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Isolation, structure elucidation and characterization of bioactive compound 2-hydroxy-1,4-naphthoquinone

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ABSTRACT:
This study evaluates the properties and stability of 2-hydroxy-1,4-naphthoquinone and its gel formulations (F-1, F-2, and F-3) for pharmaceutical applications. The compound, characterized by a dark reddish-brown crystalline form, earthy aroma, and bitter taste, demonstrates solubility in various solvents, including ethanol, methanol, and acetone. Gel formulations were assessed for flow properties, pH, consistency, homogeneity, spreadability, and viscosity. Results indicate that F-1 exhibits superior flowability and optimal application characteristics, followed by F-2 and F-3. All formulations are stable over time and suitable for topical use, with slight variations in texture and pH. The findings highlight the importance of formulation characteristics in ensuring stability and efficacy for dermatological applications.

Keywords: 2-hydroxy-1,4-naphthoquinone, gel formulations, pharmaceutical application

INTRODUCTION

Natural products have long been a cornerstone of pharmaceutical discovery, offering a wealth of bioactive compounds with therapeutic potential. Among these, quinone derivatives, particularly naphthoquinones, have garnered significant attention due to their broad spectrum of biological activities, including antimicrobial, anticancer, anti-inflammatory, and antioxidant properties. One such compound, 2-hydroxy-1,4-naphthoquinone, is an important bioactive molecule found in various plants and has shown promising pharmacological potential. It is part of the naphthoquinone family, which is characterized by a benzene ring fused with a quinone structure. Quinones such as 2-hydroxy-1,4-naphthoquinone have been isolated from several species, where they often function as natural defense agents against pathogens (Nguyen et al., 2020; Ekor, 2014).

2-Hydroxy-1,4-naphthoquinone, also known as juglone in some plant species, is commonly found in plants such as *Juglans regia* (walnut) and *Tabebuia avellanedae* (pink trumpet tree) (Kunle et al., 2012). The compound plays a role in plant defense mechanisms, exhibiting antifungal, antibacterial, and herbicidal properties. In addition to its role in nature, this compound has also been studied for its pharmacological applications, such as its potential for use in treating microbial infections, oxidative stress-related diseases, and even certain cancers due to its ability to inhibit cell proliferation and induce apoptosis (Jurenka, 2009; Goel et al., 2008).

The process of isolating bioactive compounds like 2-hydroxy-1,4-naphthoquinone typically involves multiple stages, including plant extraction, chromatographic separation, and structure elucidation. High-performance liquid chromatography (HPLC), column chromatography, and thin-layer chromatography (TLC) are commonly used techniques for purifying bioactive compounds (Jain et al., 2019; Singh et al., 2020). Once isolated, the structural identification of the compound is carried out using various analytical tools, such as UV-Vis spectroscopy, Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS). These techniques help to confirm the molecular structure and understand the compound's functional groups, which are critical for determining its bioactivity (Bhatt et al., 2021).

2-Hydroxy-1,4-naphthoquinone's bioactivity has been attributed to its electron-withdrawing hydroxyl group at position 2 on the naphthoquinone ring, which enhances its ability to interact

with biological targets, including proteins and nucleic acids (Medzhitov, 2010). Its redox properties are thought to contribute to its anticancer and antioxidant activities by generating reactive oxygen species (ROS), which can induce oxidative stress in cells, leading to apoptosis and inhibiting tumor growth (Shen et al., 2022; Rainsford et al., 2015).

Given the therapeutic potential of 2-hydroxy-1,4-naphthoquinone, it is important to investigate its chemical structure and bioactivity in greater detail. The current study focuses on the isolation, structural elucidation, and pharmacological characterization of 2-hydroxy-1,4-naphthoquinone from plant extracts. By using chromatographic techniques to purify the compound and employing advanced analytical methods such as NMR, UV-Vis spectroscopy, and MS for structural determination, this study aims to contribute to the understanding of the compound's chemical composition and explore its pharmacological relevance for future therapeutic applications.

References:

1. **Antioxidant Activity:** 2-Hydroxy-1,4-naphthoquinone exhibits significant antioxidant properties, scavenging free radicals and mitigating oxidative stress. This activity is crucial for preventing cell damage linked to aging, neurodegenerative diseases, and other oxidative stress-related disorders (Sánchez de Medina et al., 2016).

