Frontiers in Health InformaticsISSN-Online: 2676-7104 www.healthinformaticsjournal.com
2025; Vol 14: Issue 2 Open Access

Development, Standardization And Pharmacological Screening Of Nanogel Herbal Formulation

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Cite this paper as: Ankur Tripathi, Anand Chaurasiya, Dharmendra Singh Rajput, Naveen Gupta, Brajesh Sirohi (2024), Development, Standardization And Pharmacological Screening Of Nanogel Herbal Formulation. *Frontiers in Health Informatics*, *14(2) 2282-2296*

Abstract: This study investigates the formulation, evaluation, and characterization of saffron extract-based gels, emphasizing their organoleptic, physicochemical, and phytochemical properties. Saffron, a prized botanical with rich sensory attributes, was explored for its chemical composition, including the presence of compounds such as steroids, triterpenoids, saponins, tannins, and proteins. The formulation process involved blending saffron extract with various excipients, including Tween 80, Carbomer 934, and Polyvinyl alcohol, across ten different formulations (S-1 to S-10). These formulations were evaluated for key attributes such as pH, spreadability, texture, homogeneity, and stability. Results indicated that formulations S-1, S-3, S-4, S-7, and S-10 exhibited slightly heterogeneous properties, with pH values ranging from 5.5 to 6.1, and required further refinement for improved consistency. The findings highlight the complexity of creating a stable and effective saffron gel formulation, while emphasizing the importance of continuous refinement to achieve the desired quality and performance. The study contributes valuable insights into saffron's potential as both a therapeutic and cosmetic agent, advocating for further optimization in gel formulation to unlock its full potential.
Keywords: Saffron extract, Gel formulation, physicochemical properties, phytochemical analysis.

Introduction

Wound healing is a critical physiological process that involves restoring the integrity of injured tissue. This multifaceted process comprises hemostasis, inflammation, proliferation, and remodeling, which must occur in a well-orchestrated manner to achieve successful tissue repair. However, chronic wounds or delayed healing, often caused by infections, oxidative stress, or underlying health conditions like diabetes, pose significant challenges in clinical management (Guo & DiPietro, 2010).

Conventional wound therapies, while effective to some extent, are often associated with limitations, including slow healing rates, adverse drug reactions, and high costs. These challenges have led to the exploration of

advanced wound care strategies, such as nanotechnology-based drug delivery systems. Among these, nanogels have gained attention due to their small size, high surface area, biocompatibility, and ability to deliver drugs or bioactive compounds precisely to the wound site. Nanogels also allow controlled and sustained release of therapeutic agents, reducing the frequency of application and enhancing patient compliance (Kabanov & Vinogradov, 2009).

Herbal medicines, rooted in traditional practices, are recognized for their natural bioactive compounds, such as alkaloids, flavonoids, saponins, and terpenoids, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. When integrated into nanogel formulations, these phytoconstituents can synergistically accelerate wound healing by addressing multiple aspects, including infection control, inflammation reduction, and stimulation of collagen synthesis and angiogenesis (Raina et al., 2022)

Role of Herbal Medicines in Wound Healing

Herbal medicines have been extensively utilized for their wound-healing properties. Bioactive compounds present in medicinal plants, such as alkaloids, flavonoids, saponins, and terpenoids, exhibit antioxidant, antimicrobial, and anti-inflammatory effects. These phytochemicals aid in modulating oxidative stress, preventing infections, and enhancing cellular proliferation and angiogenesis, which are critical for wound repair (Sharma et al., 2020). Herbal nanotechnology integrates these benefits with advanced drug delivery systems, ensuring precise targeting and controlled release for improved therapeutic outcomes (Kabanov & Vinogradov, 2009).

Nanogels in Wound Management

Nanogels, characterized by their nano-scale size, hydrophilic nature, and high drug-loading capacity, offer significant advantages for wound treatment. They provide controlled drug release, enhance drug stability, and improve penetration at the wound site. Studies have demonstrated the potential of nanogels to deliver herbal extracts effectively, combining modern pharmaceutical techniques with traditional medicinal benefits. Their biocompatibility further ensures minimal side effects during wound healing (Madheswaran et al., 2021).

Crocus sativus in Wound Healing

Crocus sativus, commonly known as saffron, has been studied for its diverse pharmacological properties, including its wound-healing potential. Saffron contains bioactive compounds like crocin, crocetin, safranal, and picrocrocin, which exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities. Research indicates that these compounds promote collagen synthesis, angiogenesis, and epithelialization, making saffron an excellent candidate for wound management (Rezaee & Hosseinzadeh, 2013). When incorporated into nanogels, the therapeutic efficacy of saffron can be significantly enhanced due to improved stability and targeted delivery (Babaei et al., 2015).

