INTRODUCTION

The causes of skin dyschromia, particularly conditions like melasma, are multifaceted and treating them poses significant challenges. Melasma, a prevalent hyperpigmentation disorder, affects a substantial segment of the population, primarily women residing in regions experiencing elevated levels of exposure to UV radiation.\(^1,2\) Melasma is characterized by symmetrical patches of excessive pigmentation with irregular borders, commonly appearing on the cheeks, forehead, and jawline.\(^3,4\) While the exact origins of melasma is still uncertain, various Factors that can trigger it have been identified. These factors include exposure to sunlight to varying extents, pregnancy, the use of oral contraceptives and steroids, liver-related conditions, the use of photosensitizing medications, and skin inflammation.\(^5\) Post-inflammatory hyperpigmentation (PHI) and melasma are common in individuals of all phototypes of skin, with a notable prevalence among deeper-pigmented skin tones.\(^6\) The above-mentioned dermatological issues are the primary reason for individuals to seek cosmetic consultations and are the driving force behind the strong demand for effective skin-lightening treatments.\(^7\)

ABSTRACT

Stubborn skin pigmentation issues like post-inflammatory hyperpigmentation (PIH) and melasma are the primary reasons people seek cosmetic consultations. Treating these conditions topically is challenging, as it involves inhibiting various stages of production of the pigment process. A powerful tyrosinase inhibitor like, kojic acid (KA) is employed as a formulation to regulate pigmentation production by suppressing the melanogenesis process. It’s important to note that the application of KA has been approved by the Food and Drug Administration (FDA), US, for dermatological treatments. The goal of this investigation was to formulate a nanoemulsion containing kojic acid for skin delivery using an emulsification method. The characteristics of the KA nanoemulsion were thoroughly examined through techniques like fourier transform infrared spectroscopy, (FTIR) particle size analysis and transmission electron microscopy (TME). In addition, the formulation’s performance was evaluated through both ex-vivo permeation study and in-vitro release study. Analysis of the FTIR, X-ray diffraction (XRD), and differential scanning calorimetry (DSC) results revealed that kojic acid with other ingredients in the formulation did not exhibit any chemical interactions. The kojic acid nanoparticles that were produced exhibited a spherical shape and were uniformly distributed, with an average size diameter of 184 nm. In in-vitro tests, it was observed that 87.67% of the drug was released within 12 hours. Moreover, ex-vivo permeation evaluation demonstrated that 81.24% of the drug permeated the skin within 8 hours of application. The thermal stability studies confirmed the stability of the kojic acid nanoemulsion, with no signs of creaming, cracking, or phase separation in the formulation. In conclusion, the findings of this study suggest that the kojic acid nanoemulsion holds great promise as an effective means for delivering kojic acid within the upper layers of the skin for treating of facial dyschromia.

KEYWORDS: Hyperpigmentation, Nanoemulsion, Facial dyschromia, Kojic acid.

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melanin production. Kojic acid’s chemical structure consists of two hydroxyl groups and is represented.

Kojic acid offers several additional benefits beyond its depigmenting properties. It exhibits antioxidant activity, which means it shields the skin surface from the detrimental impact of ultraviolet radiation and sunlight by countering free radicals produced as a result of reactive oxygen species.\textsuperscript{16} Furthermore, kojic acid is recognized for its role as a key component in treatments for skin whitening, combating browning of the skin, and serving as an antibacterial agent.\textsuperscript{17,18} These properties make it a versatile ingredient in skincare formulations. Notably, kojic acid is also extensively utilized in various food products to prevent browning.\textsuperscript{19} Moreover, Food and Drug Administration (FDA) has authorized the utilization of kojic acid and other compounds combination in the US for the purpose of dermatological treatment.\textsuperscript{20}

The utilization of nanoemulsion as a novel drug delivery system, tailored with kojic acid, is particularly advantageous because of its compelling characteristics, such as a large surface area, and excellent biocompatible nature.\textsuperscript{17,21} Other hand, the production of kojic acid nanoemulsion was successfully achieved, and it was demonstrated that this nanoemulsion dispersion significantly enhances the topical delivery of kojic acid and a promising and potentially groundbreaking approach to creating a novel topical preparation for the treatment of hyperpigmentation disorders.\textsuperscript{22} Moreover, the study highlights that kojic acid in this nanoemulsion form offers greater effectiveness and stability which can be stored for extended periods under standard conditions.

MATERIALS AND METHODS

Materials
Kojic acid was collected from local suppliers and supplied by SGT University. Transcutol P, and vitamin E were taken from Merck Pvt. Ltd. Mumbai, India. Polyvinyl alcohol (PVA), xanthan gum, castor oil (CO), and tween 80 were taken from Sigma Aldrich Chemicals Pvt. LTD.