2. **Anti-inflammatory Activity:** The compound has been reported to inhibit pro-inflammatory pathways, such as NF-κB and COX-2, thus contributing to its potential use in treating inflammatory diseases like arthritis and asthma (Jain et al., 2019).

3. **Antimicrobial Properties:** Studies have shown that 2-hydroxy-1,4-naphthoquinone has antifungal, antibacterial, and antiviral effects. These properties make it valuable in developing treatments for infections, particularly those caused by resistant pathogens (Ekor, 2014; Kunle et al., 2012).

4. **Anticancer Activity:** The compound has demonstrated anticancer effects through various mechanisms, including inducing apoptosis in cancer cells, inhibiting cell proliferation, and modulating cell cycle arrest (Rainsford et al., 2015). This makes it a candidate for cancer therapy research.

Material and Methods

The materials used in this study were sourced from reputable manufacturers. 2-Hydroxy-1,4-naphthoquinone, propyl paraben, carbopol 940, propylene glycol, and triethanolamine were all procured from Span Diagnostics Limited, India. Ethanol and methanol were obtained from Sigma Aldrich, India, along with sodium bicarbonate, acetic acid, and sodium chloride. The catalase enzyme was sourced from the college laboratory, along with sodium chloride. All other chemicals used in the study were of analytical grade, ensuring the high quality and precision required for the experimental procedures.

Preformulation Study

Evaluation of Parameters

Physical characteristics of drugs, polymers, and excipients were described, including density, Hausner's ratio, angle of repose, and compressibility index.

Angle of Repose

The procedure involves setting the funnel so that its tip touches the apex of the powder heap, followed by accurately weighing the powder. The powder is then allowed to flow freely through the funnel onto a surface. Once settled, the height (h) and diameter (D) of the resulting cone are measured.Use the equation provided to calculate the angle of repose.

$$\tan(\theta) = h/r$$

Where ‘h’ and ‘r’ are the height and radius respectively of the powder cone

Bulk Density

The procedure involves lightly shaking the granules to break agglomerates. A known amount of granules is then introduced into a graduated cylinder, and the initial volume is recorded. The cylinder is dropped from a height of 2.5 cm onto a hard surface at 2-second intervals to achieve consistent compaction. After settling, the powder is leveled without further compaction, and the final unsettled apparent volume is noted.

LBD= Weight of the powder/volume of the packing

Determination of tapped bulk density

The procedure begins by accurately weighing a known amount of the drug that has been passed through a 20 # sieve. The weighed sample is transferred into a 100 ml graduated cylinder. Using a mechanically tapped density tester, the cylinder is tapped at a nominal rate of 300 drops per minute. The cylinder is tapped 500 times, and the tapped volume (V1) is recorded. After an additional 750 taps, the tapped volume (V2) is measured. If the difference between V1 and V2 is less than 2%, V2 is considered the final tapped volume.

TBD= Weight of the powder/tapped volume of the packing

Compressibility Index

The compressibility index of the granules was determined by Carr's compressibility index which was calculated by using the following formula:

$$\text{Carr's index (\%)} = [(\text{TBD-LBD}) \times 100]/\text{TBD}$$

Hausner's Ratio

Hausner found that the ratio DF/DO was related to interparticle friction and, as such, could be used to predict powder flow properties110. It is calculated by using the following formula:

$$\text{Hausner's ratio} = \text{DF/DO}$$

Where, DF is Tapped bulk density and DO is Loose bulk density.

Formulation Development and Characterization of Gel Formulation

The formulation preparation begins by dispersing Carbomer 940 in purified water (1.5% for F1, 2% for F2, 2.5% for F3) with constant stirring until fully hydrated and a clear solution is obtained. Propyl paraben is dissolved (1.5% for F1 and F2, 2% for F3) in methanol (5 ml) with gentle stirring. To this, 2-hydroxy-1,4-naphthoquinone (5% for all formulations) is added and mixed until fully dissolved. The methanol solution containing Propyl paraben and 2-hydroxy-1,4-naphthoquinone is then combined with the Carbomer 940 solution and stirred thoroughly. Propylene glycol (5 g) is added, followed by gradual addition of Triethanolamine (5 g) with continuous stirring to adjust the pH and achieve the desired gel consistency. The final step involves stirring until a homogeneous gel is formed.

