DEVELOPMENT AND CHARACTERIZATION OF KOJIC ACID LOADED DRUG DELIVERY SYSTEM FOR THE TREATMENT OF SKIN DISORDERS

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Abstract

Background: For the effective hyperpigmentation treatment potent melanin synthesis inhibitors such as kojic acid was used.

Objectives: The objective of the study is to develop and characterize kojic acid loaded nanoemulsion, for hyperpigmentation treatment in cosmetic and dermatological products.

Methods: Kojic acid loaded nano emulsion was formulated by high-speed homogenization method. It was characterized by transmission electron microscopy (TEM), in addition to the evaluation of the release profile and permeation profile.

Results: The FTIR, XRD and DSC results indicated the entrapment of Kojic acid in nanoemulsion without any chemical interaction with other excipients. The optimized formulation yielded the polydispersity index 0.2, particle size 184 nm, and pH 5.62. The TEM analysis of optimum formulation of kojic acid showed that the particles were small spherical shaped. The in vitro drug release studies and ex vivo permeation evaluation of formulation showed that 87.67 % of release at 12 h and 81.24 % of permeation of the drug at 8 h of application respectively. The stability studies were carried out for 90 days at 25°C/60% and 40±2°C /75±5% RH. At 25°C stability studies indicated that Kojic acid nanoemulsion was stable but at 40°C slight yellowish color appears in the formulation.

Conclusion: The findings revealed that the proposed Kojic acid nanoemulsion is a promising platform and suitable for topical application in the treatment of skin disorders.

Key words: Kojic acid, nanotechnology, nanoemulsion, hyperpigmentation.

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INTRODUCTION

Hyperpigmentation is a common, usually harmless condition of skin disorder in which patches of skin become uneven localized or caused diffused darkening of the skin. This darkening occurs when an excess of melanin, the brown pigment that produces normal skin color, forms deposits in the skin [1]. This skin disorder may occur congenitally or inherently. Also, excessive exposure of harmful ultraviolet light and intake of certain drugs or chemicals may as well contribute to the skin condition. Despite the availability of various treatment options, clinical management of hyperpigmentation presents a challenge for dermatologists.

Kojic acid is a potent tyrosinase inhibitors which blocks tyrosinase and inhibit overproduction of melanin, to inhibit the production of excess pigment in the human skin [2]. Kojic acid can be used as an active ingredient for tyrosinase related skin problems such as hyperpigmentation. Kojic acid can help scavenge and counteract skin-damaging free radicals caused by exposure to things such as UV damage and pollution. This not only helps improve overall skin tone as well good general anti-aging ingredient.[3]

Since the last decade, several strategies for developing drug delivery systems have contributed to the improvement of topical formulations, thereby improving the therapeutic index of drugs, increasing their effectiveness and reducing their toxicity [4,5]. The uniqueness of nanoemulsions that resist physical destabilization due to aggregation and gravitational separation can improve the potent activity of the nanoemulsion active ingredients. Kojic acid is highly water-soluble leads to low penetrability and also susceptible to photodegradation [6]. To enhance permeation of the active ingredients into the skin and increase the photostability, an oil-in-water (O/W), transcutol P in nanoemulsion was used as a potential carrier system.

The aim of this study was to develop, characterize and evaluate oil in water nanoemulsion to present the individual as well as combined advantages like sustained release profile, increase penetration of the hydrophilic agents, impart photostability and reduce skin irritancy [7]. The study also focused on including smart excipients in formulation thus allowing reduction of drug concentration without affecting the therapeutics and also lowering the side effects. The results suggest that the Kojic acid nanoemulsion can be an efficient topical delivery system to treat hyperpigmentation.

MATERIALS AND METHODS:

Materials

Kojic acid was purchased from Local suppliers (Cosmetic grade) and supplied by SGT University. Transcutol P, Vitamin E were purchased from Merck Pvt. Ltd. Mumbai, India. Polyvinyl alcohol (PVA), xanthan gum, Castor oil (CO), Tween 80 were purchased from Sigma Aldrich Chemicals PVT. LTD. Bangalore, India. All reagents were of analytical grade as per the requirement.

Methods:

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

Physicochemical characterization

Droplet Size

Dynamic light scattering method was used to measure the droplet size and size distribution of molecules in the nanoemulsions system. Particle size and polydispersity index of the kojic acid-loaded nanoemulsion formulation was analyzed by using a droplet size analyzer (Zetasizer Nano ZS; Malvern Instruments, Malvern), scattered at an angle of 173° (temperature 25°C). The droplet size (20–200 nm) was analyzed based on the intensity weighed distribution.

