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PREFORMULATION PROFILING OF MORIN HYDRATE: A RATIONAL APPROACH TOWARD THE DEVELOPMENT OF LIPID-BASED NANOCARRIER SYSTEMS FOR ENHANCED DERMAL DELIVERY

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ABSTRACT

Background: Morin hydrate is a naturally occurring polyphenolic flavonoid with notable antioxidant, anti-inflammatory, and free radical scavenging activities. However, its pharmaceutical application is limited by poor aqueous solubility, high crystallinity, and low physicochemical stability, necessitating detailed preformulation characterization to develop effective lipid-based drug delivery systems. **Materials and methods:** A comprehensive preformulation study of morin hydrate was conducted using physicochemical and analytical characterization techniques. UV-visible spectrophotometry was employed for the wavelength determination and calibration curve analysis. Physicochemical behavior was studied by melting point, partition coefficient, and solubility studies. Functional group compatibility and thermal properties were studied using Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC), respectively. The crystalline properties were further characterized by X-ray diffraction (XRD) analysis. **Results:** Morin hydrate exhibited characteristic absorption maxima at 265 nm and 385 nm; the latter was selected for quantitative analysis due to its higher sensitivity and reduced interference. The calibration curve showed excellent linearity, consistent with the Beer-Lambert law. The melting point (285 ± 2 °C) indicated a high crystalline purity and thermal stability. Partition coefficient analysis showed moderate lipophilicity, appropriate for lipid-based formulations. FTIR spectra confirmed the presence of characteristic functional groups and the absence of chemical incompatibility, while DSC and XRD analyses revealed a highly crystalline, thermally stable structure. Solubility studies indicated the highest solubility in oleic acid as compared to other lipid carriers. **Conclusions:** The results suggest that morin hydrate is a suitable candidate for incorporation into nanostructured lipid carrier systems, owing to its favorable physicochemical and biopharmaceutical properties, which enhance solubility, stability, and dermal bioavailability.

KEYWORDS: Morin hydrate, Preformulation, NLCs, solubility, Flavonoid, and Topical drug delivery.

1. INTRODUCTION

Morin hydrate (3,5,7,2',4'-pentahydroxyflavone) is a notable naturally occurring bioflavonoid within the flavonol category, characterised by five hydroxyl groups situated on the A, B, and C rings.[1,2] It is predominantly extracted from species in the Moraceae family, such as Maclura pomifera (Osage orange), Morus alba (white mulberry), and Psidium guajava (guava), as well as from several other plants and fruits.[3] Morin hydrate is present in dietary sources such as tea, coffee, red wine, seaweed, and cereal grains. Historically used in traditional medicine, particularly in Thai and Chinese practices, this yellowish pigment has garnered considerable interest in contemporary pharmacology owing to its favourable safety profile and low cytotoxicity.[2]

The pharmacological versatility of morin hydrate is extensive, including potent antioxidant, anti-inflammatory, anticancer, neuroprotective, cardioprotective, and hepatoprotective activities.[3–5] The therapeutic effectiveness is intrinsically linked to its distinctive chemical structure, characterised by a flavone

backbone bearing five phenolic hydroxyl groups. These functional groups allow the molecule to operate as an effective natural radical scavenger, stabilise reactive oxygen species (ROS), and regulate essential cellular signalling pathways, including NF-κB, Nrf2, MAPK, and JAK/STAT.[3]

Despite its promising therapeutic potential, the clinical application of morin hydrate is severely restricted by its poor biopharmaceutical properties.[4,6] Classified as a BCS class IV molecule, it exhibits extremely low aqueous solubility (approximately 28.0–28.7 µg/mL) and limited intestinal permeability.[7–9] These restrictions are particularly important in topical drug delivery systems, where limited permeability and insufficient water solubility limit efficient drug deposition at the site of action. Conventional topical formulations' homogeneous distribution is impeded by their strong crystalline structure and limited solubility, which reduces the availability of medications.[3,10]