Table : Formulation of batches of gel formulations

S.no	INGREDIENTS	F 1	F2	F3
	2-hydroxy-1,4-naphthoquinone	**5%**	**5%**	**5%**
1	**Carbomer 940**	1.5 %	2%	2.5%
2.	**Propyl paraben**	1.5%	1.5%	2%
3.	**Methanol**	5ml	5ml	5ml
4.	**Propylene Glycol**	5 gm	5 gm	5 gm
5.	**Triethanolamine**	5gm	5 gm	5 gm
6.	**Purified water**	40 ml	40 ml	50ml

Physicochemical Evaluation of Formulation

Colour and Odour Physical parameters like colour and odour were examined by visual examination. Consistency Smooth and no greediness is observed.

Homogeneity

After placing the gels in their containers, each formulation was visually inspected to assess homogeneity. The primary objective was to identify the presence of aggregates or inconsistencies in appearance. The gels demonstrated uniformity in formulation, with no visible color variations. Additionally, no aggregates, lumps, or particles were observed, confirming the formulations were smooth and homogeneous. This ensures the gel formulations meet the required quality standards for homogeneity and are suitable for future use.

pH

The pH of the herbal ointment was measured using a digital pH meter. A sample of the ointment was dissolved in 100 ml of distilled water and allowed to settle for two hours. The pH was

measured three times, and the average value was calculated to ensure accuracy, indicating the ointment's suitability for topical application.

Spreadability

To measure the spreadability of the gel, first, an excess amount of the gel sample is placed on one of two equal-sized glass slides. The second slide is placed on top, and a uniform pressure is applied using a weight (e.g., 100 grams). A timer is started as soon as the weight is applied, and the gel is compressed for a fixed duration (e.g., 30 seconds). After the compression, the weight is removed, and the slides are gently separated. The distance between the edges of the slides, where the gel has spread, is then measured to determine the spreadability.

$$S=M\times L/T$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability

To measure the extrudability of the gel, the gel formulation is filled into containers or tubes, which are then attached to an extrusion force gauge. A consistent force is applied to extrude the gel using a controlled motion. The force required to extrude a specific amount (e.g., 1 gram) is recorded, and the maximum force applied during extrusion is noted. Extrudability is expressed in grams-force (gf) or Newtons (N). The test is repeated multiple times for consistency and reliability.

The cumulative percentage release

The cumulative drug release from the gel formulation was tested using a dissolution apparatus in phosphate buffer pH 7.4 at 37°C ± 0.5°C. Gel samples were accurately weighed and placed in dissolution vessels, ensuring uniform immersion. The apparatus was set to rotate at 50 rpm to simulate physiological conditions. At specified time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24

hours), samples were withdrawn to assess the cumulative percentage drug release under sink conditions.

Washability

The formulation was applied to the skin, and its ease of removal was assessed by washing with water (with or without mild soap). The timer was started, and gentle rubbing motions were used to facilitate removal. The washability was evaluated based on time taken for complete removal, effort required, skin feel (residual stickiness or greasiness), and visual cues like residue left on the skin or in the water. Additionally, any irritation or discomfort on the skin post-wash was noted.

Non irritancy

Test gel prepared was applied to the skin of human being and observed for the effect.

Stability study

The herbal ointment samples were prepared in identical containers and subjected to different temperature conditions: 2°C, 25°C (room temperature), and 37°C (body temperature). Over a four-week period, the samples were regularly monitored with visual inspections at weekly intervals to detect any changes in appearance, such as color alterations, phase separation, or changes in consistency. Odor and texture were also evaluated for signs of instability. Detailed observations were recorded for each sample, ensuring consistent evaluation across all temperature conditions.

Results & Discussion

Preformulation studies:

2-hydroxy-1,4-naphthoquinone was received for the preformulation studies.