This study aims to develop a nanogel herbal formulation specifically designed for wound healing. The objectives include its standardization, evaluation of physicochemical and stability parameters, and pharmacological screening to validate its therapeutic potential. The findings will contribute to the growing field of herbal nanotechnology and offer an advanced, cost-effective solution for wound care.

Material & Methods

Material Used

The formulation of herbal gels involves various materials, including active ingredients like Crocus sativus and excipients such as gelling agents, hydroxypropyl methylcellulose (HPMC), and triethanolamine for gel consistency. Solvents like ethanol and purified water are used to dissolve components, while glycerin and Tween 80 serve as humectants and emulsifiers, respectively. Analytical reagents like Fehling's solution, Molisch's reagent, Benedict's qualitative reagent, Barfoed's reagent, NaOH solution, ferric chloride solution,

Mayer's reagent, Dragendorff's reagent, Hager's reagent, and the Lieberman–Burchard reagent are used for qualitative phytochemical testing during formulation and standardization.

Instrument Used

The formulation and evaluation of herbal gels require a variety of instruments to ensure product quality, stability, and efficacy. Key equipment includes analytical balances for accurate measurements, mixer/blenders, homogenizers, and emulsifying equipment for uniform mixing, and heating and mixing equipment for preparation. Instruments like pH meters, viscometers or rheometers, and stability chambers are essential for assessing physical and chemical properties. Supporting tools such as beakers, flasks, pipettes, stirring rods, funnels, burettes, and petri dishes aid in laboratory procedures. Additional equipment like desiccators, centrifuge tubes, test tubes, drying ovens, and pH electrodes ensure thorough analysis and testing during gel formulation.

Collection And Authentication Of Plant Material

The selected plant material Crocus sativus threads was purchased from local market of Bhopal India. The specimens were identified and authenticated by the Department of Botany and their herbarium was deposited.

Soxhlet extraction

The Soxhlet extraction of saffron involves weighing 10 g of saffron threads and placing them in the thimble of a properly assembled Soxhlet extractor connected to a round-bottom flask containing 100 ml of ethanol as the solvent. The ethanol is heated using a heating mantle, vaporized, and condensed in the condenser, allowing it to drip onto the saffron for cyclic extraction over several hours until the solvent becomes saturated with saffron compounds. The extract is then concentrated using a rotary evaporator to remove ethanol, filtered through filter paper to eliminate solids, and stored in a dark, airtight container to preserve its bioactive properties. This process ensures efficient extraction of saffron compounds, suitable for research in food, pharmaceutical, or cosmetic applications, with safety precautions adhered to during solvent handling and heating.

Macroscopic studies

The selected crude drugs were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc.

Physicochemical Evaluation

Determination of Ash Value

Ash content is a crucial parameter in the physicochemical analysis of food and agricultural products, reflecting the inorganic residue remaining after the complete combustion of organic matter. It provides valuable insights into the mineral composition, particularly the inorganic salts present in the sample. The analysis requires a crucible (porcelain, quartz, or metal), a muffle furnace capable of 550-600°C, a desiccator for cooling and drying, and an analytical balance for accurate measurements. The procedure involves weighing the sample, preheating the crucible in the muffle furnace to remove residues, and cooling it in a desiccator. The weighed sample is evenly placed in the crucible and subjected to gradual heating in the furnace until complete combustion is achieved. After cooling the crucible in the desiccator, the ash content is determined by weighing the crucible with the ashed sample, with the weight increase corresponding to the ash content. This method ensures accurate and reliable determination of mineral residues.

Calculation

$$\text{Ash Content (\%)} = \frac{Weight\ of\ Ash}{Weight\ of\ Sample} \times 100$$

Determination of Ash Value

The precise determination of total ash content involves evaluating both naturally occurring minerals and added impurities. This process requires materials such as a crucible (porcelain, quartz, or metal), a muffle furnace capable of 550-600°C, a desiccator for cooling, an analytical balance for accurate measurements, and an ashing dish for uniform burning. The procedure begins with accurately weighing a representative sample and preheating the crucible in the muffle furnace to remove organic residues, followed by cooling in a desiccator. The weighed sample is transferred into the crucible, ensuring even distribution, and subjected to combustion in the furnace at around 550-600°C until fully ashed. The cooled crucible is reweighed, with the weight increase indicating the total ash content. This method ensures reliable results for mineral and impurity analysis.