To overcome this limitation, the development of lipid-based drug delivery systems has emerged as a strategic approach.[5,6] Nanotechnology platforms—including solid lipid nanoparticles (SLNs), nanomicelles, and microemulsions—offer a sophisticated approach to enhance the solubility and bioavailability of hydrophobic polyphenols.[5,6] These systems provide several advantages, such as better solubilization, improved skin penetration, controlled drug release, and protection against environmental degradation.[11]

Preformulation studies are the cornerstone of any successful pharmaceutical formulation, whether conventional or nanocarrier-based.[5,7] A comprehensive understanding of the drug's solubility, partition coefficient, nature, and degradation kinetics across various physiological media is essential for selecting appropriate excipients and optimising the delivery vehicle.[7,9] In this research work, the various preformulation parameters, such as solubility, melting point, crystallinity, partition coefficient, and stability—all crucial for logical formulation design—are systematically assessed. Additionally, compatibility studies employing analytical methods such as Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR) are essential for assessing potential interactions between the medicine and excipients.

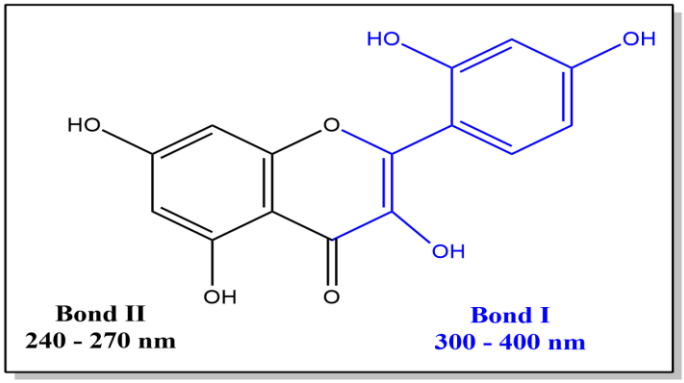

Figure 1: Structure of Morin Hydrate.

2. MATERIALS AND METHODS

2.1 Material

Morin hydrate (≥98%) analytical grade obtained from *Sigma–Aldrich, USA*, Glyceryl Monostearate (Loba Chemie, Mumbai, India), castor oil, Oleic acid, olive oil, Ethanol, Methanol, and Stearic acid. All are Pharmaceutical standards grade.

2.2 Preparation of stock solution

Precisely weighed 10 mg of morin hydrate was placed in a 10 mL calibrated volumetric flask and diluted with 10 mL of methanol to prepare a primary stock solution (Stock I) of 1000 µg/mL. The stock was subsequently diluted with methanol to prepare an operational stock solution (Stock II) of 10 µg/mL for further tests.[12]

2.3 Determination of the wavelength of maximum absorbance (λmax)

The UV-visible absorption spectra of Stock II were acquired in full-scan mode (200–800 nm), with methanol as the blank. We determined the wavelength of maximum absorbance (λ_max) from the acquired spectrum using the instrument's software.[12]

2.4 Calibration Curve Preparation

Stock II was serially diluted to provide six calibration standards with concentrations of 10, 20, 40, 60, and 80 µg/mL. Absorbance measurements for each standard were recorded at the specified λ_max of 385 nm in fixed-wavelength mode. A calibration curve was constructed by plotting absorbance against concentration, with the procedure performed in quintuplicate to ensure reliability and statistical validity. Linear regression analysis yielded the line equation and the coefficient of determination (R^2), which were used to validate the approach.

2.5 Solubility Studies

The solubility of morin hydrate in pure solvents was studied using saturated solutions with excess compound. These solutions were sonicated and agitated for at least 2

hours. To reach solid–liquid equilibrium, they were placed in a thermostatic bath for 48 hours.[13] The mixtures were filtered through a microporous membrane to remove particulates before sampling. Absorbances of the diluted solutions were measured using a Shimadzu 2100 UV–Visible spectrophotometer across a wavelength range of 385 nm, with concentrations determined by previously established calibration curves for all solvents.