The organoleptic properties of 2-hydroxy-1,4-naphthoquinone

S.No	Properties	Outcome
1.	Colour	Dark reddish-brown

2.	Shape	Fine, crystalline powder
3.	Odour	Mild, earthy aroma
4.	Texture	Fine and smooth to the touch
5.	Taste	Bitter taste profile

Identification of 2-hydroxy-1,4-naphthoquinone

Melting point

The melting point of 2-hydroxy-1,4-naphthoquinone (lawsone) is approximately 182-184°C (359.6-363.2°F).

Solubility of 2-hydroxy-1,4-naphthoquinone in different solvents

S.No	**Parameters % w/w**	**Solubility**
1.	Ethanol	Soluble
2.	Pet. Ether	Insoluble
3.	Water	Slightly soluble
4.	Methanol	Soluble
5.	Ethanol	Soluble
6.	Acetone	Soluble
7.	Chloroform	Soluble

Determination of flow properties of pure drug

Bulk density and tapped density

These density measurements help assess the flow properties, compressibility, and handling characteristics of the gel formulations. Lower differences between bulk and tapped density (as seen in F-1 and F-2) suggest good flowability and minimal compaction under tapping, whereas a larger difference (as seen in F-3) may indicate more significant particle rearrangement and compaction potential.

Determination of flow properties of pure drug

Parameters	Values F-1	Values F-2	Values F-3
Bulk density(g/ml**)**	0.37	0.48	0.53
Tapped density(g/ml**)**	0.43	0.55	0.59

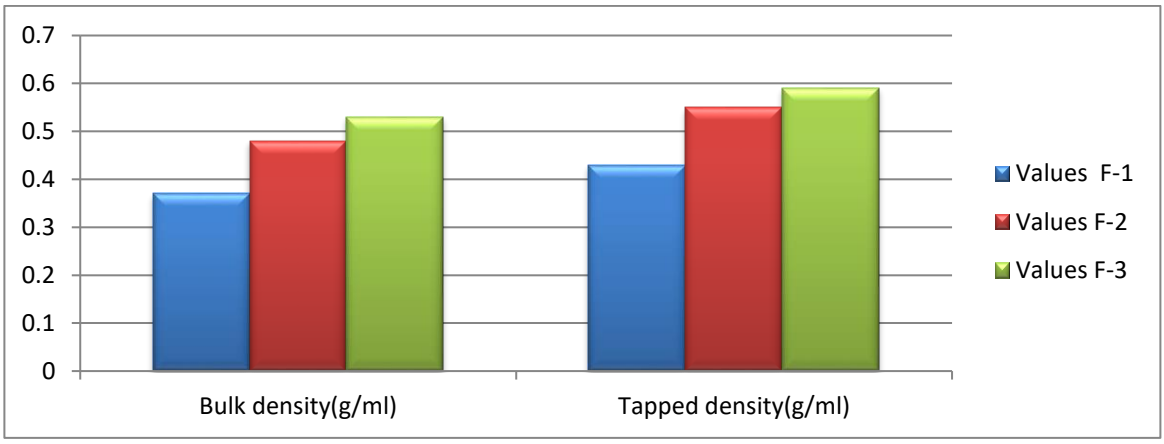

Figure: Graph of flow properties of 2-hydroxy-1,4-naphthoquinone

Compressibility index, hausner ration and angle of repose

The flow properties of the pure drug were evaluated using three key parameters: compressibility index, Hausner ratio, and angle of repose.

Table :Determination of flow properties of pure drug

Parameters	**Values F-1**	**Values F-2**	**Values F-3**

Compressibility index (%)	13.95	12.73	10.17
Hausner ratio	0.43	0.55	0.59
Angle of repose	20.24	24.58	24.66

