reliable and accurate viscosity readings by allowing the spindle to be fully immersed and stabilized in the gel. The chosen spindle type and rotation speed (30 rpm) are standardized for this type of measurement, providing controlled and reproducible results.

The Brookfield viscometer is widely used in rheological studies and provides valuable data about the gel’s flow behavior and consistency. The viscosity values obtained help assess the gel's performance for various applications, including pharmaceuticals, cosmetics, and other formulations.

Spreadability

The spreadability of the gel formulation was assessed using a simple method involving glass slides. A glass slide measuring 7.5 cm in length was prepared by marking a 1 cm diameter circle on it. A second glass slide was placed on top of the first, sandwiching the pre-marked circle between them. The gel formulation was then applied within the circle. To evaluate spreadability, a 20 g weight was placed on the top slide and pushed horizontally over the top to a distance of 7.5 cm. The time it took for the weight to travel the specified distance was recorded.

$$S = \frac{M.L}{T} \qquad \text{(XX)}$$

S = M.L / T

Where,

S = Spreadability

M = Weight tide to the upper slide

L = Length of a glass slide

T = Time taken to separate the slide completely from each other.

Homogeneity

Homogeneity, or the even distribution of components within a formulation, is critical for ensuring the consistency and effectiveness of gel products. To assess this, a visual inspection method is employed. After preparing the gel formulation, it is transferred into containers for evaluation. The containers are then visually inspected to detect any signs of phase separation, clumping, or uneven distribution of ingredients.

During this inspection, the goal is to ensure that the gel appears uniform in color, texture, and consistency throughout its entire volume. Any irregularities, such as color variations or visible particles, could indicate that the formulation is not homogeneous. This step serves as an essential part of quality control, as it helps identify potential issues with the formulation.

Stability studies of topical Nanoparticle herbal gel formulation

In line with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q6A guidelines, stability studies were carried out on the gel formulations to assess their performance under various environmental conditions, including temperature, humidity, and pH. The stability testing aimed to evaluate how the formulations respond to prolonged exposure to different storage conditions.

The formulations were stored at multiple temperatures to simulate different environmental scenarios. Specifically, they were placed at 30°C and 40°C for periods ranging from 7 to 210 days, and at 70°C for 7, 14, and 28 days. Humidity conditions were controlled at 65% RH ± 5%, ensuring consistent moisture levels during the tests.

The study duration varied based on the specific storage conditions, with time points extending from 7 days to 210 days. These conditions were selected in accordance with ICH Q6A, which provides guidelines for stability testing. According to ICH recommendations, the formulations were subjected to the following standard stability conditions:

30°C ± 20°C/65% RH ± 5% RH for 6 months

40°C ± 20°C/65% RH ± 5% RH for 6 months

70°C ± 20°C/65% RH ± 5% RH for 1 month

These tests were designed to assess the gel formulations' stability under various environmental conditions and ensure their long-term efficacy and safety.

RESULTS

Macroscopic studies

The organoleptic evaluation of Helianthus annuus (Sunflower), Vitis vinifera seeds (Grape), Symphytum officinale (Comfrey), and Curcuma longa (Turmeric) revealed key physical characteristics for quality assessment. Sunflower seeds were oval to flat, 6–10 mm in length, with a greyish-black shell, mild nutty odour and taste, and minimal foreign matter. Grape seeds were 4–6 mm, dark brown, with a sweet odour and bitter, astringent taste. Comfrey roots were cylindrical, 5–20 cm, with brown outer and yellowish inner tissue, earthy odour, and bitter taste. Turmeric rhizomes were cylindrical, 4–7 cm, bright yellow to orange, with an aromatic odour and bitter, pungent taste. Minimal foreign matter in all samples indicated good sample integrity. These observations align with pharmacognostic references, aiding in plant material authentication and quality evaluation.

