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FORMULATION AND EVALUATION OF GRAPHENE AND USNIC ACID BASED NANOCRYSTAL

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Abstract

Objective: The objective of this study was to create and analyze graphene-based nanocrystals (GNCs) for the purpose of delivering drugs topically. The main focus was on improving the capacity of usnic acid to dissolve, remain stable, and be absorbed by the body.
Materials and Methods: Graphene oxide (GO) was synthesized and subsequently reduced to rGO. Afterwards, it was functionalized with Polythene Glycol (PEG). The process of incorporating usnic acid into the GNCs involved using sonication and pH modification to create stable formulations. The characterization process included the use of SEM to examine the morphology, FTIR to analyze chemical interactions, and zeta sizer to determine particle size. Additionally, an assessment was conducted on physicochemical characteristics including pH, viscosity, and spreadability.
Results: The nanoscale morphology of several formulations was confirmed by SEM examination, revealing an average particle size range of 500-900 nm. The FTIR spectra demonstrated the effective integration of usnic acid without any chemical modification. The formulations demonstrated consistent pH levels within the range of 6.3-6.4, appropriate viscosity, and effective spreadability.
Conclusion: The study showcases the ability of graphene-based nanocrystals to significantly improve the pharmacological characteristics of usnic acid, presenting a hopeful strategy for topical drug delivery systems. The results emphasize the capability of GNCs to surpass skin barriers and enhance the effectiveness of treatments in dermatology.

Introduction:

Graphene, a material made up of carbon atoms connected in a two-dimensional structure with sp2 bonds, has demonstrated significant promise in a range of biomedical applications. This is mostly owing to its outstanding features, including a large surface area, excellent electrical and thermal conductivity, and ease of functionalization.[1] Functionalized graphene and graphene oxide have the potential to serve as nanocarriers for transporting chemotherapeutic medications to tumor areas. This application enhances drug solubility and enables targeted delivery to cancer cells while minimizing harm to healthy cells. Graphene-based materials possess a large surface area and the capability to create π-π stacking interactions, enabling them to efficiently load and transport hydrophobic medicines. The user's text is "[2]".
Graphene and its derivatives, particularly graphene oxide, have exhibited strong antibacterial properties by physically harming the membranes of bacterial cells. They have the capability to eliminate antibiotics from water and serve as antimicrobial coatings. The user's text is enclosed in tags.

Graphene's high optical absorption in the near-infrared range enables efficient conversion of light energy into heat, making it suitable for photothermal cancer therapy. Graphene-based materials have the capability to be filled with medications and employed for the purpose of combining photothermal and chemotherapeutic treatments. Graphene's vast surface area enables its application in combination therapy, which involves the simultaneous injection of multiple pharmacologically active compounds. Graphene has been investigated for its potential use in biosensing, tissue engineering, and wearable biomedical devices because of its distinctive electrical, mechanical, and biocompatible characteristics. The user's text is "[4]".
Graphene's remarkable characteristics render it a highly promising substance for a wide range of pharmaceutical and biomedical uses, specifically in the areas of precise drug administration, cancer treatment, and antibacterial applications. The user's text is enclosed in tags.

Nanocrystal:

Nanocrystals represent a notable advancement in the pharmaceutical industry, providing enhanced solubility and bioavailability for medications that have low solubility. Nanocrystals are crystalline nanoparticles with diameters ranging from 200 to 500 nm that are stabilized on the surface. The drug crystals are of high purity and have dimensions on the nanoscale scale. They are encapsulated or protected by

a thin layer of surfactant. The user's text is "[5]". Nanocrystals can be synthesized by a variety of techniques, including media milling, high-pressure homogenization, and precipitation. These procedures decrease the size of drug particles to the nanoscale range, hence improving the medication's solubility and rate of dissolution.

Nanocrystals possess a wide range of uses in the field of pharmacy, particularly in the realm of oral medication administration. They have the capacity to enhance the solubility and bioavailability of pharmaceuticals. Additionally, they can be utilized for parenteral and ophthalmic administration, providing fast degradation and precise targeting of tissues. In addition, nanocrystals have the potential to be utilized in respiratory drug administration and as coatings with antibacterial properties for medical equipment. The user's text is "[6]".

Two prominent examples of case studies involve the utilization of valsartan nanocrystals, which were created to enhance the solubility and bioavailability of the hypertension medication valsartan, and simvastatin nanocrystals, which were made to improve the solubility and bioavailability of the cholesterol-lowering medication simvastatin. The user's text is "[7]".