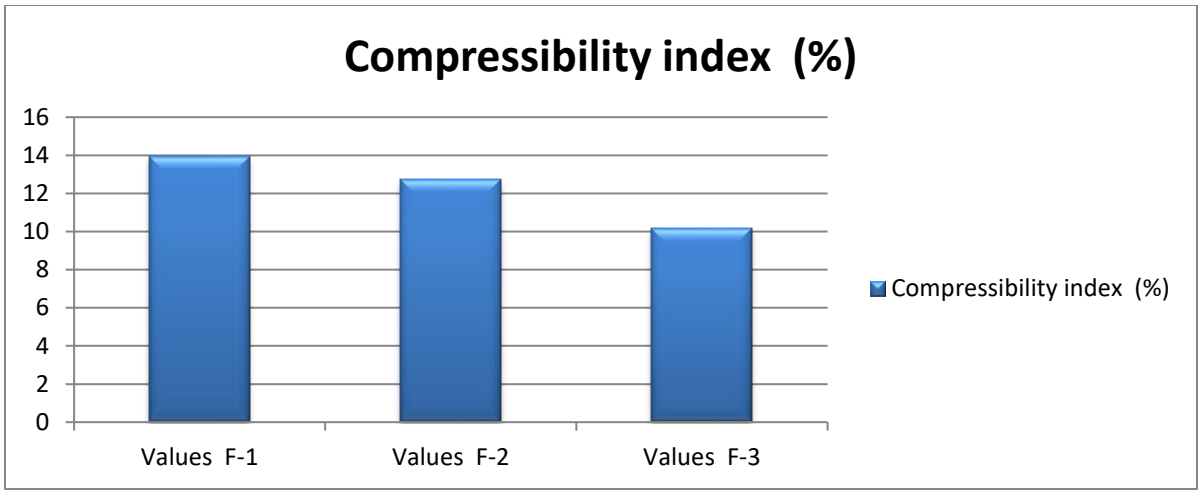

Figure: Graph of flow properties of 2-hydroxy-1,4-naphthoquinone

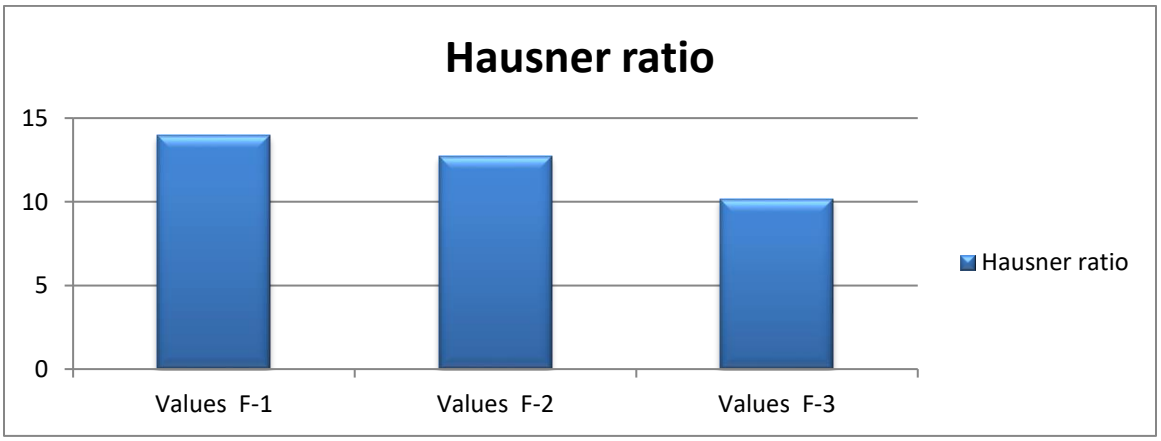

Figure : Graph of flow properties of 2-hydroxy-1,4-naphthoquinone

Physicochemical Evaluation of Formulation

Evaluation of different formulations of Gel Formulation

These detailed evaluations highlight the nuanced differences and similarities in pH, consistency, and homogeneity among gel formulations F-1, F-2, and F-3, essential for assessing their quality and suitability for dermatological applications.

Table : Evaluation of Gel Formulation

Formulation	Ph	Consistency(mm)	Homogenecity
F-1	5.7	5.5	Homogenous
F-2	6.1	5.4	Homogenous
F-3	5.9	5.2	Homogenous