2.5 Melting Point

The melting point of morin hydrate was determined using a melting point apparatus (Electrothermal IA9100) to evaluate the thermal stability of morin hydrate. Finely powdered morin hydrate was introduced into three glass capillaries, ensuring uniform packing to minimise packing-induced variations, and placed in the melting point apparatus. The samples were subjected to gradual heating (2 °C/min) from ambient temperature to 400 °C, with melting initiation and completion temperatures recorded precisely using a digital temperature sensor.

2.6 Partition coefficient

The partition coefficient (log P) of a compound indicates its distribution strength between two immiscible solvents and serves as a primary measure of its lipophilicity, which influences how easily a drug can penetrate biological membranes[9] The shake-flask method was used to determine the partition coefficient (log P) of pure MH and the MHNS formulation. A sample (MH) was added to a separating funnel containing a 1:1 mixture of water and n-octanol (5 mL each). The separating funnel containing the sample was shaken for 30 min and then equilibrated for up to 12 h. Thereafter, the aqueous phase was collected, and the MH content was determined by UV at 385 nm. The log P was calculated using the following equation.[14]

The partition coefficient was calculated using the following equation.

$$\log P = \log\left(\frac{C_{octanol}}{C_{water}}\right)$$

where $C_{octanol}$ and C_{water} represent the equilibrium concentrations of morin hydrate in the n-octanol and aqueous phases, respectively.

2.8 Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was utilised to identify the functional groups of pure morin hydrate, an essential preformulation step to verify its chemical stability before nanocarrier development. This technique relies on the absorption of molecular vibrations in the infrared spectrum (4000–400 cm^{-1}), yielding a molecular fingerprint for identifying hydroxyl, carbonyl, and aromatic groups, which are critical for evaluating drug-excipient compatibility. Pure morin hydrate (2 mg) was thoroughly combined with dry KBr (200 mg) and crushed into discs utilising a Shimadzu IR-Affinity-1 spectrophotometer (Japan) at a resolution of 4 cm^{-1} with 32 scans.[15]

2.9 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was used to characterise the thermal properties of pure morin hydrate, providing a critical preformulation evaluation of its melting behaviour and thermal stability. The analysis employed a DSC 404 F3 Pegasus® equipment (NETZSCH, Germany) functioning under inert conditions. Samples of 5–10 mg of pure morin hydrate were precisely weighed into standard aluminium pans and securely sealed to prevent moisture contamination. Thermal scans were conducted from 30 to 400°C at a heating rate of 10°C/min in a dry nitrogen atmosphere (50 mL/min flow rate), utilising an empty aluminium pan as a reference.[16][17]

2. 10 X-ray powder diffraction (XRD)

X-ray powder diffraction (XRD) was employed to assess the crystalline properties and solid-state features of pure morin hydrate during preformulation investigations. An investigation was performed using a D8 Advance X-ray diffractometer (Bruker, Germany) with Cu Kα radiation (λ = 1.5406 Å), operating at 40 kV and 40 mA. Approximately 150–250 mg of pure morin hydrate powder was precisely placed in the sample container and levelled to ensure uniform surface exposure without preferred orientation. Diffraction patterns were acquired throughout a 2θ range of 5° to 50°, with a step size of 0.02° and a scan rate of 2° per minute, culminating in a total acquisition time of 2 hours. Raw diffractograms were analysed via Bruker DIFFRAC.EVA® program for phase detection and crystallinity evaluation, compared with reference standards.[18]