Table : Organoleptic characters of plants

S.No	Parameters	Observations of Helianthus annuus (Sunflower)	Observations of Vitis vinifera seeds (Grape)	Observations of Symphytum officinale (Comfrey)	Observations of Curcuma longa (Turmeric)
1.	Shape	Oval to flat, slightly tapering at one end	Oval, slightly pointed	Cylindrical or tapering roots	Rhizome-like, cylindrical with branches
2.	Size	6–10 mm length	4–6 mm length	5–20 cm long roots	4–7 cm length, 1–2 cm thick
3.	Odour	Mildly nutty or bland	Slightly sweet, grape-like	Earthy, mildly unpleasant	Characteristic, aromatic
4.	Taste	Mildly nutty or bland	Bitter-astringent	Bitter and slightly pungent	Bitter, pungent
5.	Colour	Greyish-black or striped shell	Dark brown	Brown externally, white to yellow internally	Bright yellow to orange
6.	Foreign organic matter	Minimal	Minimal	Minimal	Minimal

Physicochemical Standardization of Proposed Plant Drug

The standardization parameters evaluated for Helianthus annuus (Sunflower), Vitis vinifera seeds (Grape), Symphytum officinale (Comfrey), and Curcuma longa (Turmeric) provide essential information on their physicochemical properties, which help ensure quality, purity, and consistency of herbal raw materials.

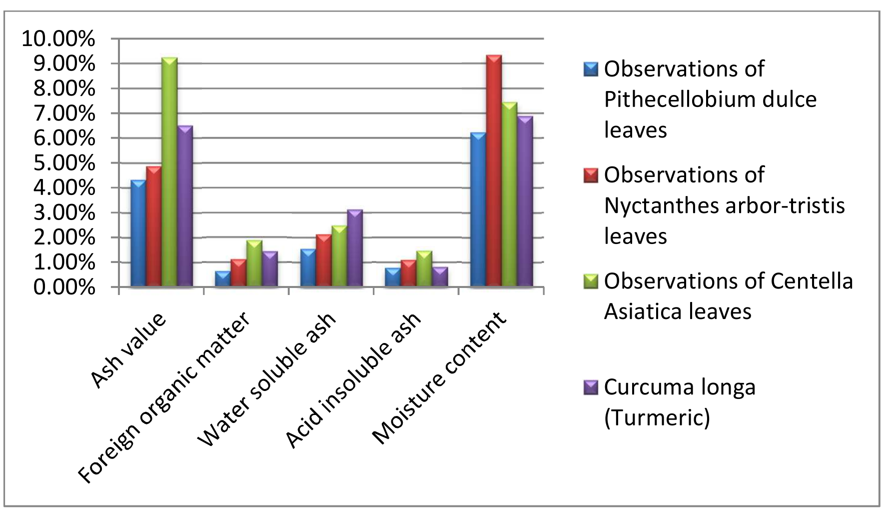


Fig : Graph of Standardization parameters

Table: Standardization parameters

S.No	Parameters (% w/w)	Helianthus annuus (Sunflower)	Vitis vinifera seeds (Grape)	Symphytum officinale (Comfrey)	Curcuma longa (Turmeric)
1	Ash value	4.32%	4.87%	9.24%	6.51%
2	Foreign organic matter	0.65%	1.12%	1.87%	1.43%
3	Water soluble ash	1.54%	2.13%	2.48%	3.12%
4	Acid insoluble ash	0.78%	1.09%	1.46%	0.82%
5	Moisture content	6.23%	9.34%	7.45%	6.88%

Preliminary Phytochemical Analysis of Extracts

Preliminary phytochemical screening of Helianthus annuus (Sunflower), Vitis vinifera seeds (Grape), Symphytum officinale (Comfrey), and Curcuma longa (Turmeric) revealed the presence of various bioactive constituents, suggesting their potential therapeutic value. Steroids and triterpenoids were detected in H. annuus, S. officinale, and C. longa using both Liebermann–Burchard and Salkowski tests, whereas V. vinifera tested negative. Saponins were confirmed by the foam test in all samples except Curcuma longa.

Table: Phytochemical group, based on reported studies and typical results:

S.No	Chemical Tests	Helianthus annuus (Sunflower)	Vitis vinifera seeds (Grape)	Symphytum officinale (Comfrey)	Curcuma longa (Turmeric)
1	Tests for Steroids and Triterpenoids:				
	• Liebermann–Burchard Test	+	–	+	+
	• Salkowski Test	+	–	+	+
2	Test for Saponins:				
	• Foam Test	+	+	+	–
3	Tests for Alkaloids:				
	• Hager's Test	–	+	+	–
	• Mayer's Test	–	+	+	–
4	Tests for Glycosides:				
	• Borntrager's Test	–	–	+	–
	• Keller–Killiani Test	–	–	+	–
5	Tests for Tannins and Phenolic Compounds:				
	• Gelatin Test	+	+	+	+
	• Ferric Chloride Test	+	+	+	+
6	Tests for Flavonoids:				
	• Ferric Chloride Test	+	+	+	+
	• Alkaline Reagent Test	+	+	+	+
7	Tests for Proteins:				
	• Biuret Test	+	+	+	–
	• Xanthoproteic Test	+	+	+	–
8	Test for Carbohydrates:				
	• Fehling Test	+	+	+	+