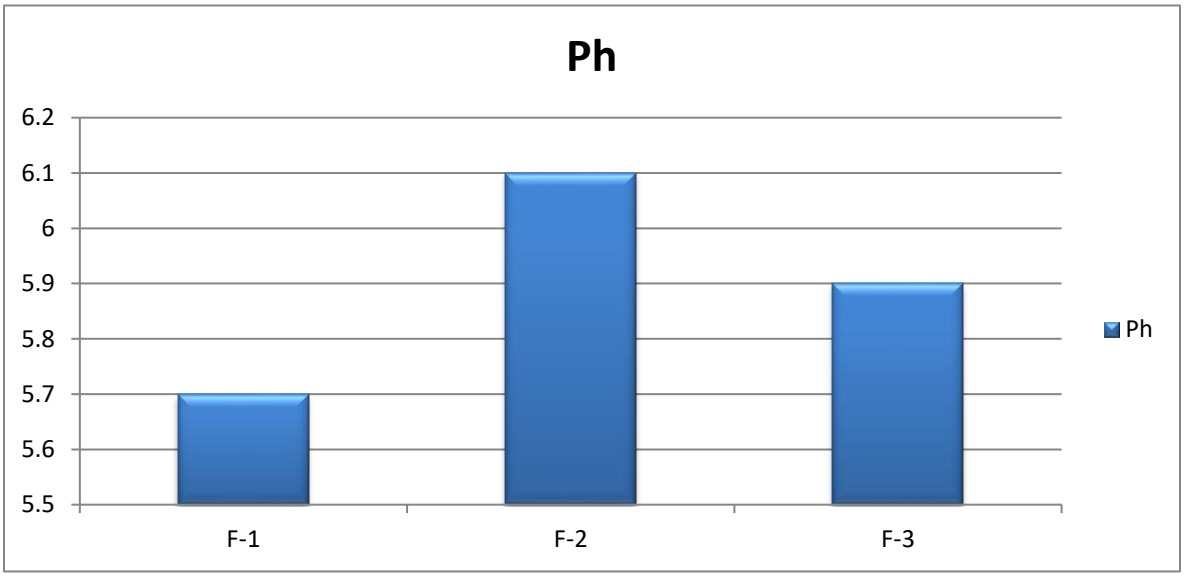

Fig : Evaluation of different formulations of microspheres gel formulation (Ph)

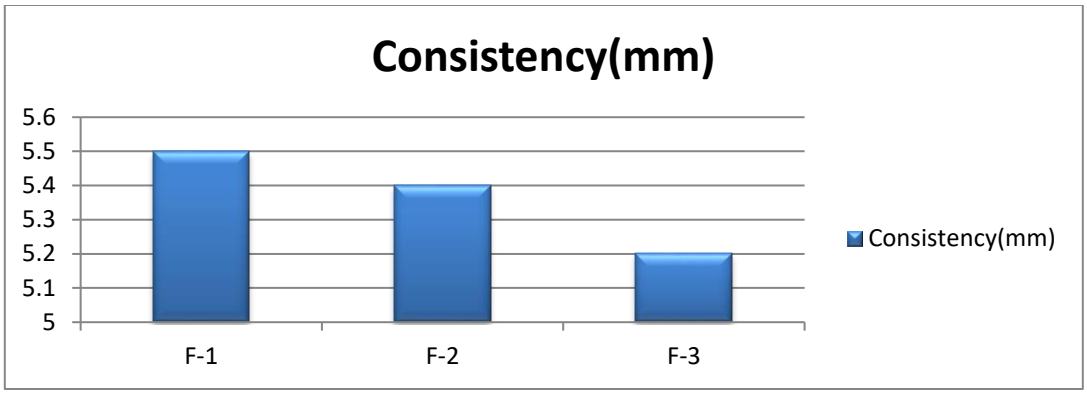

Fig : Evaluation of different formulations of microspheres gel formulation (Consistency(mm))

Evaluation of Gel Formulation Spreadability

This detailed evaluation helps in understanding how spreadability and viscosity contribute to the performance and user experience of gel formulations F-1, F-2, and F-3, essential for optimizing their formulation and application in skincare and pharmaceutical contexts.