2.11 Screening of lipids

The solubility of morin hydrate in different liquid lipids (paraffin oil, Castor oil, oleic acid, olive oil, and Isopropyl myristate) was determined by adding an excess amount of morin hydrate to 2 ml of different oils in 2ml glass vials.[19] The glass vials were tightly sealed and continuously stirred to reach equilibrium for 48h at 37±2 °C in a mechanical shaker. After that, liquid lipid and morin hydrate mixtures were centrifuged at 10,000 rpm for 15 min in a high-speed centrifuge (Sigma-3K30, Osterode am Harz, Germany) at 37±2 °C. The supernatant was separated, dissolved in methanol, and the saturation solubility was analysed by UV at 385 nm.[20]

For the selection of solid lipid, an accurately weighed amount (1g) of the solid lipids (stearic acid, glyceryl monostearate, acetyl palmitate, and cholesterol) was taken and heated to 5℃ above their melting point with continuous stirring using a magnetic stirrer with a hot plate (Remi Instrument Ltd., Mumbai, India) at 200 rpm. Morin hydrate was added in increments of 1mg until it was completely dissolved in the molten solid lipids, and the amount of solid lipids required to solubilise morin hydrate was calculated. The experiment was repeated in triplicate, and the results were presented as the mean (mg/g) ± SD.[17]

3. RESULT

3.1 Determination of wavelength of maximum absorbance:

The wavelength of maximum absorption (λmax) for morin hydrate was determined by UV-visible spectrophotometry to define appropriate conditions for quantitative analysis in preformulation experiments. Solutions with absorbance values below 1.0 were analysed in the 200-400 nm range to verify adherence to Beer 's-Lambert law. Morin hydrate (10 µg/mL in methanol) exhibited significant absorption maxima at 261 nm (primary λmax, Band II; π→π* transition) and 385 nm (secondary peak, Band I; n→π* transition), as shown in Figure 2. The 385 nm wavelength was chosen for its highest intensity and reduced interference from nanocarrier excipients.

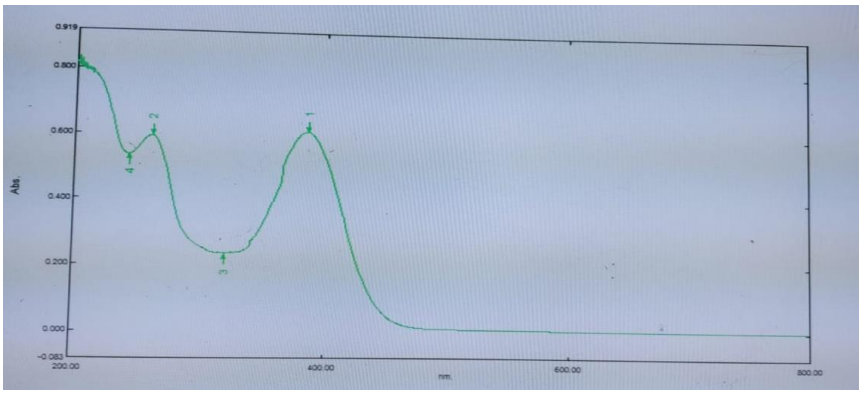

Figure 2: Maximum absorbance (λmax) of morin hydrate.

3.2 Standard curve

Quantitative UV-visible spectrophotometric analysis requires the development of a reliable calibration curve to define the concentration-response relationship, ensuring compliance with Beer 's-Lambert law across the analytical range. Calibration standards (10, 20, 40, 60, and 80 µg/mL) were prepared from a 1 mg/mL methanolic stock solution of morin hydrate, subsequently diluted with methanol. Absorbance was quantified at the specified λmax of 385 nm utilising a UV-visible spectrophotometer (Shimadzu UV-1800) in fixed-wavelength mode (slit width 1 nm, 1 cm quartz cuvette), with quintuplicate measurements for each concentration to assess procedure accuracy.