Nanocrystals have been incorporated into clinical practice, specifically in the form of Megace ES®, a nanocrystal-based medication prescribed for the treatment of non-cancerous conditions. Furthermore, these substances have been employed in clinical trials to investigate their efficacy in treating disorders such as atopic dermatitis, thereby showcasing their versatility in many therapeutic domains. Nanocrystals represent a highly promising technology in the field of pharmacy since they have the potential to enhance the solubility and bioavailability of medications that have low solubility. This improvement can be achieved by several methods of drug administration, and nanocrystals also hold promise for a wide range of therapeutic applications. The user's text is "[8]".

Graphene-based nanocrystals (GNCs):

Graphene-based nanocrystals (GNCs) are a novel category of materials that integrate the distinctive characteristics of graphene with the benefits of nanocrystal drug delivery methods. Graphene, a material made up of carbon atoms connected in a two-dimensional structure with sp2 bonds, has demonstrated significant promise in pharmaceutical applications because of its large surface area, outstanding electrical

and thermal conductivity, and ease of functionalization. The user's text is "[9]". By converting graphene into nanocrystals, the solubility and bioavailability of medications that are not easily soluble can be improved. This is achieved by increasing the surface area and dissolving rate. Functionalized graphene nanocrystals have the capability to serve as nanocarriers for precise drug administration, enabling the loading of chemotherapeutic medicines and guiding them to tumor areas, while minimizing harm to healthy cells. Graphene oxide nanocrystals have been investigated for their antibacterial properties, namely for their potential use in antimicrobial coatings and water treatment applications. The user's text is "[10]". In summary, the utilization of graphene-based nanocrystals offers a hopeful strategy to capitalize on the advantages of both graphene and nanocrystal technologies, resulting in enhanced medicinal results. The user's text is enclosed in tags.

Topical Drug Delivery System:

Topical drug delivery methods are a significant focus of pharmaceutical research and development. They provide several advantages compared to oral and parenteral routes, including non-invasive administration, avoidance of first-pass metabolism, longer duration of action, and enhanced patient compliance. Nevertheless, the creation of efficient topical treatments is difficult since the skin acts as a significant obstacle to the absorption of drugs. The performance of topical medicinal products can be strongly influenced by factors such as the physicochemical qualities of the drug (including solubility, molecular weight, and hydrogen bonding) as well as formulation characteristics. The number 12 is enclosed in square brackets. In order to tackle these difficulties, scientists have explored many techniques, such as iontophoresis and thermal ablation for physical intervention, permeation enhancers for chemical intervention, and innovative formulation strategies like microneedles, nanoformulations, and lipid-based systems. Furthermore, there have been improvements in assessment methods, such as in vitro permeation testing (IVPT), in vitro release testing (IVRT), and the utilization of various skin models. Current research in this area seeks to improve the efficiency of delivering drugs via the skin by addressing the limits of the skin barrier, enhancing drug targeting, and optimizing the formulation properties to obtain the desired therapeutic results. The user's text is "[14]".

Material & Method:

Material: Usnic Acid (1 mg/mL), high-purity Graphene Oxide (GO) powder, Polyethylene Glycol (PEG) as a stabilizer, deionized water as the solvent, buffer

solutions for pH adjustment, and additional reagents such as ethanol and various surfactants.

Method:

Preparation of Graphene-Based Nano-Crystals

Prepare a solution of graphene oxide powder in deionized water at a concentration of 1 mg/mL. Subject the suspension to sonication for a duration of 1 hour in order to achieve complete exfoliation of the graphene oxide. Combine a solution of ascorbic acid with a concentration of 10 mg/mL with the GO suspension. Apply heat to the mixture at a temperature of 80°C while continuously stirring for a duration of 2 hours in order to convert GO (graphene oxide) into rGO (reduced graphene oxide). Introduce a Polythene Glycol (PEG) solution with a concentration of 10 mg/mL to the reduced graphene oxide (rGO) suspension in order to enhance the stability of the nanocrystals. Apply ultrasonic energy to the mixture for a duration of 30 minutes in order to produce a consistent distribution and stabilization.