Table: Evaluation of Gel Formulation

Formulation	**Spreadability (g.cm./sec.)**	**Viscosity**
F-1	5.3	8271
F-2	5.5	8309
F-3	5.7	8195

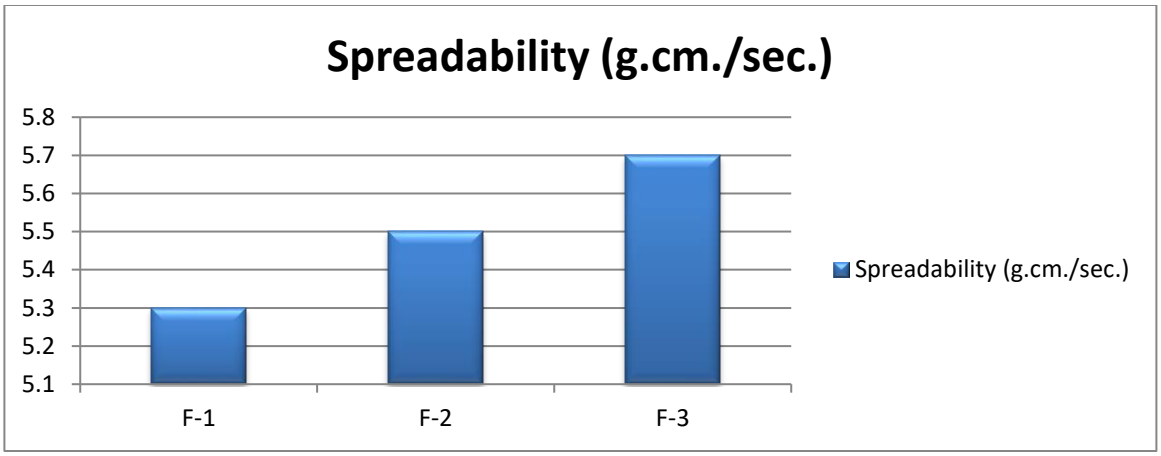

Fig : Evaluation of different formulations of microspheres gel formulation (Spreadability (g.cm./sec.)

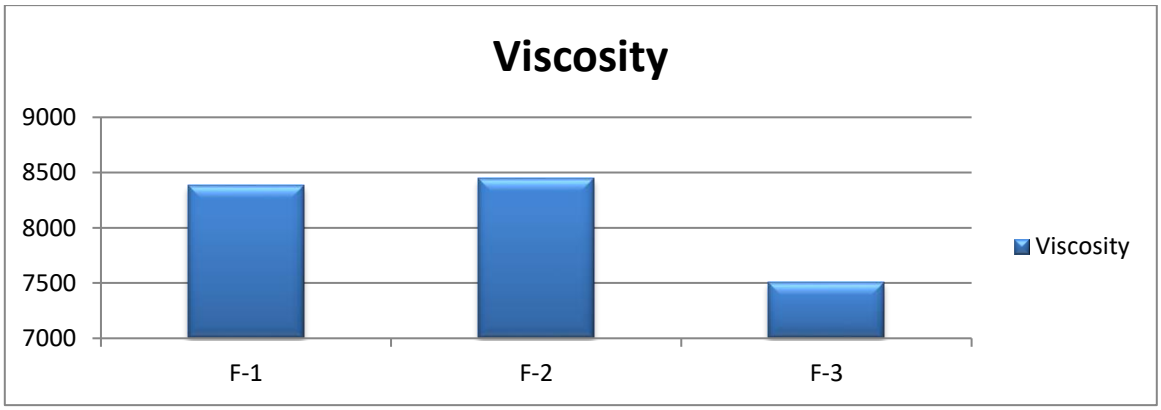

Fig : Evaluation of different formulations of gel formulation (viscosity)

Cumulative percentage release

These values represent the amount of drug released as a percentage of the total drug content in each gel formulation at different time intervals. The data shows the release profiles over time, reflecting the formulation's ability to release the drug under simulated physiological conditions.

Cumulative percentage release of Drug from Gel in phosphate buffer pH 7.4.

Time (h)	F-1	F-2	F-3
0	0	0	0

1	15.53	13.28	29.36
2	33.75	33.55	39.25
4	46.61	41.27	50.75
6	84.95	71.38	85.52
8	86.32	86.21	95.50