Calculation

$$\text{Total Ash Content (\%)} = \frac{Weight\ of\ Ash}{Weig\quad of\ Sample} \text{x}100$$

Determination of acid insoluble ash

The determination of acid-insoluble ash involves assessing the mineral content that remains undissolved in dilute hydrochloric acid, providing insights into impurities like silica or other insoluble substances. The process requires materials such as a crucible, muffle furnace (550-600°C), desiccator, analytical balance, ashing dish, and dilute hydrochloric acid (HCl). The procedure begins with weighing the sample and completing the total ash determination process. The resulting ash is treated with HCl to dissolve soluble salts, then filtered to separate the insoluble ash. The residue is washed with hot water, dried, and ashed in a muffle furnace until a constant weight is achieved. After cooling in a desiccator, the residue is weighed, with the resulting weight increase representing the acid-insoluble ash content. This method ensures precise evaluation of mineral impurities.

Calculation:

$$\text{Acid Insoluble Ash Content (\%)} = \frac{Weight\ of\ Acid\ Insoluble\ Ash\ Content}{Weight\ of\ Sample} \text{x}100$$

Determination of water soluble ash

The determination of water-soluble ash involves evaluating the mineral components that dissolve in water, providing insight into the solubility and purity of the sample. The process requires materials such as a crucible, muffle furnace (550-600°C), desiccator, analytical balance, ashing dish, and water. The procedure begins by accurately weighing a representative sample, followed by the total ash determination process. The resulting ash is treated with water to dissolve water-soluble components, then filtered to separate these components from the insoluble ash. The residue is washed with water, dried, and ashed in a muffle furnace until a constant weight is achieved. After cooling in a desiccator, the residue is weighed, and the increase in weight corresponds to the water-soluble ash content. This method is essential for determining the solubility characteristics of a material.

Calculation:

$$\text{Water-Soluble Ash Content (\%)} = \frac{Water{-}Soluble\ Ash\ Content}{Weight\ of\ Sample} \text{x}100$$

Determination of loss on drying

Loss on drying (LOD) is a crucial parameter for determining the moisture content of a sample, essential in industries like pharmaceuticals, food, and materials testing. The process involves using an analytical balance, drying oven or moisture analyzer, and a desiccator. First, a representative sample is weighed, then dried using either the drying oven method (placing the sample in a pre-weighed dish and drying at 105-110°C until constant weight is achieved) or the moisture analyzer method (following the instrument's instructions). After drying, the sample is cooled in a desiccator to prevent moisture absorption, then reweighed. The difference in weight before

and after drying indicates the moisture content, providing insights into the material's stability, quality, and processing conditions.

Calculation:

Loss on Drying (%) = $\frac{Initial\ Weig\quad - Final\ Weigh}{Initial\ Weigh}$ x100

Moisture content

Moisture content is a vital parameter for quality control, product development, and process optimization in industries such as food, pharmaceuticals, and materials testing. The determination process involves using an analytical balance, drying oven or moisture analyzer, and a desiccator. First, accurately weigh the sample, then choose a drying method: the drying oven method (dry the sample in a pre-weighed dish at 105-110°C until constant weight) or the moisture analyzer method (follow the analyzer's instructions). After drying, place the sample in a desiccator to cool, preventing moisture absorption. Finally, reweigh the sample. The decrease in weight represents the moisture content.

Prelimnary phytochemical analysis of extracts

Preliminary phytochemical analysis is an essential step in identifying and characterizing the chemical compounds present in plant extracts, which may have various biological activities and contribute to the medicinal properties of the plants. One of the key tests for alkaloids involves using Mayer's reagent, Dragendorff's reagent, Wagner's reagent, and Hager's reagent. In Mayer's test, a yellow or cream-colored precipitate indicates alkaloids, while Dragendorff's reagent forms an orange or red-brown precipitate. Wagner's reagent yields a reddish-brown precipitate, and Hager's reagent forms a yellow or orange precipitate, all of which signify the presence of alkaloids.

For carbohydrates, tests such as Molisch's, Fehling's, and Benedict's reagents are used. In Molisch's test, a violet ring at the junction of the plant extract and sulfuric acid indicates carbohydrates. Fehling's and Benedict's reagents detect reducing sugars; Fehling's test produces a brick-red precipitate when reducing sugars are present, while Benedict's test forms a colored precipitate ranging from green to brick-red upon heating. Glycosides are identified using the modified Borntrager's reagent test, where a pink, red, or violet color in the ammoniacal layer suggests their presence.

Phytosterols and triterpenoids can be detected using Liebermann, Libermann-Burchard, and Salkowski reagents. In Liebermann's test, a color change from violet to blue or green indicates these compounds, while the Libermann-Burchard test results in a green color. Salkowski's reagent test produces a red-brown color in the presence of triterpenoids. Protein and amino acids are tested with Millon's, Ninhydrin, and Biuret reagents. A brick-red precipitate from Millon's reagent indicates proteins, while Ninhydrin's test produces a blue or purple color for amino acids. Biuret's test forms a violet color in the presence of proteins.