Transmission electron microscopy (TEM) measurement

The size and morphology of the optimized nanoemulsion were investigated using microscopy technique by TEM (JEOL JEM-1400Flash; JEOL, Tokyo, Japan). The sample was homogenized in deionized water. A formvar coated copper grid was arranged on top of a drop of diluted sample and left at room temperature (25°C) for 3 minutes. It was then stained using 2% phosphotungstic acid for 2 minutes and air dried before analysis.

Fourier transform infra-red spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) spectra of the samples of KA, and physical mixture of optimum formulation components were evaluated were recorded over the range of 400–4,000 cm^{-1} on a Thermo Nicolet Nexus, Smart Orbit spectrometer using the potassium bromide disc method.

Differential scanning calorimetry (DSC) analysis

DSC technique was examined to analysis of the crystallinity degree and thermal behaviours studies of KA-nanoemulsion [8]. Prior to heating, about 5 mg of KA, PVA & xanthan gum mixture, and physicochemical mixtures of optimum formulation components, were subjected to an aluminium oxide pan with an empty pan used as a reference. DSC was set at 20 to 300 °C temperature range by scanning rate of 20 °C/min under N_2 flow.

X-ray diffractions (XRD) analysis

X-ray diffraction (XRD) patterns were used to determine the crystal structure of the samples and the measurements were done at room temperature and running at 2 h from 1° to 100°, with a 0.04° step size and 1 s step time, on an XRD diffractometer (Shimadzu, Tokyo, Japan) using CuKα radiation (λ 1.5406 Å) at a current of 30mA and a voltage of 40 kV.

pH Measurement:

The pH of the Kojic acid formulation was determined using a pH meter (Mettler Toledo). The measurements of the pH of the formulation were performed in triplicate and the average values were calculated. Calibration with standard buffer solutions at pH 4.00, 7.00, and 10.00 was performed before pH measurements were taken [9].

In-vitro release study

In vitro drug release studies were carried out in phosphate buffer 0.2 M pH 7.4 using dialysis bag and magnetic stirrer. The dissolution studies were performed using 50 mL of beaker at 37 ± 0.5 °C at 100 rpm. The sample (2 mL) was withdrawn at predetermined time intervals (0, 0.25,0.5, 1, 2, 4, 6, 8 and 12 h) and replaced with same volume of fresh dissolution medium. The withdrawn sample filtered through 0.45 µm Whatman filter paper. The filtered solutions were taken and assayed by using UV-Vis Spectrophotometer (Shimadzu 1800, Japan) at a wavelength of 268 nm [10].

***Ex -vivo* permeation study**

Ear skin of pig was obtained from slaughterhouse. It was kept deep freeze -80°C and then fatty layer was removed carefully. Pig skin was then stabilized in Franz diffusion cell (Logan instrument Corporation, NJ USA) containing phosphate buffer pH 7.4 in both donor and accepter compartment. Phosphate buffer from both the side was removed and then 1ml of formulation suspended in PBS 0.2 M pH 7.4 as a controlled group containing drug equivalent was placed in donor compartment of capacity 5 ml and acceptor compartment capacity 11ml was filled with phosphate buffer 0.2 M pH 7.4. Drug was permeating through an area of 0.785cm^2. The 2 ml of samples from acceptor compartment was removed past appropriate time intervals and was replaced with equal volume of phosphate buffer pH 7.4 media to maintain sink condition. Samples were analyzed at 268nm using UV –visible spectrophotometer.

Formulation Stability Test:

The optimized kojic acid nanoemulsion was subjected to stability as per ICH guidelines (ICH Q1 R2). The formulations were stored at different temperatures and humidity conditions 25 ºC / 60 ± 5% RH and accelerated temperature (40 ºC / 75 ± 5% RH) for a period of three months. The optimized formulations stored in amber color spray bottles were evaluated for parameters like appearance, pH, drug release and viscosity over a period of 3 months.

RESULTS AND DISCUSSION:

Droplet Size

The particle size of the optimized kojic acid nanoemulsion was 184 nm with a polydispersity index of 0.201 presenting the monodispersity in the globule size distribution. Nanoemulsions with a droplet size ranging between 100 and 200 nm are more favourable for cosmeceutical purpose. The particle size and polydispersity are depicted in Figure 1.