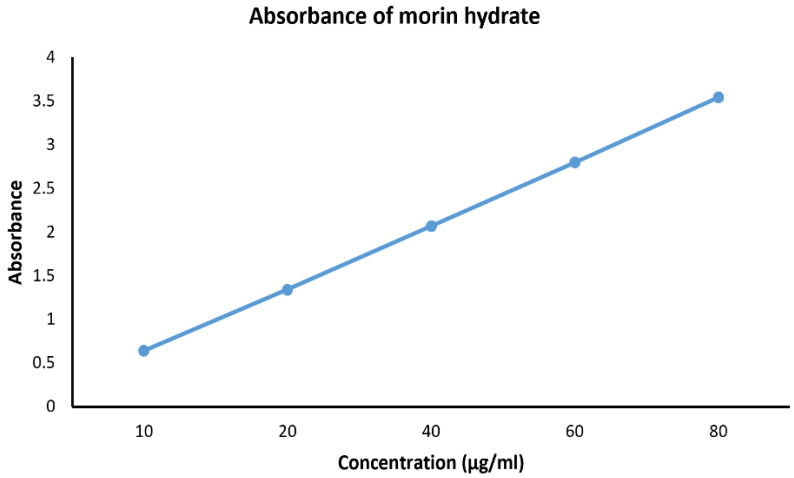

Figure 3: Standard curve of Morin hydrate.

3.3 Melting Point

The melting point of morin hydrate was determined using a melting point apparatus (Electrothermal IA9100) to evaluate the thermal stability of morin hydrate. Finely powdered morin hydrate was introduced into three glass capillaries, ensuring uniform packing to minimise packing-induced variations, and placed in the melting point apparatus. The samples were subjected to gradual heating (2 °C/min) from ambient temperature to 400 °C, with melting initiation and completion temperatures recorded precisely using a digital temperature sensor. Morin hydrate exhibited a sharp melting transition at 285 ± 2 °C (range: 283-286 °C across triplicates), characteristic of its high crystalline purity and absence of polymorphic impurities. This value demonstrates excellent concordance with pharmacopeial standards (285-287 °C).

3.4 Solubility study

The solubility profile of morin hydrate was studied in water, ethanol, methanol, and n-hexane based on the polarity. Solubility of Morin hydrate in water was found

to be (0.28 mg/ml, 28 mg/ml, 48 mg/ml, and 0.18 mg/ml, respectively), which has low water solubility because of its polyphenolic structure, which possesses relatively few ionic groups and considerable intermolecular hydrogen bonding, thereby restricting its solubility in water. In contrast, solubility was much higher in polar organic solvents, with methanol having the highest solubilising power, followed very closely by ethanol. The increased solubility in alcohols is probably because they can form hydrogen bonds with the hydroxyl groups of morin hydrate and so increase the dispersion of the molecules.

3.5 Partition Coefficient

Morin hydrate exhibited a partition coefficient (Log P) of 2.05 ± 0.7, indicating moderate lipophilicity. The molecule exhibited a preferential affinity for the organic (n-octanol) phase over the aqueous phase, indicating its partial hydrophobicity attributable to the flavonoid backbone, despite the presence of several hydrophilic hydroxyl groups. The determined Log P value indicates that morin hydrate has a balanced hydrophilic-lipophilic profile, facilitating passive diffusion through biological membranes. From a formulation standpoint, the moderate Log P value supports the suitability of morin hydrate for incorporation into lipid-based delivery systems such as SLNs and NLCs. This equilibrium enables effective solubilization in lipid matrices and optimal interaction with aqueous biological environments, hence improving medication penetration and retention in dermal layers.

3.6 FTIR studies

Fourier transform infrared (FTIR) spectroscopy was utilised to clarify the molecular vibrational characteristics of pure morin hydrate and confirm the absence of chemical impurities during preformulation assessment. The spectrum exhibited distinctive absorption bands typical of the flavonoid structure, including a broad hydroxyl (–OH) stretching vibration centred around 3400 cm^{-1}, associated with phenolic and enolic groups, as well as a sharp peak at 1660 cm^{-1} corresponding to the carbonyl (C=O) stretch of the chromone ring.