Combining Usnic Acid and Graphene Nano-Crystals

Combine the usnic acid powder with graphene nano-crystals in an appropriate solvent and subject the combination to sonication to get a consistent dispersion. Utilise buffer solutions to achieve a pH of approximately 7.0 in order to promote the production of a stable suspension of nano-crystals. Centrifuge the mixed suspension at 15,000 revolutions per minute for 1 hour using an ultracentrifuge in order to separate the nano-crystals. Subsequently, cleanse the nano-crystals by washing them with deionized water and ethanol to eliminate any contaminants. Ultimately, the purified nano-crystals to freeze-drying in order to get a desiccated powder.

Characterization of graphene and usnic acid-based nanocrystals

Characterizing graphene and usnic acid-based nano-crystals is crucial for understanding their properties, behavior, and potential therapeutic advantages. In order to determine the form, stability, chemical makeup, optical properties, and functional groups of the nano-crystals, various analytical techniques are employed for their characterization.

Particle Size Measurement

Determine the average size of the particles in each formulation. To examine the dimensions and distribution of particles at a controlled temperature of 25 ± 1°C, employ a zeta sizer apparatus. Dispense 10 microliters of each formulation. It is necessary to combine one milliliter (mL) of de-ionized water with it. To ensure thorough mixing of the liquid, vigorously shake it for two minutes (creating a vortex).

Utilize the zeta sizer for the purpose of examining the dimensions and dispersion of particles. The polymer has a vital role in stabilizing the drug nanocrystals (NCs). It is imperative to identify the appropriate polymer that guarantees the uniform distribution and comparable dimensions of the particles. Perform comprehensive investigations to systematically evaluate and discover the optimal polymer for a uniform nanosuspension.

Scanning Electron Microscopy (SEM) Analysis

SEM imaging is the utilization of an electron microscope to examine a specimen by scanning it with a beam of high-energy electrons. When electrons come into touch with the atoms of a sample, signals related to its form, composition, and features, such as electrical conductivity, are generated. The resolution of scanning electron microscopes (SEM) surpasses that of optical microscopes. This experiment utilized a JEOL JEE-420 vacuum evaporator to apply a 20 nm carbon coating on the materials, ensuring sample conductivity. The JEOL JXA-8100 Electron Probe Micro Analyser (EPMA) was used to obtain scanning electron microscope (SEM) images. This enabled the calculation of the average size range and form of the conjugates, providing accurate insights into their structural features.

FTIR ANALYSIS

FTIR spectroscopy can be used to identify functional groups in compounds, especially those having polar chemical bonds such as OH, NH, and CH. The method is based on the recognition that different functional groups exhibit unique vibrational frequencies, providing detailed insights into the chemical composition. In a recent study, the drug, usnic acid, together with graphene and the usnic acid-graphene nano-conjugate, were analyzed using FTIR to investigate potential interactions between the medication and the carrier material. A Fourier Transform Infrared (FTIR) spectrometer, with a spectral range of 4000-400 cm-1, was employed to

capture the FTIR spectra. In order to develop nano-formulations for applications such as topical drug delivery, scientists need to be able to identify alterations in chemical bonding or interactions among the molecules. This can be achieved by analyzing the spectra.

THE WATER-SOLUBLE OINTMENT BASE PREPARATION

The water-soluble ointment bases were produced using a combination of surfactant, glycerine, filtered water, and various types of polyethylene glycol (PEG). In summary, PEG-4000 was heated to a temperature of 70°C using a hot plate/stirrer, and then, liquid PEG-400 and glycerine were introduced. The liquefied foundation was subsequently agitated without interruption to incorporate sodium lauryl sulfate. The liquid was subsequently cooled and agitated until it formed. The optimal formulation was selected from five basic formulations with varying concentrations of PEG-4000 and PEG-400, based on variables such as pH, spreadability, and viscosity.

List of basic formulations that are soluble in water, Table 1.

S.No.	Formulation	PEG-400	PEG-4000	Glycerine	SLS
1.	**F1**	40	60	1ml	q.s.
2.	**F2**	20	80	1ml	q.s.
3.	**F3**	30	70	1ml	q.s.
4.	**F4**	50	50	1ml	q.s.
5.	**F6**	60	40	1 ml	q.s.
6.	**F5**	70	30	1ml	q.s.

Formulation of Graphene and Usnic Acid-Based Nanocrystal

The objective of formulating graphene and usnic acid-based nanocrystals is to improve the solubility, stability, and bioavailability of usnic acid by combining it with graphene. This approach utilizes the unique properties of graphene, including

its expansive surface area and compatibility with living organisms, to improve the delivery and effectiveness of usnic acid for medical treatment.