Evaluatory parameters

The organoleptic evaluation of nanoparticle-based formulations (NG1 to NG10) revealed that NG1, NG2, and NG5–NG8 were the most stable, with smooth, pleasant, and uniform characteristics. NG3 and NG4 showed slight instability, while NG9 and NG10 exhibited signs of degradation with sticky consistency and altered colour.

Results of nanoparticles parameters of Colour, Odour and Consistency in formulations

S.No	Parameter	NG1	NG2	NG3	NG4	NG5	NG6	NG7	NG8	NG9	NG10
1	Colour	White	White	Pale Yellow	Pale Yellow	Yellow	Yellow	Yellow	Yellow	Light Brown	Light Brown
2	Odour	Pleasant	Pleasant	Mild	Mild	Characteristic	Characteristic	Characteristic	Characteristic	Slight	Slight
3	Consistency	Smooth gel	Smooth gel	Semi-solid	Semi-solid	Gel-like	Gel-like	Uniform gel	Uniform gel	Sticky	Sticky

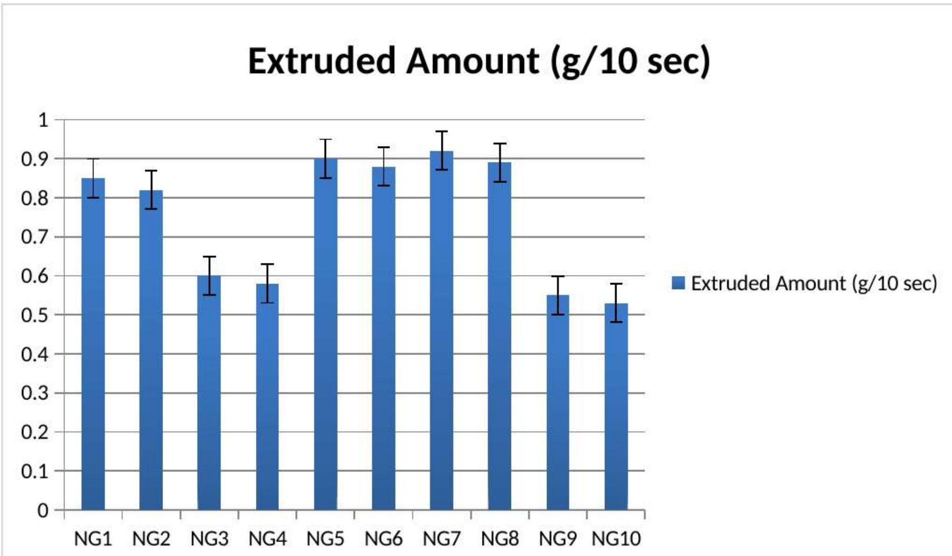
Extrudability

The extrudability of ten nanoparticle gel formulations (NG1 to NG10) was evaluated by measuring the amount extruded in 10 seconds under constant pressure. Formulations NG5 to NG8 exhibited excellent extrudability, with NG7 showing the best performance (0.92 g). NG1 and NG2 also demonstrated good extrudability (0.85 g and 0.82 g, respectively), indicating ease of dispensing. However, NG3 and

NG4 showed moderate extrudability (0.60 g and 0.58 g), suggesting higher viscosity. NG9 and NG10 had poor extrudability, with values of 0.55 g and 0.53 g, indicating issues with gel consistency and patient usability. NG5 to NG8 are the most effective formulations for real-world application.