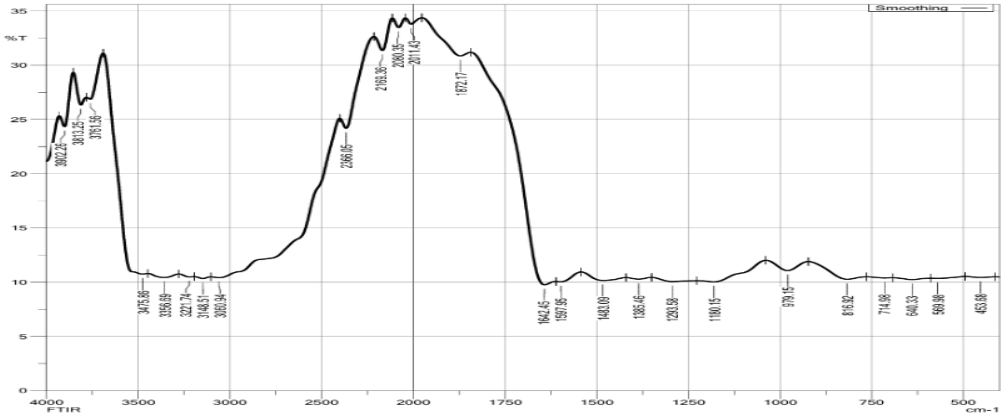

Figure 3: FTIR Spectra of morin hydrate.

3.7 DSC Analysis

DSC of morin hydrate was performed to evaluate its thermal stability and polymorphic purity in preformulation studies. The thermogram displayed a significant exothermic peak at approximately 285°C, indicating the melting transition of crystalline morin hydrate, in agreement with the literature, which reports a melting point of 285–300°C. This distinct, symmetrical peak, commencing at approximately 280°C and exhibiting negligible shoulder, signifies exceptional purity and the lack of substantial amorphous material or hydration-related phenomena below 200°C.

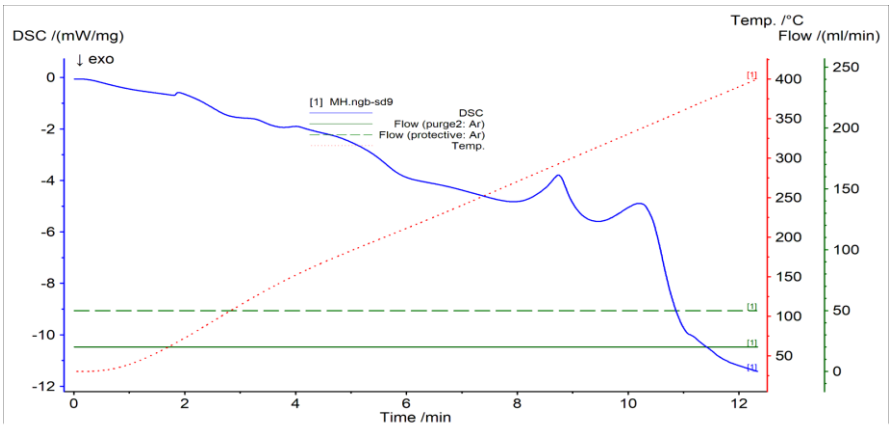

Figure 4: DSC of Morin hydrate.

3.8 X-ray diffraction (XRD) studies

Powder X-ray diffraction (PXRD) of morin hydrate was performed to verify its crystalline structure and polymorphic identity as a component of preformulation characterisation. The diffractogram displayed very strong Bragg diffraction peaks at 2θ values of approximately 10.2°, 14.2°, and 25.4°, as well as significant reflections at 8.3°, 16.7°, 28.4°, and 31.6°, indicating a highly organised crystalline lattice characteristic of the anhydrous hydrate form.