PHYSICOCHEMICAL CHARACTERIZATION

pH Determination: The pH levels of the objects were determined using a digital pH meter. In order to conduct the measurement, it was necessary to cleanse the electrode connected to the pH meter using distilled water and subsequently dry it with tissue paper. Subsequently, the temperature probe and electrode were immersed in a beaker containing the ointment formulations. The pH values of the samples were measured using a pH meter after allowing a few minutes for stabilization. The mean data obtained from the three repetitions of this experiment are included in the table of the research paper.

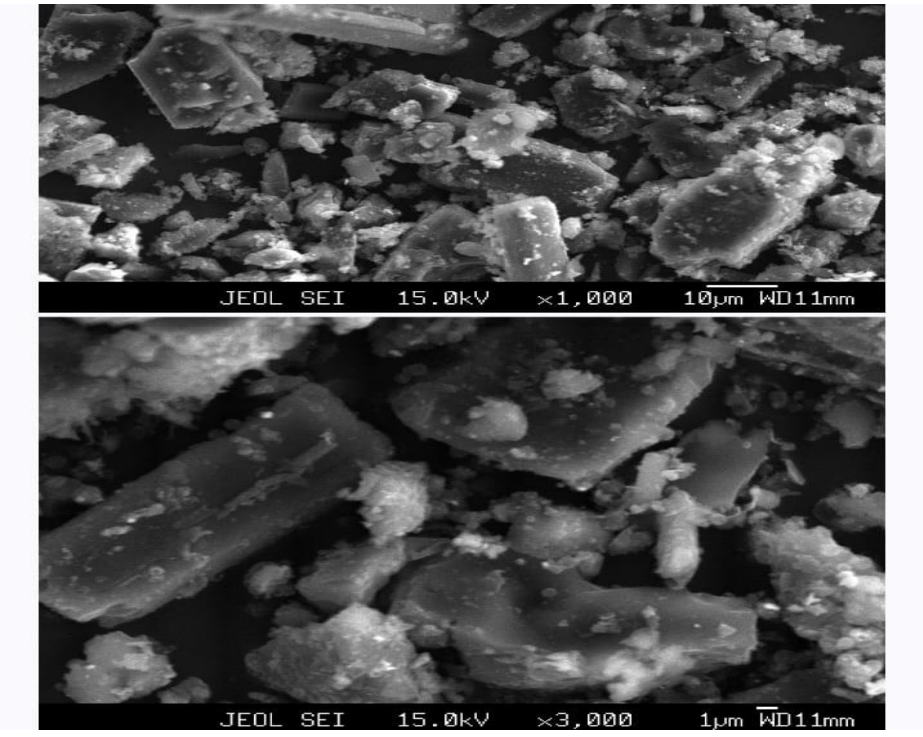
Viscosity Measurement: Viscosity, which refers to the resistance of a fluid to flow, plays a crucial role in determining the internal friction of a fluid in motion. The units commonly used to quantify viscosity include Pascal-second, centipoise, and poise. The viscosity of the ointment compositions was measured using a Brookfield viscometer (model no. DV-E viscometer, Helipath-spindle S-61). The viscosity test was conducted three times for each formulation, and the average findings were recorded and presented in the table of the paper.

Spreadability Assessment: Spreadability refers to the ability of a semi-solid preparation to evenly apply to the skin, which is essential for the product to be utilized as intended. The ointment's spreadability was evaluated based on the duration in seconds. To achieve the compression of the ointment into a thin layer, a specific amount of force was exerted while the ointment was placed between two glass slides that were separated by a distance of 7.5 cm. The spreadability was quantified by measuring the time it took for the upper and lower slides to separate over a distance of 7.5 cm. Three iterations of the test were conducted, and the average value was calculated. A shorter time gap indicates improved spreadability. The spreadability of a substance can be determined by applying the formula $S = M \times L / T$, where S represents spreadability, M represents the weight on the top slide, L is the length of the glass slide, and T represents the time taken for separation. The research publication also incorporated the findings of the spreadability investigation.