Methods
The nanoemulsion was developed using an emulsification technique. PVA was dissolved in distilled water and heated at 80°C to dissolve it. The oil phase was prepared by mixing castor oil, and vit. E. Aqueous phase was obtained by blending PVA, kojic acid and xanthan gum, then tween 80 and transcutol P added with it. Oil phase was poured into the aqueous phase drop by drop while heated at 30°C and stirred at 700 to 800 rpm by a magnetic stirrer. Then, the mixture was further homogenized by a homogenizer. Finally, the mixture was sonicated to get proper nano size, using a probe sonicator.

Physicochemical Characterization

FTIR spectroscopy
To explore potential interactions among the components of the formulation, an fourier-transform infrared (FTIR) analysis was carried out. This analysis was conducted at room temperature and covered the spectral range from 4000 to 400 cm\(^{-1}\). It involved examining both kojic acid (KA) and a physical mixture of KA nanoemulsion. The FTIR analysis was performed using a Bruker FTIR spectrometer, KBr and disk method was applied. This method involves mixing the sample with KBr and pressing it into a disk for analysis.\textsuperscript{23}

Differential scanning calorimetry
To confirm the melting points of kojic acid and the physical mixtures of the optimized formulation were subjected to differential scanning calorimetry (DSC) analysis. In this analysis, 5 mg samples were heated under a specified atmosphere. The temperature range for the analysis was from 20 to 300°C. Sealed aluminum pans were used to contain the samples, and alumina was used as a reference material.

X-ray diffraction analysis
To determine the sample’s crystallinity, an X-ray diffraction (XRD) diffractometer, manufactured by Shimadzu in Tokyo, Japan, was employed. This functioned at a voltage of 40 kV and a current of 50 mA, using CuK(α) radiation with a 0.154 nm wavelength. XRD analysis involved scanning the samples at a rate of 2 degrees per minute within scanning range 4° < 2θ < 20 < 70° at room temperature.

Particle size determination
To assess the mean particle size and polydispersity index (PDI) the prepared nanoemulsion, dynamic light scattering (DLS) was employed. This analysis was conducted using a Zeta-sizer, Malvern. Measurements were performed and scattered at an angle 173° (temperature 25°C). Three measurements were performed separately on each prepared sample.\textsuperscript{24}

Transmission electron microscopy
Transmission electron microscopy (TEM) is widely utilized to assess the aggregation state, shape, and size of nanoparticles in detail. In this study, a TEM instrument (JEOL JEM-1400Flash; JEOL in Tokyo, Japan) was employed to obtain TEM micrographs of the nanoparticles.

In-vitro release studies
Kojic acid’s release profile from the nanoemulsion formulation was assessed through dialysis bag method. In this method, phosphate buffer (0.2M, pH 7.4) was chosen as in-vitro release media. The procedure involved placing approximately 1-mL of the formulation into a dialysis bag. This bag was then immersed in 50 mL phosphate buffer at 37°C and stirred using a magnetic stirrer. About 2 mL samples were withdrawn from the release media at specific time intervals and replaced with the same amount of that fresh media. The content of kojic acid in these samples was determined by analyzing them using a UV-visible spectrophotometer at a wavelength of 268 nm.

Ex-vivo permeability studies
The study investigated the kojic acid permeation from the nanoemulsion formulation was assessed through dialysis bag method. In this method, phosphate buffer (0.2M, pH 7.4) was chosen as in-vitro release media. The procedure involved placing approximately 1-mL of the formulation into a dialysis bag. This bag was then immersed in 50 mL phosphate buffer at 37°C and stirred using a magnetic stirrer. About 2 mL samples were withdrawn from the release media at specific time intervals and replaced with the same amount of that fresh media. The content of kojic acid in these samples was determined by analyzing them using a UV-visible spectrophotometer at a wavelength of 268 nm.
both compartments, ensuring that the dermal surface faced the donor compartment. About 1-mL of phosphate buffer was transferred in the receptor compartment at 37°C and 5 mL of kojic acid (KA) nanoemulsion was placed onto the donor compartment. Over a period of up to 8 hours, 2 mL of withdrawn samples were replaced with the same amount of fresh receptor media at predetermined intervals of time. The obtained samples were analyzed at 268 nm wavelength using a UV-visible spectrophotometer to determine their kojic acid content. The data collected, specifically the mean cumulative percentage of kojic acid, was plotted against time.

Thermodynamic stability of nanoemulsion
The nanoemulsion formulation's thermodynamic stability was assessed through two methods: the centrifugation test and the freeze-thaw cycles.

In this centrifugation test, nanoemulsion samples were centrifuged for 5 minutes at 3500 rpm. After this, the samples were examined for phase separation or instability.

In the freeze-thaw stability test, the nanoemulsion samples were placed in test tubes and subjected to tests. For each cycle the samples were frozen for 24 hours at 4°C, and then thawed for 24 hours at 40°C. After this cycle, formulations were carefully observed for phase separation, as this could signify instability.