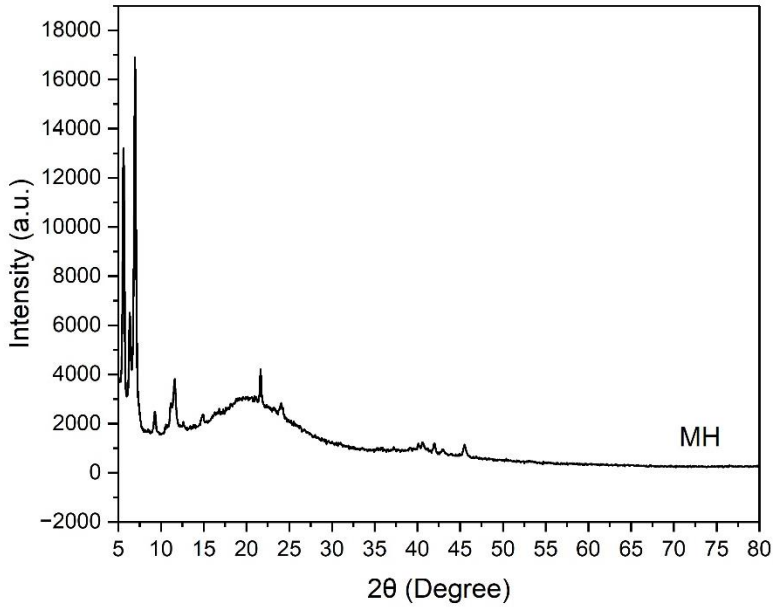

Figure 5: XRD of pure morin hydrate.

3.9 Screening of lipids

The solubility of morin hydrate in liquid lipids is markedly affected by lipid polarity and molecular structure. Solubility of morin hydrate in different liquid lipids was found to be, respectively, oleic acid > olive oil > castor oil> isopropyl myristate, as shown in Figure 6. Oleic acid exhibits the greatest solubilising capacity, followed by olive oil, isopropyl myristate and castor oil, where isopropyl myristate demonstrates negligible solubility. The enhanced solubility in oleic acid results from its unsaturated nature and strong affinity for polyphenolic compounds, thereby improving the molecular dispersion of morin hydrate. The low solubility of isopropyl myristate is due to its ester functionality, which enhances its interaction with medications compared with nonpolar alternatives such as paraffin oil. Furthermore, the solubility of olive oil is associated with its triglyceride composition and minor polar elements that enhance drug partitioning.

In solid lipids, the solubility of morin hydrate varies with the crystalline structure and melting behaviour of lipid matrices. Glyceryl monostearate has the highest solubilization capacity, followed by stearic acid, while cholesterol and acetyl palmitate show lower incorporation. The amphiphilic nature of glyceryl monostearate promotes moderate solubility, whereas the rigid crystalline form of cholesterol limits drug incorporation. The findings suggest that lipid type and structural characteristics are crucial in determining the solubility behaviour of morin hydrate, which is vital for the development of a nanocarrier system.

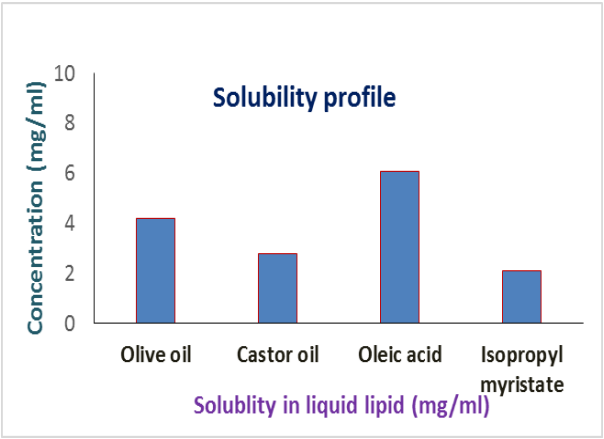

Figure 6: Solubility profile of morin hydrate in different liquid lipids.