For phenolic compounds and tannins, the Ferric Chloride reagent test produces a blue-black or green color, indicating phenolic compounds, while the Lead Acetate reagent test forms a white or yellow precipitate, signaling tannins. Flavonoids are detected using Shinoda's reagent, where a red color suggests their presence. Oils and fats can be identified with the Oily Spot test, which leaves a translucent spot on filter paper, confirming their presence. Finally, saponins are tested by shaking the extract with water; persistent foam or a stable foam layer indicates the presence of saponins.

Formulation of Nanogel

Formulating a saffron extract gel involves combining key ingredients like saffron extract, Tween 80, Carbomer 934, Polyethylene glycol, Polyvinyl alcohol, Triethanolamine, and purified water. The process begins with accurately weighing the ingredients. Carbomer 934 is dispersed in purified water, followed by the addition of Tween 80 for stability. Saffron extract is then mixed into the dispersion, and hydration forms the gel structure.

The gel is neutralized with Triethanolamine to achieve the desired pH, and Polyethylene glycol and Polyvinyl alcohol are added for homogeneity. The mixture is thoroughly blended, and quality control tests for consistency, viscosity, and microbial stability are conducted. After successful testing, the gel is packaged with labeling and storage instructions. Adherence to safety and regulatory guidelines ensures a high-quality final product.

Table: Formulation Table Composition gel

Ingredients (g)	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
Saffron extract	80	80	80	80	80	80	80	80	80	80
Tween 80	-	2	-	2	-	3	-	4	-	-
Carbomer 934	2	-	3	-	2	-	2	-	-	-
Polyethylene glycol	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Polyvinyl alcohol	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Triethanolmine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Purified Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

q.s means "quantity sufficient

Formulated Gel

Evaluation of Gel

pH

To evaluate the pH, a pH meter is preferred for its precision, although pH indicator strips can also be used. Begin by preparing the gel sample, potentially diluting it to ensure homogeneity. Calibrate the pH meter with standard buffer solutions to ensure accuracy. Immerse the pH electrode into the gel sample, allow the reading to stabilize, and record the value. The pH directly impacts ingredient stability, making pH evaluation essential for gel formulation quality.

Spreadability

Spreadability is an essential quality in evaluating topical formulations like gels, creams, and lotions. It determines how easily a product spreads on the skin, influenced by the formulation's texture and rheological

properties. Visual observation is often the first step, with a smooth and uniform appearance indicating good spreadability.

Extrudability

Extrudability is a key characteristic in the evaluation of topical formulations, particularly gels, and refers to the ease with which a product can be dispensed from its container or packaging. This property is crucial for user convenience, ensuring that the product can be easily and uniformly extruded or dispensed when applied to the skin.

Viscosity

Viscosity is often measured using a viscometer, an instrument designed to assess the resistance of a fluid or gel to shear forces. Different types of viscometers, such as rotational viscometers or capillary viscometers, may be used based on the gel's characteristics.

Homogeneity

In the context of gels, homogeneity is particularly important to ensure consistent application and the desired properties. Visual assessment is the simplest method to evaluate homogeneity. The absence of visible clumps, aggregates, or phase separation indicates good homogeneity. Regular visual checks during and after the manufacturing process are essential.

Stability study:

Stability studies of topical herbal gel formulations were conducted in adherence to the recommendations outlined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), specifically ICH Q6A. The primary objective of these studies was to evaluate the stability of the formulations under varying environmental conditions, encompassing temperature, humidity, and pH.

Stability Testing Conditions:

1. **Temperature and Humidity Conditions:**
    - The formulations underwent storage at different temperatures:
        - 30°C and 40°C for 7, 14, 28, 70, 140, and 210 days.
        - 70°C for 7, 14, and 28 days.
    - Humidity conditions were maintained at 65% RH ± 5%.
2. **Stability Duration:**
    - The stability assessment spanned time points ranging from 7 to 210 days, contingent upon the specific storage conditions.
3. **ICH Recommendations:**
    - The selected temperature and humidity conditions align with the ICH Q6A guidelines for stability testing.
    - The specified conditions include:
        - 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.

By adhering to these rigorous stability testing conditions, the study aimed to provide a comprehensive understanding of the formulations' robustness and integrity under simulated environmental challenges. The assessment covered a range of temperatures and humidity levels, ensuring that the herbal gel formulations would maintain their quality, efficacy, and physical attributes over an extended period. The data generated from these stability studies will be instrumental in determining the formulations' shelf life and storage recommendations,

ultimately contributing to the formulation's regulatory compliance and successful market introduction

Results

Macroscopic studies:

Saffron's allure extends beyond the visual, beckoning the olfactory senses with its distinct and captivating fragrance.