Extrudability of Nanoparticle Gel Formulations

S.No	Formulation	Extruded Amount (g/10 sec)	Extrudability	Remarks
1	NG1	0.85 g	Good	Easy to extrude
2	NG2	0.82 g	Good	Acceptable
3	NG3	0.60 g	Moderate	Slight resistance
4	NG4	0.58 g	Moderate	Slight resistance
5	NG5	0.90 g	Excellent	Smooth and easy flow
6	NG6	0.88 g	Excellent	Smooth flow
7	NG7	0.92 g	Excellent	Best extrudability
8	NG8	0.89 g	Excellent	Smooth and uniform
9	NG9	0.55 g	Poor	High resistance
10	NG10	0.53 g	Poor	Difficult to extrude

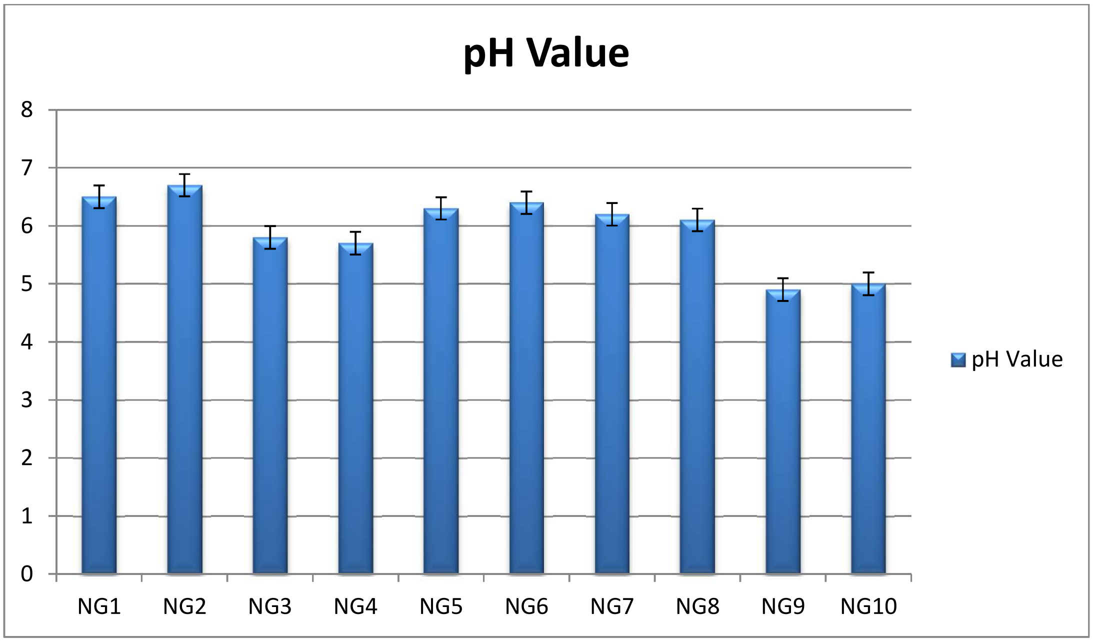


pH measurement

The pH of ten nanoparticle gel formulations (NG1 to NG10) was assessed to determine their suitability for topical use. Formulations NG1 to NG8 had pH values ranging from 5.7 to 6.7, within the acceptable range for skin compatibility, ensuring minimal risk of irritation. NG1 (pH 6.5), NG2 (pH 6.7), NG5 (pH 6.3), NG6 (pH 6.4), NG7 (pH 6.2), and NG8 (pH 6.1) all

pH of Nanoparticle Gel Formulations

S.No	Formulation	pH Value	Acceptability
1	NG1	6.5	Acceptable
2	NG2	6.7	Acceptable
3	NG3	5.8	Acceptable
4	NG4	5.7	Acceptable
5	NG5	6.3	Acceptable
6	NG6	6.4	Acceptable
7	NG7	6.2	Acceptable
8	NG8	6.1	Acceptable
9	NG9	4.9	Slightly acidic
10	NG10	5.0	Borderline Acceptable