4. DISCUSSION

Morin hydrate (3,5,7,2',4'-pentahydroxyflavone) is a notable naturally occurring bioflavonoid within the flavonol category, characterised by five hydroxyl groups situated on the A, B, and C rings.[1,2] is predominantly extracted from species in the Moraceae family, such as Maclura pomifera (Osage orange), Morus alba (white mulberry), and Psidium guajava (guava), as well as several other herbs and fruits.[3] Morin hydrate exhibits broad pharmacological activity, including significant anti-inflammatory, anticancer, cardioprotective, antioxidant, hepatoprotective, and neuroprotective properties.[3–5] Although it has promising therapeutic potential, the clinical utilisation of morin hydrate is significantly limited by its poor biopharmaceutical

properties. Classified as a BCS class IV compound, it demonstrates markedly low water solubility (about 28.0–28.7 µg/mL), which also restricts topical drug delivery systems, where inadequate water solubility and restricted permeability restrict effective drug deposition at the site of action. The robust crystalline structure and limited solubility hinder homogeneous distribution in conventional topical formulations, thereby diminishing the medication's availability.[7–9]

This research presents a rigorous preformulation characterisation of morin hydrate, highlighting its physicochemical and biological limitations and their consequences for formulation development. UV-visible spectroscopy research verified unique absorption maxima associated with $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, typical of flavonoid structures. The use of 265 nm for analytical quantification ensured increased sensitivity and reduced interference, affirming its appropriateness for formulation research.

The melting point determination (285 ± 2 °C) indicates a highly crystalline, pure chemical, as further validated by DSC and XRD analyses. The pronounced exothermic peak observed in DSC thermograms and the identification of distinct Bragg diffraction peaks in PXRD confirm the absence of amorphous material and polymorphic transitions. The crystallinity guarantees chemical stability but also leads to poor water solubility, a significant constraint on bioavailability.

The partition coefficient (log P = 2.05 ± 0.7) indicates moderate lipophilicity, reflecting a balanced hydrophilic-lipophilic profile. This trait is beneficial for transdermal drug delivery, as it facilitates membrane penetration and compatibility with lipid matrices. The presence of multiple phenolic hydroxyl groups leads to pH-dependent ionisation, which may influence drug partitioning and permeability in physiological settings.

The FTIR study verified the structural integrity of morin hydrate, with typical hydroxyl and carbonyl functional groups preserved, indicating no degradation or chemical interactions during preformulation processing. This is essential for ensuring compatibility with excipients in later formulation phases.

Solubility investigations revealed variable solubility among lipid carriers, with the highest solubility observed in oleic acid. This discovery is especially important for selecting lipid components in nanostructured lipid carrier systems because solubilization capacity directly affects drug loading and stability. The comparatively diminished solubility in other oils, such as castor oil and isopropyl myristate, underscores the imperative for meticulous excipient evaluation.

Morin hydrate exhibits a broad range of beneficial pharmacological properties; however, preformulation studies consistently reveal considerable biopharmaceutical challenges, particularly inadequate aqueous solubility and stability, indicating the need for novel delivery methods. In this regard, lipid-based delivery systems, especially nanostructured lipid carriers (NLCs), emerge as a promising solution. These systems enhance the solubility of morin hydrate, protect against physicochemical degradation, and improve dermal permeation and retention, thereby potentially augmenting its therapeutic efficacy in topical applications.

5. CONCLUSION

The present preformulation study comprehensively explored the physicochemical properties of morin hydrate, emphasising its high crystallinity, moderate lipophilicity, and restricted water solubility. Analytical and solid-state characterisation validated its structural integrity, thermal stability, and the absence of polymorphic impurities. The solubility and partition coefficient support its suitability for lipid-based delivery systems, especially SLN or NLCs. These findings provide a solid scientific basis for the systematic development of new formulations to enhance the solubility, stability, and dermal bioavailability of morin hydrate.

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ONFLICTS OF INTEREST: No Conflict of Interest.

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