Table : Organoleptic characters of plants Rosmarinus officinalis (rosemary) leaves

S.No	Parameters	Observations of Crocus sativus (saffron)
1.	Shape	Typically has a thread-like shape. It is long and slender
2.	Size	High-quality saffron threads are long and uniform in size
3.	Odour	Saffron has a distinct and aromatic fragrance
4.	Taste	It has a slightly bitter taste, and its pungent
5.	Colour	The color of saffron threads is a crucial indicator of quality. High-quality saffron has a deep red color.
6.	Foreign organic matter	Good quality saffron should be free of any foreign organic matter, such as dirt, insects, or other plant material.

Physicochemical Standardization of Proposed Plant Drug

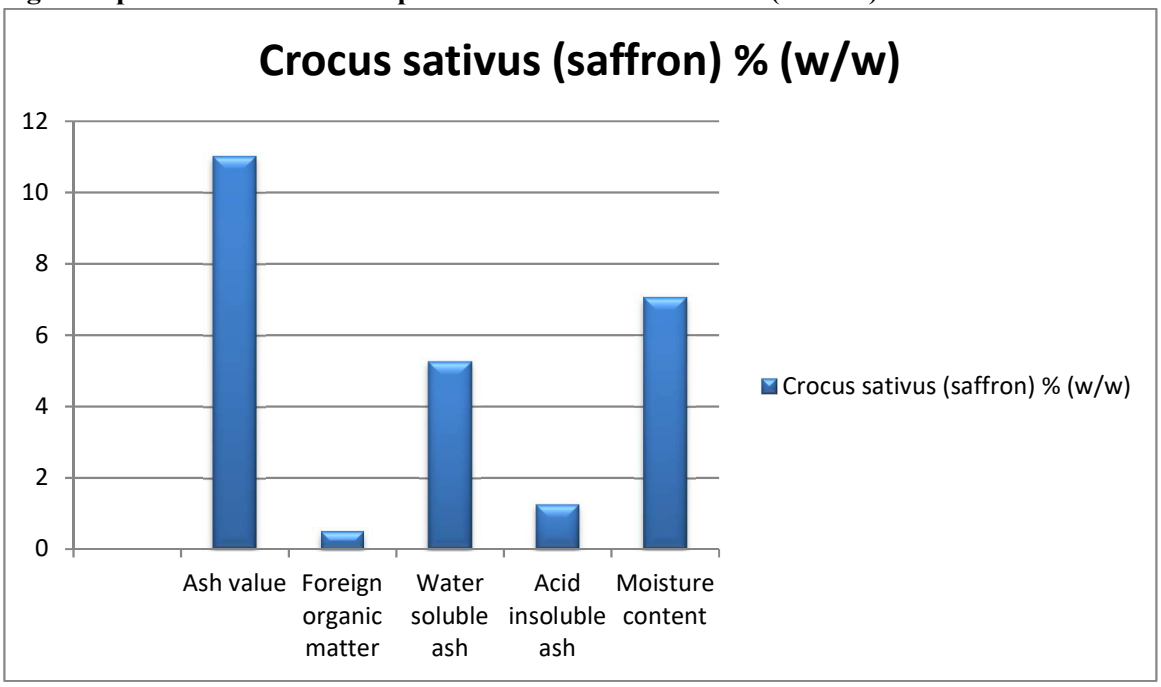
The following physicochemical properties were determined by normal technique using the powdered plant material of Crocus sativus (saffron).

Table. Standardization parameters of Crocus sativus (saffron)

S.No	Parameters % (w/w)	Crocus sativus (saffron) (% w/w)
1	Ash value	11.02
2	Foreign organic matter	0.5
3	Water soluble ash	5.25
4	Acid insoluble ash	1.25

5.	Moisture content	7.05
6.	Extractive value	1.5g (15%)

Fig : Graph of Standardization parameters of Crocus sativus (saffron)



Preliminary Photochemical Analysis of Extracts:

In the analytical exploration of Crocus sativus, commonly known as saffron, an array of chemical tests has been conducted on its ethanol extract to unravel the intricate composition and potential bioactive constituents. These tests serve as a scientific tapestry, weaving together the presence or absence of specific compounds, offering insights into the chemical profile of saffron.

Table : Phytochemical Profile of Crocus sativus (saffron) Extract.