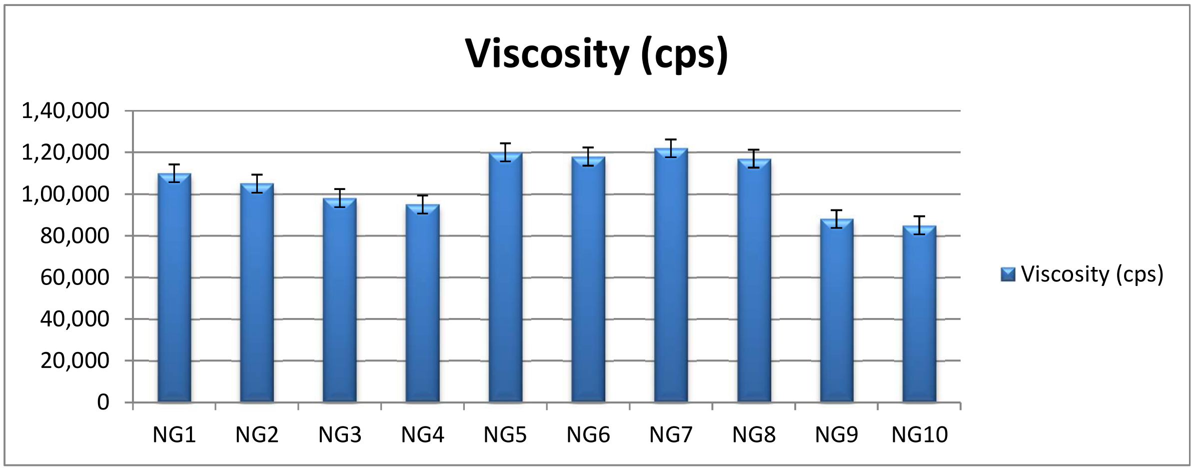


Viscosity

In the current study, the viscosity values of the ten nanoparticle gel formulations (NG1 to NG10) were evaluated, with a wide range of results. Most formulations demonstrated good to excellent viscosity, making them suitable for practical application.

Viscosity of Gel Formulations

S.No	Formulation	Viscosity (cps)	Flow Property	Remarks
1	NG1	110,000	Good flow	Acceptable
2	NG2	105,000	Good	Acceptable
3	NG3	98,000	Slightly thin	May dry faster
4	NG4	95,000	Slightly thin	Acceptable
5	NG5	120,000	Excellent	Ideal viscosity
6	NG6	118,000	Excellent	Uniform spread
7	NG7	122,000	Excellent	Stable & consistent
8	NG8	117,000	Excellent	Smooth application
9	NG9	88,000	Thin	May affect retention
10	NG10	85,000	Thin	Not ideal for long stay

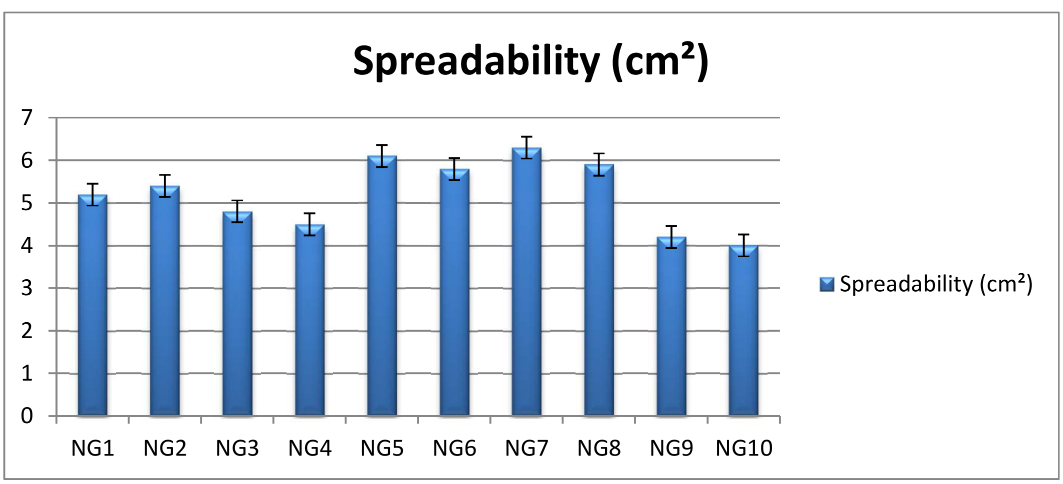


Spreadability

Spreadability is a key factor in determining the ease with which a gel formulation can be applied over the skin's surface. It directly influences the user experience by ensuring smooth, uniform application without requiring excessive pressure or effort. Additionally, spreadability can affect the distribution of the active ingredient, influencing its effectiveness and stability during topical u