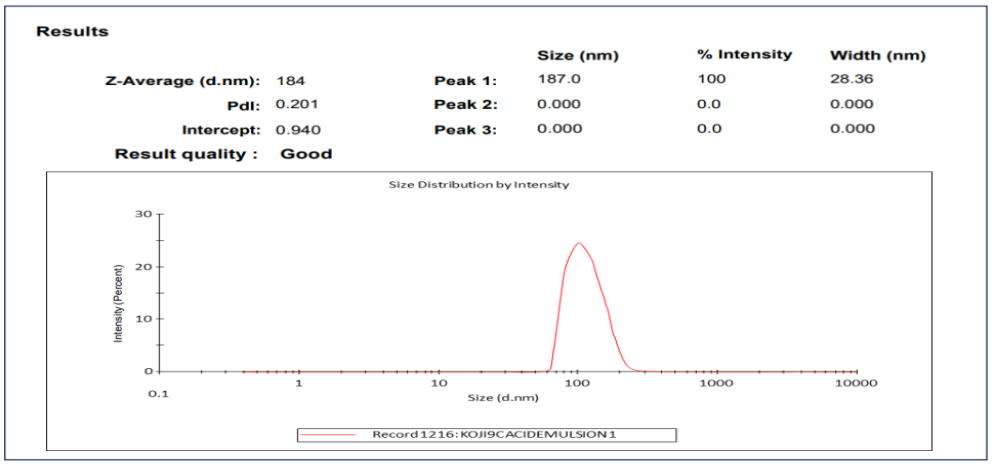

Figure 1: Particle size and PDI of the kojic acid loaded nanoemulsion

Transmission electron microscopy (TEM) measurement

The TEM image of kojic acid nanoemulsion are shown in Figure 2. The spherical shape of the oil droplets were distributed uniformly and homogenously throughout the formulation without any aggregation in the system. Moreover, the droplet size analyzed from TEM corresponded with the size obtained from the Zetasizer analysis.

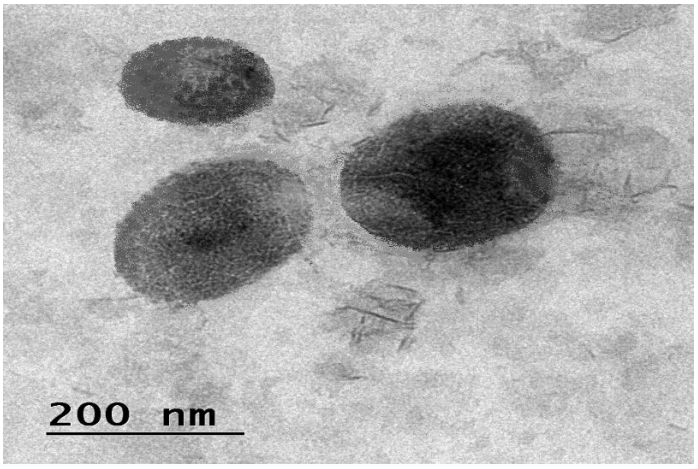

Figure 2: TEM image of the kojic acid loaded nanoemulsion

Fourier transform infra-red spectroscopy (FTIR)

FTIR technique is an important and efficient method for the quick assess of encapsulated active ingredient (drug) and investigating the existence of chemical interaction between drug and formulation components during the preparation process of the drug formulation [11]. FTIR spectra for physical mixture of kojic acid, and free KA drug are shown in Figure 3.

In the FTIR spectrum of KA- physical mixture, characteristic peaks related to C=O and C=C groups of KA were still recognisable. The results proved that there was no chemical interaction between KA and other formulation ingredients.

The results of FTIR analyses of pure KA, and physical mixture of kojic acid were summarised as follows:

The FTIR spectrum of KA represented absorption bands at 3263 cm^{-1} and 3145.9 cm^{-1} due to O-H groups stretching vibrations, also peaks at 2925 cm^{-1} and 2840.2 cm^{-1} (aliphatic C-H stretching), 1610-1660 cm^{-1} (C=O stretching of ring), 1579-1630 cm^{-1} (C=C stretching)

The FTIR spectrum of Physical mixture of kojic acid showed an absorption band at 3200-3570 cm^{-1} (O—H stretching vibration of the hydroxy group), the bands at 2850-3000 cm^{-1} due to asymmetric and symmetric C–H stretching vibrations of CH2 and CH3 groups, 1699.7 cm^{-1} due to C=O carbonyl stretch, the peaks at 1558 & 1654.9 cm^{-1} are attributed to the C=C stretching vibration of PVA