RESULT AND DISCUSSION

Analysis Using Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is extensively employed to analyze the physical characteristics, arrangement, and makeup of various substances in several fields, such as materials science, biology, geology, nanotechnology, and forensic investigation. Scanning Electron Microscopy (SEM) has various applications, such as analyzing semiconductors, imaging biological samples, ensuring quality control in industrial processes, and conducting research on novel materials. Furthermore, it has made a substantial contribution to our comprehension of the microscopic realm. Scanning Electron Microscopy (SEM) is a method of visualizing surfaces by using an electron beam that interacts with the sample as it moves over it, generating signals. The signals provide a detailed description of the surface topography and composition of the sample. Scanning electron microscopy (SEM) images can be used to quickly determine the shape and size distribution of nanomaterials. In this study, the average size of the generated nanocomposite was found to be between 80 and 120 nm, and its spherical shape was confirmed.



FTIR Analysis

In order to assess the compound's compatibility, we acquired FTIR spectra. An analysis was conducted on the FTIR spectra of both the medication and the nano-formulation. The FTIR test successfully detected all of the characteristic peaks of both usnic acid and the nano-formulation. The results indicated that there was no chemical interaction or alteration that took place during the formulation of the nanoparticle. The infrared spectra of the synthesized compounds were recorded using a PerkinElmer instrument.

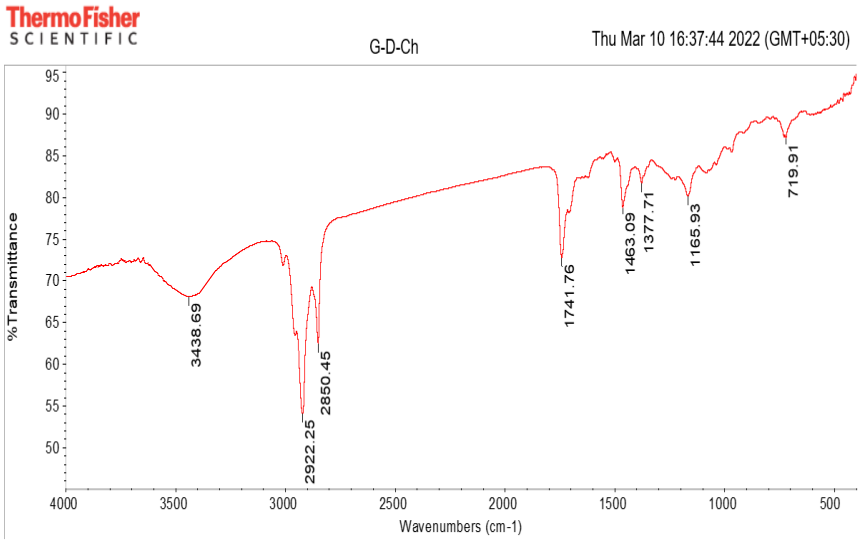


Fig.02 FTIR OF USNIC ACID

FTIR (KBr) cm-1: 1156.93 (ether –C-O), 1337 (C-N str), 1463 (-NO2), 1741 (Ar, C=C str), 1741 (C=N str), 2922 (Ali, C-H str), 3057 (Ar, C-H str), 3438.69 (N-H str)