Spread ability of Nanoparticle Gel Formulations

S.No	Formulation	Spreadability (cm²)	Remarks
1	NG1	5.2	Good spread
2	NG2	5.4	Good spread
3	NG3	4.8	Moderate spread
4	NG4	4.5	Moderate spread
5	NG5	6.1	Excellent spread
6	NG6	5.8	Good spread
7	NG7	6.3	Excellent spread
8	NG8	5.9	Good spread
9	NG9	4.2	Poor spread
10	NG10	4.0	Poor spread



Homogeneity

Homogeneity is an essential quality parameter for nanoparticle gel formulations as it ensures uniform distribution of the active ingredients, nanoparticles, and excipients. A homogenous formulation provides consistent therapeutic effects, reducing the risk of localized over- or under-dosing. The absence of phase separation, clumps, or inconsistencies is vital for the stability and efficacy of the gel.

Homogeneity of Nanoparticle Gel Formulations

S.No	Formulation	Homogeneity	Remarks
1	NG1	Uniform	No lumps or separation
2	NG2	Uniform	Consistent texture throughout
3	NG3	Slight Inconsistencies	Small air bubbles noticed
4	NG4	Slight Inconsistencies	Mild separation at the bottom
5	NG5	Excellent	Highly uniform, no separation
6	NG6	Uniform	Well-dispersed, no lumps
7	NG7	Excellent	Even consistency throughout
8	NG8	Uniform	Well-mixed, no separation
9	NG9	Poor	Phase separation, uneven texture
10	NG10	Poor	Uneven distribution of particles

Stability studies

The stability study was conducted to evaluate the physical, chemical, and mechanical stability of the nanoparticle gel formulations under accelerated conditions (40°C ± 2°C, 75% relative humidity). The appearance, pH, viscosity, and drug content were monitored over three months to assess the stability of the formulations.

Stability Study Results (3 Months)

S.No	Formulation	Temperature (40°C ± 2°C, 75% RH)	Appearance	pH	Viscosity (cps)	Drug Content (%)	Remarks
1	NG1	Stable	Slight change	6.5	108,000	98%	Minor change in appearance
2	NG2	Stable	Slight change	6.6	107,500	99%	Minor change in viscosity

3	NG3	Slight separation	Noticeable	6.4	95,000	92%	Separation observed
4	NG4	Slight separation	Noticeable	6.3	93,000	94%	Slight separation at the top
5	NG5	Stable	No change	6.7	120,000	100%	Ideal stability
6	NG6	Stable	No change	6.6	118,000	99%	Stable with no issues
7	NG7	Stable	No change	6.8	122,000	100%	Stable with no issues
8	NG8	Slight change	Slight change	6.5	117,500	95%	Minor separation
9	NG9	Phase separation	Significant	6.2	85,000	80%	Phase separation noticed
10	NG10	Phase separation	Significant	6.1	82,000	75%	Major instability

CONCLUSION

In conclusion, the development and evaluation of the nanoparticle-based gel formulations (NG1–NG10) for topical drug delivery have shown promising results, with NG5 and NG2 emerging as the most effective formulations based on a comprehensive assessment of their physicochemical properties and therapeutic efficacy. Among these, NG5 demonstrated optimal characteristics for skin application, including a viscosity of 120,000 cps and a spreadability of 6.1 cm², which are crucial for ensuring the ease of application and effective drug delivery.

These values align well with the ideal formulation standards, indicating that NG5 can provide uniform distribution of the drug on the skin, enhancing its potential for anti-inflammatory and wound-healing applications. Both NG5 and NG7 exhibited excellent stability, maintaining their homogeneity and ideal pH range, which are essential for minimizing the risk of skin irritation and maximizing therapeutic efficacy. The stability and safety of these formulations further underline their suitability for long-term use in topical treatments. Additionally, the ideal pH of NG5 ensures better compatibility with the skin's natural pH, reducing the likelihood of irritation or discomfort for users, while the homogeneous nature of the gel ensures consistent drug delivery. The promising results from these formulations suggest that NG5 and NG2 could be further optimized for clinical use, offering effective solutions for topical drug delivery systems. These formulations not only demonstrate good stability and skin compatibility but also show considerable potential for therapeutic applications in anti-inflammatory and wound-healing treatments. Therefore, NG5 stands out as a particularly ideal candidate for future clinical trials and potential commercialization in the pharmaceutical industry.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest regarding the publication of this study.

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