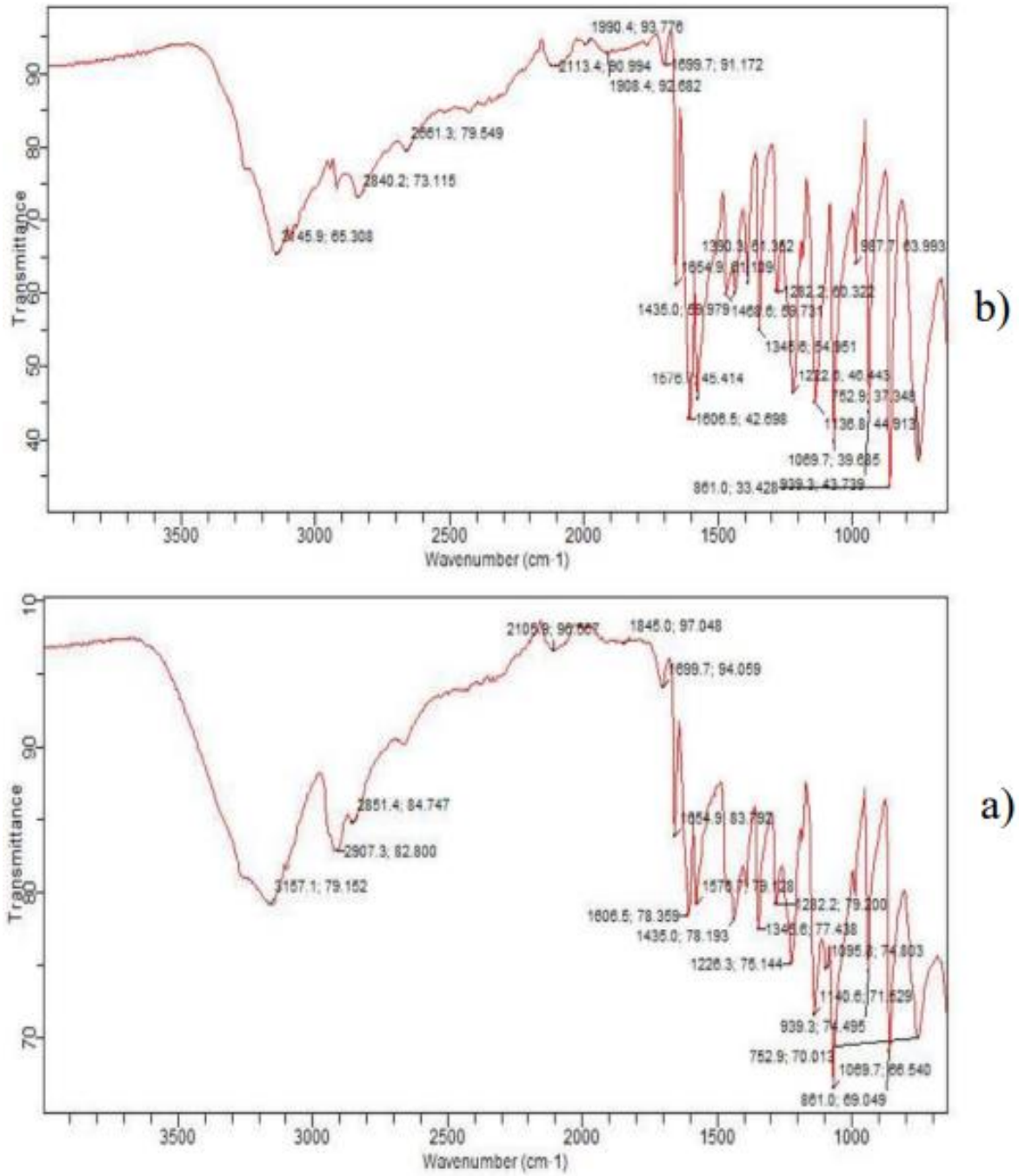

Figure 3: FTIR Spectrum of a) pure kojic acid b) physical mixture of Kojic acid

Differential Scanning Calorimetry (DSC) Analysis

DSC analysis was used to characterize the enthalpy changes and melting temperature and recrystallization behaviour, unloaded and loaded nanoparticles [12]. DSC thermograms of the pure KA, PVA & Xanthan gum mixture, and the physical mixture are shown in Figure 4. The DSC thermograms of KA, and PVA & Xanthan gum mixture exhibited a single sharp endothermic peak at about 158.81 °C and 323.04 °C, respectively, which confirms the extreme crystalline nature of them.