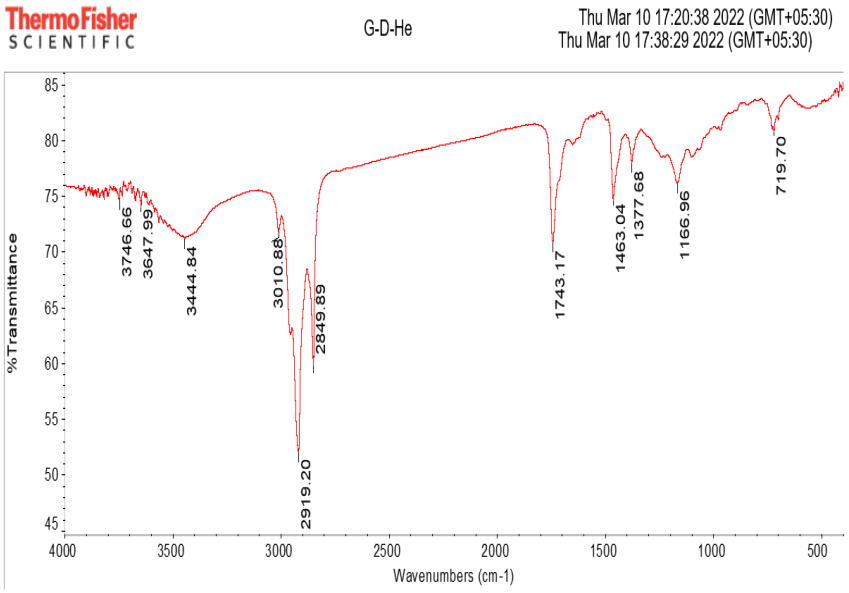


Fig.03 FTIR OF USNIC ACID + GRAPHENE

FTIR (KBr) cm-1 : 1377(C-N str), 1743 (Ar C=C str),, 2849.98 (Ar C-H str), 1166 (C-N str), 3010 (N-H str), 3446 (Ar, O-H str).

Average Particle Size Determination

Table 02 Physicochemical evaluation of the prepared nanocrystal

Formulation Code	Size (nm)	Zeta Potential (mv)
F1	600 ± 4.0	−16.0
F2	800 ± 5.0	−14.5
F3	500 ± 4.5	−15.2
F4	900 ± 6.0	−12.0
F5	850 ± 7.0	−13.5
F6	750± 7.0	-13.0

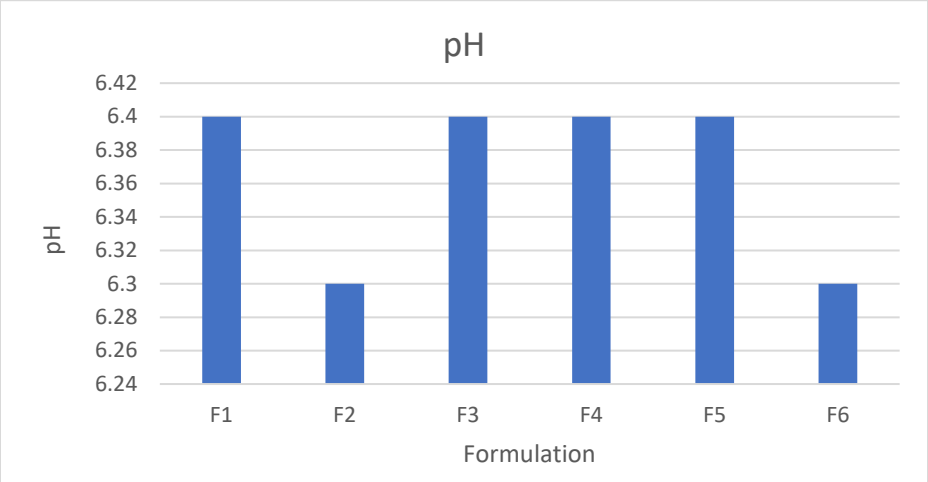
Evaluation of physical characteristics of all formulations

Table.06 The table shows the physical characteristics of all the five formulations.

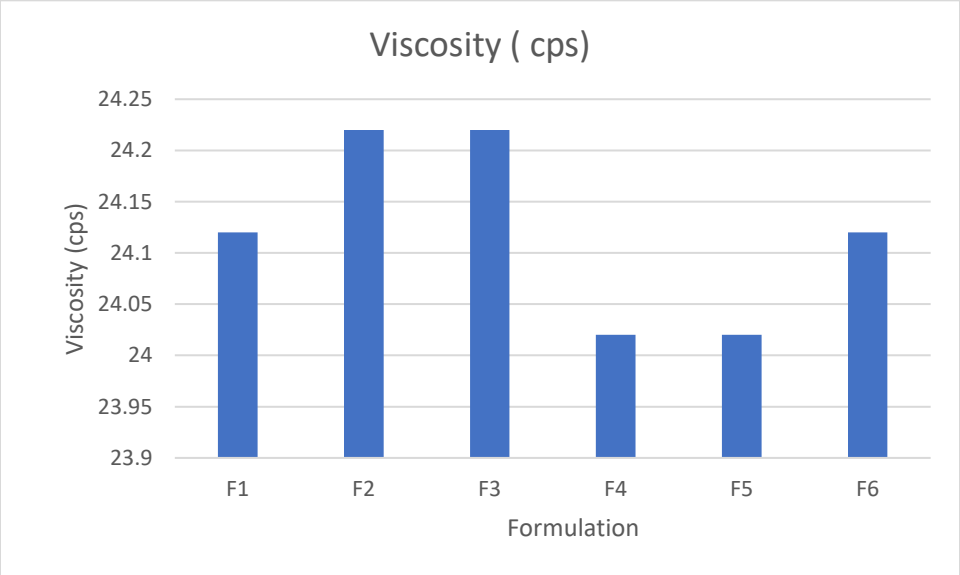
Formulation	pH	Viscosity (cps)	Spread ability(gm.cm/sec)
F1	6.4±0.2	24.12±0.14	28.73±0.52
F2	6.3±0.2	24.22±0.16	27.21±0.17
F3	6.4±0.2	24.22±0.16	30.11±12
F4	6.4±0.2	24.02±0.14	29.11±12
F5	6.4±0.2	24.02±0.16	31.11±12
F6	6.3±0.2	24.12±0.16	32.12±12