RESULTS AND DISCUSSION

FTIR Spectroscopy
Figure 1 of FTIR spectra reveals distinctive absorption peaks across the 4000 to 400 cm⁻¹ range for kojic acid and the physical mixture within the formulation. In kojic acid's spectrum, strong peaks at 3263 and 3145.9 cm⁻¹ signify hydroxyl group (-OH) stretching, while 2925 and 2840.2 cm⁻¹ represent aliphatic carbon-hydrogen (C-H) stretching. The range of 1610 to 1660 cm⁻¹ exhibits peaks related to carbonyl group (-C=O stretching). For FTIR spectrum of the physical mixture, a principal absorption peak at 3200 to 3570 cm⁻¹ indicates hydroxyl group (-OH) stretching, while 2850 to 3000 cm⁻¹ showcases asymmetric and symmetric carbon-hydrogen (C-H) stretching vibrations.

The analysis of the physical mixture indicated that the mixture retained all the characteristic peaks of kojic acid. This finding suggests that there were no chemical interactions or alterations during the preparation of the nanoemulsion. It further demonstrates that kojic acid remained stable within the formulation, validating its suitability for the intended application.

Differential Scanning Calorimetry
As shown in Figure 2, both KA and the physical mixture displayed endothermic melting peaks. KA exhibited a melting peak at 155.29°C, while the physical mixture displayed peaks at 154.50, 210.32, 223.34 and 324.70°C. These findings indicate that endotherm peak of kojic acid at 154.50°C remained present in the thermogram peaks of the KA-physical mixture. This observation suggests that Kojic Acid did not undergo any transition from crystalline form during the preparation of the physical mixture.

X-ray Diffraction Analysis
The patterns of XRD of KA and KA nanoemulsion's physical mixture are presented in Figure 3. XRD peaks of the kojic acid show at the positions of 14.3132, 19.2311, 21.5541, 25.298, 27.5791, 30.9434, 36.147, 37.4011, 39.0766°. The physical mixture exhibited peaks at around 20 = 14.2387, 19.2234, 21.5255, 25.2407, 27.5741, 30.9403, 36.0642, 37.3857, 39.0068°. KA is present within the physical mixture in its crystalline form, as has been shown previously. In contrast, the peaks indicate that result did not show any phase change for the physical mixture.

Particle Size Analysis
Figure 4 presents the results for PDI and the prepared formulation's mean particle size. The nanoemulsion was found to have PDI of 0.201 and a mean particle size of 184 nm. This PDI value falls within the range recognized as acceptable for a homogenous size distribution. Typically, up to 0.3 PDI values are optimal according to studies, but up to 0.5 values are generally acceptable. The low PDI of 0.201 suggests that the nanoemulsion formulation maintains a consistent and uniform particle size distribution, which is crucial for its effective use in various applications.

Transmission Electron Microscopy
Figure 5 displays the TEM micrograph of the sample. The nanoparticles exhibited a monodispersed distribution and a
narrow size distribution, and they appeared spherical in shape. This observation underscores the stability of the nanoemulsion, as there was no evidence of particle agglomeration. The nanoemulsion maintained a high level of stability, which is essential for its intended application.

**In-vitro Release Study**
The study indicated that the kojic acid formulation achieved a maximum drug release of 87.67% within a 12-hour period. The release rate, as shown in Figure 6, indicates a sustained release of kojic acid from the optimized nanoemulsion formulation. This sustained release property underscores the modified release character of the nanoemulsion, suggesting its potential utility in controlled and prolonged delivery of kojic acid, particularly in skin care or dermatological applications.

**Ex-vivo Permeation Study**
Figure 7 depicts the permeation profile of kojic acid through pig skin using the nanoemulsion. The ex-vivo permeation study conducted over 8 hours demonstrated a skin permeability of 81.24%. The results indicate that the nanoemulsion facilitates increased kojic acid permeation through the skin layers while retaining more in the skin's upper layers after application. This property suggests the potential for reduced application frequency and lower dosages, with the added benefit of minimizing systemic side effects when compared to traditional topical treatments.

**Thermodynamic Stability of Nanoemulsion**
The nanoemulsion formulation successfully passed the thermodynamic stability tests, including the freeze-thawing test and centrifugation test. These tests were conducted to ensure that the nanoemulsion formulations remain thermodynamically stable and free from issues like creaming, cracking, and phase separation that can occur in nanoemulsions.

**CONCLUSION**
In conclusion, we have successfully formulated an efficient and cost-effective approach for synthesizing kojic acid nanoemulsion. Our comprehensive analysis, including FTIR, DSC, and XRD, indicates that kojic acid is compatible with the components of the nanoemulsion. The experimental results demonstrated that the nanoparticles were uniformly sized and spherical shape. Furthermore, both in-vitro release and ex-vivo permeation studies confirmed that the kojic acid nanoemulsion exhibited a controlled and sustained release of kojic acid while
retaining the drug effectively in the skin. This study suggests that the nanoemulsion could be a superior choice for delivering kojic acid, particularly for addressing facial dyschromia, compared to other drug delivery methods.

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