S.no	Chemical Tests	*Crocus sativus (saffron) Extract*
		Ethanol
1.	Tests for Steroids and Triterpenoids:	
	• Liebermann's 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 Polysaccarides:	
	• Molish Reaction	-

Where + is Present and – is Absent

Physical evaluation and characterization of formulated nanogel

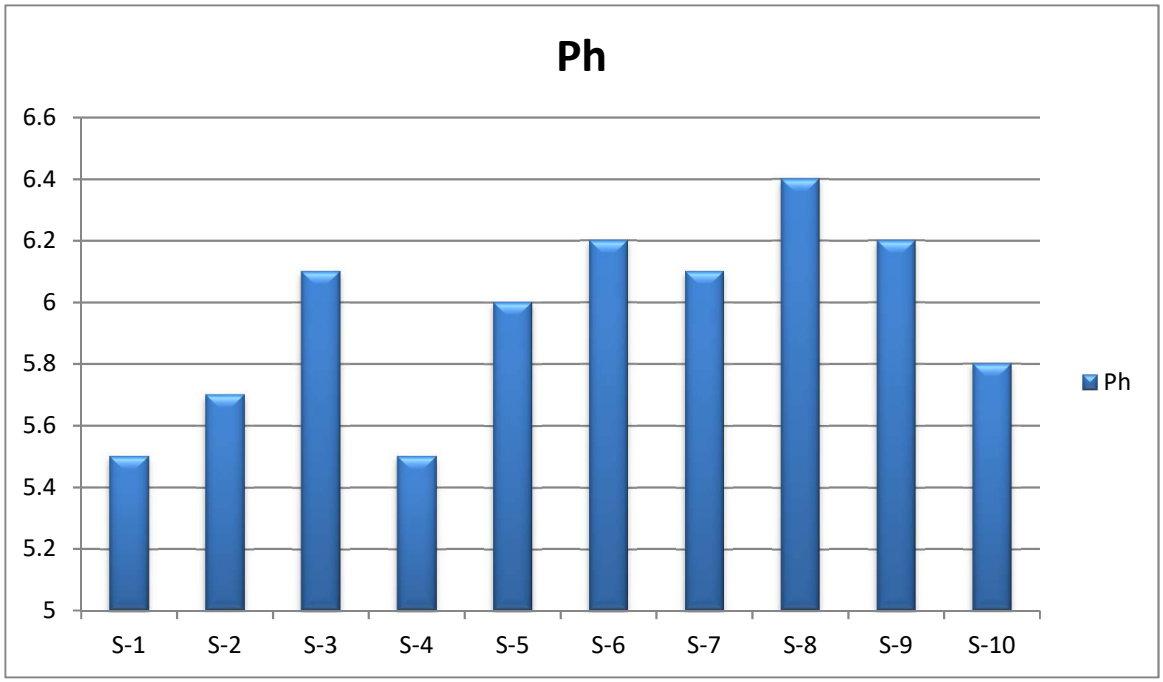
Evaluation of Gel Formulation

In the realm of pharmaceutical sciences, the art of formulation is a delicate dance between precise measurements and the pursuit of optimal characteristics. Our quest for excellence in gel formulations led us to a meticulous evaluation of ten distinct formulations, each identified by the label S-1 through S-10. This evaluation, conducted with an unwavering commitment to precision, encompassed the critical parameters of pH, consistency, and homogeneity.