Physical analysis of the graphene-based nanocrystal topical gel

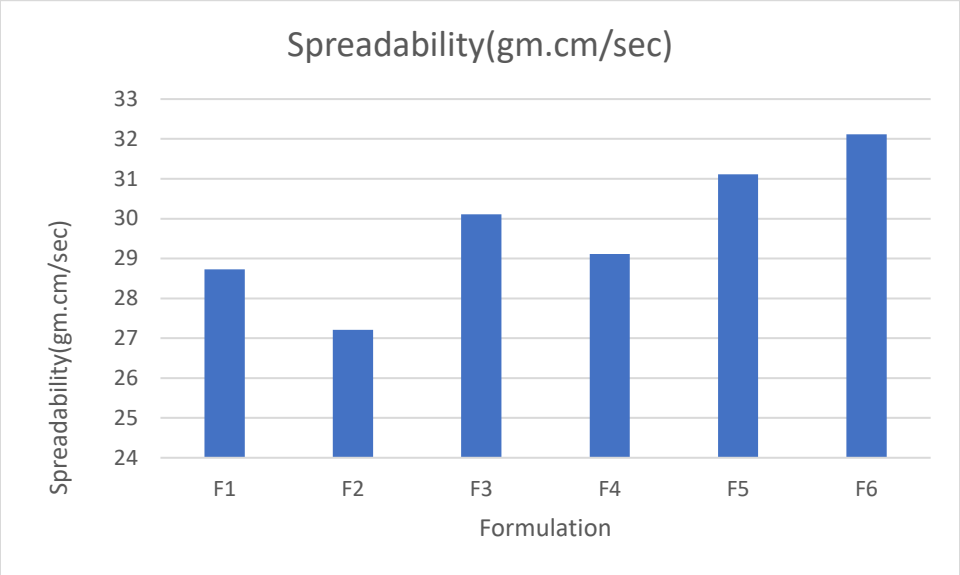
The formulations consisted of Black Brown, and the nanocrystal base, which had just been prepared, was transparent (Table 5.3). Throughout the 30-day observation period, there were no alterations in the odor, appearance, or color of any of the formulations, ranging from F1 to F6, in the base. This indicates that the created formulas were stable while stored at different temperatures, specifically 8 and 40 ◦C. **pH:** It was noticed that the interval between 6.3 and 6.4 when subjected to varying storage circumstances, remained stable for a duration of 30 days. The composition and pH of the base, held at 8 ◦C for one month, did not exhibit any substantial alteration. The specific specifics are more crucial than the control (base) for the duration of one month.



Viscosity: The viscosity of the composition, which was kept in the latter case for 30 days, was found within range.



Spreadability: The distribution of formulation and base was studied. Both formulations have a good spread



Conclusion

Overall, the development and analysis of graphene-based nanocrystals (GNCs) for the purpose of delivering drugs via the skin is notable progress in the field of pharmaceutical research. The study effectively showed that GNCs can improve the solubility, stability, and bioavailability of usnic acid, a promising natural chemical with medicinal potential. The nanocrystals were synthesized using a systematic approach involving GO reduction, functionalization with PEG, and integration of usnic acid. These nanocrystals demonstrated desirable physicochemical features, such as steady pH levels, suitable viscosity, and exceptional spreadability. The formulations were proven to have nanoscale shape and chemical integrity using SEM and FTIR investigations. The results indicate that GNCs have the potential as efficient carriers for transdermal delivery of usnic acid, which could enhance the effectiveness of treatment and improve patient outcomes in dermatological disorders. Further investigation should prioritize the optimization of these formulations for clinical use and the exploration of their wider therapeutic implications in topical drug delivery systems.

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