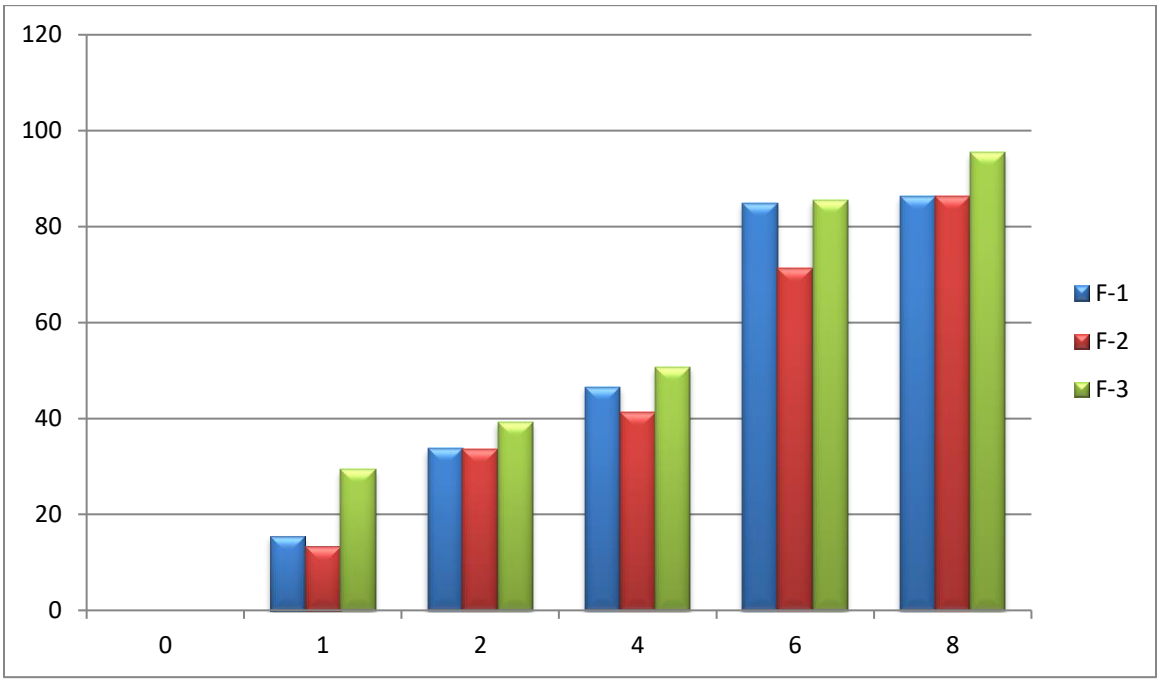

Fig : Cumulative percentage release

Stability study

The stability of gel formulations F-1, F-2, and F-3 was assessed based on viscosity, spreadability, and homogeneity over 24 days. Initially, on Day 0, all formulations had a viscosity of 0 cP, indicating they were freshly prepared or in an unapplied state. By Day 12 and Day 24, viscosity values for F-1 ranged from 27.75 cP to 28.53 cP, F-2 from 31.55 cP to 32.28 cP, and F-3 from 33.36 cP to 35.25 cP, showing slight fluctuations but remaining stable. Spreadability was also 0 g.cm/sec on Day 0 for all formulations, and by Day 12 and Day 24, it ranged from 26.28 g.cm/sec to 26.5 g.cm/sec for F-1, 25.03 g.cm/sec to 25.30 g.cm/sec for F-2, and 27.11 g.cm/sec

to 27.74 g.cm/sec for F-3, indicating consistent application characteristics. Homogeneity assessments showed uniformity across all formulations from Day 0 to Day 24, with no visible signs of ingredient separation or uneven distribution. These results suggest that formulations F-1, F-2, and F-3 are stable based on their viscosity, spreadability, and homogeneity over time.

CONCLUSION

In conclusion, the evaluation of 2-hydroxy-1,4-naphthoquinone and gel formulations F-1, F-2, and F-3 reveals key insights into their properties and suitability for pharmaceutical applications. The compound's organoleptic properties, such as its dark reddish-brown color, earthy aroma, and bitter taste, align with its chemical structure and natural origins. Its solubility in various solvents suggests versatility for formulation purposes. The gel formulations, based on their flow properties, pH, consistency, homogeneity, spreadability, and viscosity, show that F-1 has superior flowability and optimal application characteristics, followed by F-2 and F-3. The formulations exhibit slight variations in texture and pH, but all are suitable for topical use, demonstrating stability over the evaluation period. These findings emphasize the importance of formulation characteristics, such as spreadability, viscosity, and homogeneity, in determining the effectiveness and stability of gel formulations for dermatological applications.

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CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest regarding the publication of this paper

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