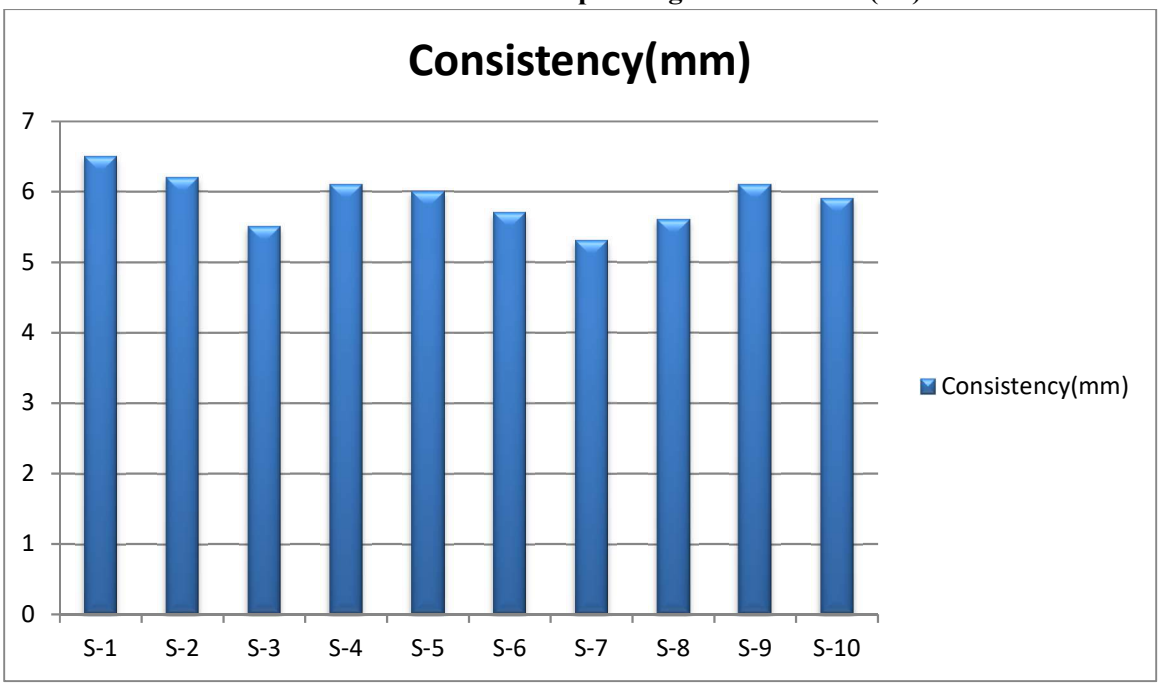
Table. Evaluation of Gel Formulation

Formulation	Ph	Consistency(mm)	Homogenecity

S-1	5.5	6.5	Slightly Homogenous
S-2	5.7	6.2	Homogenous
S-3	6.1	5.5	Slightly Homogenous
S-4	5.5	6.1	Slightly Homogenous
S-5	6.0	6.0	Homogenous
S-6	6.2	5.7	Homogenous
S-7	6.1	5.3	Slightly Homogenous
S-8	6.4	5.6	Homogenous
S-9	6.2	6.1	Homogenous
S-10	5.8	5.9	Homogenous



Evaluation of different formulations of microspheres gel formulation (Ph)



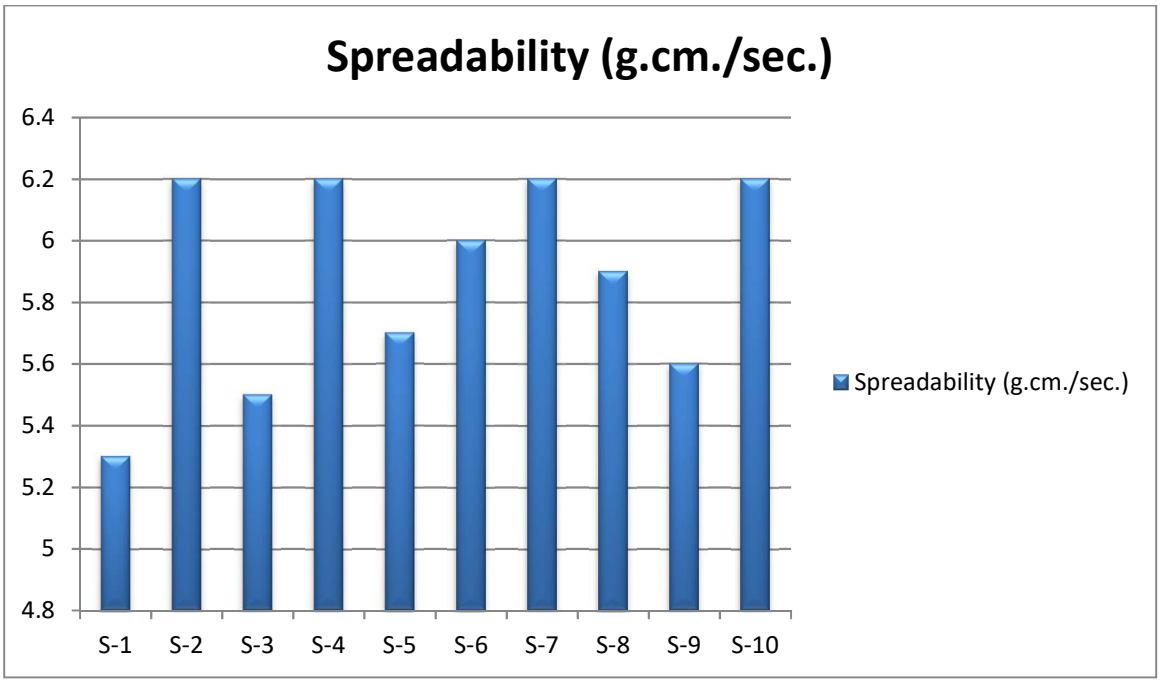
Evaluation of different formulations of microspheres gel formulation (Consistency(mm)

Evaluation of Gel Formulation

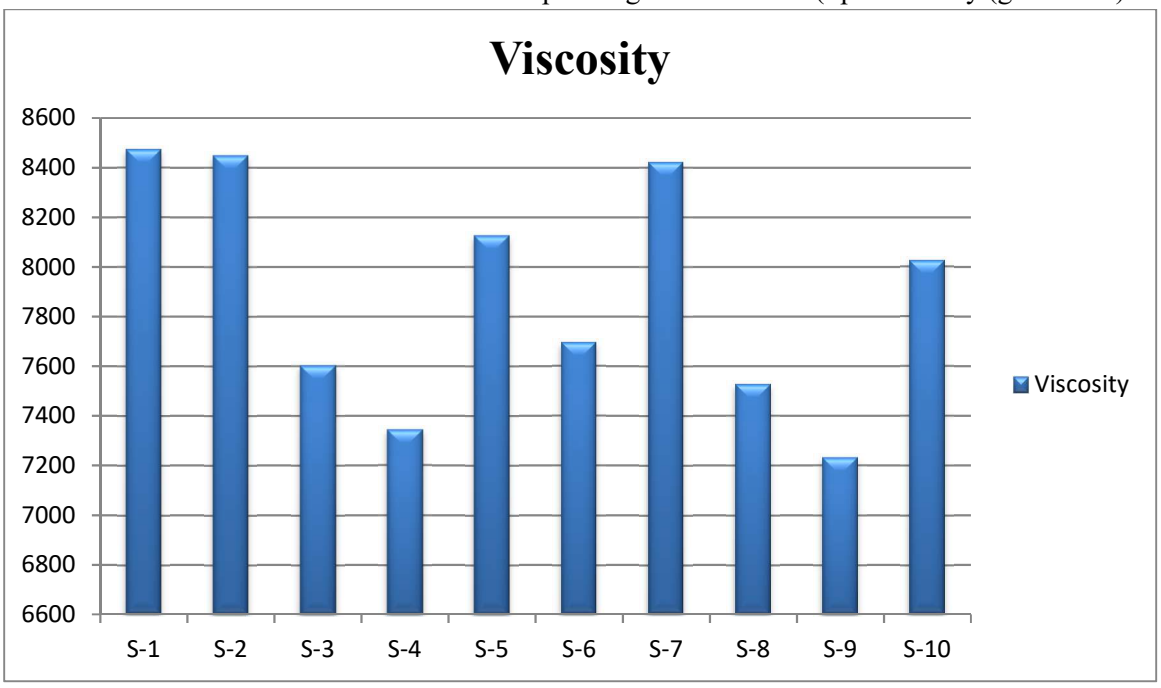
In the alchemy of pharmaceutical formulation, the evaluation of gel formulations extends beyond the parameters of pH and consistency to delve into the realms of spreadability and viscosity. These two facets, akin to the fluid choreography of a ballet, elucidate the formulations' ability to seamlessly integrate into the canvas of application.

Table. Evaluation of Gel Formulation

Formulation	Spreadability (g.cm./sec.)	Viscosity
S-1	5.3	8472
S-2	6.2	8448
S-3	5.5	7602
S-4	6.2	7342
S-5	5.7	8125
S-6	6.0	7694
S-7	6.2	8421
S-8	5.9	7526
S-9	5.6	7231
S-10	6.2	8024



Evaluation of different formulations of microspheres gel formulation (Spreadability (g.cm./sec.)



Evaluation of different formulations of nanogel gel formulation (Spreadability (g.cm./sec)

Conclusion

In conclusion, the comprehensive evaluation of saffron, both organoleptically and through its physicochemical and chemical properties, highlights its multifaceted identity as a valuable botanical resource with significant culinary and therapeutic applications. The detailed chemical tests underscore saffron's rich phytochemical profile, laying the foundation for further exploration of its pharmacological and medicinal properties. The formulation analysis reveals that while several formulations show promising attributes, they remain slightly

heterogeneous and require refinement for enhanced consistency and uniformity. This underscores the continuous effort needed in pharmaceutical formulation development to achieve the perfect balance of pH, consistency, and homogeneity, ultimately striving for excellence in creating effective and high-quality products. The pursuit of perfection in this intricate process reflects the ongoing dedication to improving and fine-tuning formulations for optimal performance and user satisfaction.

Further Investigations: The observed effects warrant further investigation into the specific mechanisms underlying the treatment's efficacy in wound healing. Exploring factors such as cellular responses, inflammation, angiogenesis, and tissue remodeling could provide deeper insights into the therapeutic mechanisms at play. Additionally, future studies may explore the clinical relevance of these observed outcomes, potentially leading to the development of a novel therapeutic intervention for wound healing.

Acknowledgment:

I would like to express my heartfelt gratitude to all those who have supported me throughout the course of this study.

Conflict of Interest:

The authors declare that there are no conflicts of interest associated with this research.

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