Thermal analysis of physical mixture by DSC indicated endothermic peak at 154.50 °, 210.32 °, 223.34 ° and 324.70 °C. The melting endotherm of the KA at 158.81 °C was present in the thermograms of KA- physical mixture structure, which demonstrates that KA has not changed from crystalline to amorphous form.

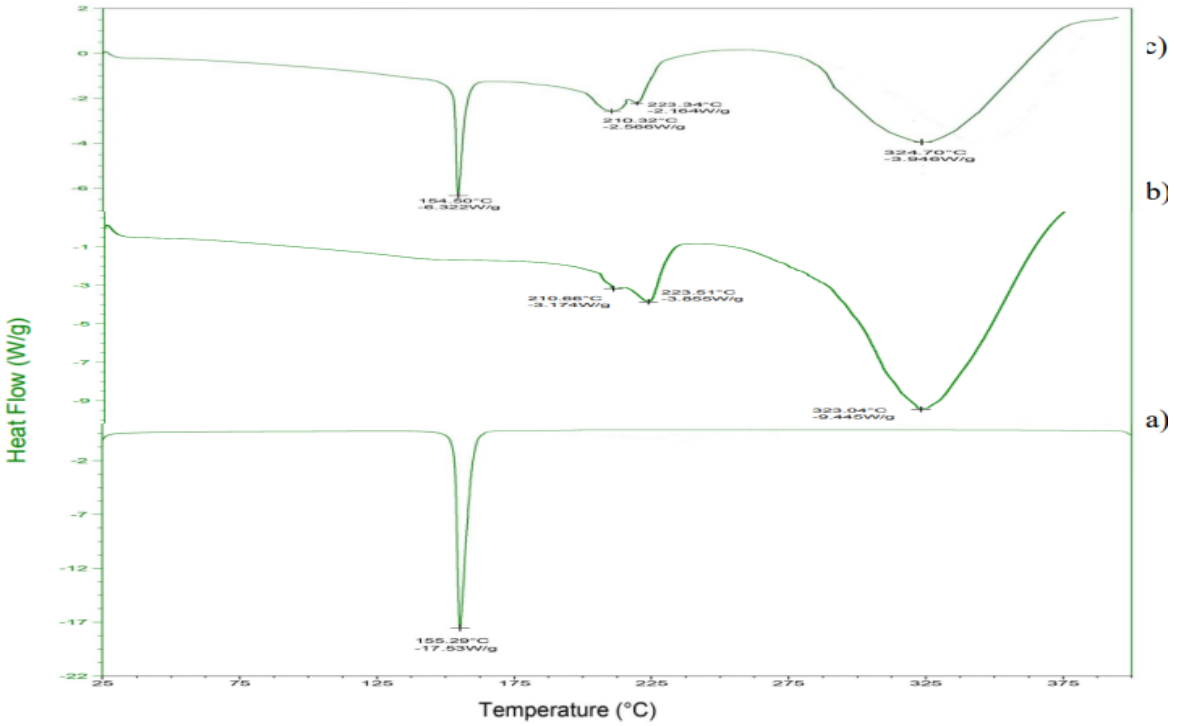

Figure 4: DSC images of a) pure kojic acid b) PVA & xanthan gum mixture and c) physical mixture of Kojic acid

X-ray diffractions (XRD) Analysis

The XRD patterns of the prepared pure KA, PVA & xanthan gum mixture, and physical mixture of optimum formulation are shown at Figure 5, respectively. In the XRD pattern of the pure KA, significant diffraction peaks were detected at 2θ: 14.3132°, 19.2311°, 21.5541°, 25.298°, 27.5791°, 30.9434°, 36.147°, 37.4011°, 39.0766° that this represents the crystalline nature of KA. PVA & xanthan gum mixture showed a crystalline diffractogram with sharp peaks at 2θ: 19.6113°, 22.6495°, 40.6474°. These sharp and distinct peaks for KA and PVA & xanthan gum mixture demonstrated the highly crystalline nature of them.

The PXRD pattern of physical mixture exhibited the characteristic peaks at 14.2387°, 19.2234°, 21.5255°, 25.2407°, 27.5741°, 30.9403°, 36.0642°, 37.3857°, 39.0068°, 44.7915°.

The XRD patterns of physical mixture exposed both the characteristic peaks of the pure KA and PVA & xanthan gum mixture, depicted the same peaks at the same position. The ten characteristic peaks for KA and PVA & xanthan gum mixture were observed also at physical mixture in Figure, which indicates that KA present within the physical mixture in its crystalline form and did not result in any phase change for the physical mixture.

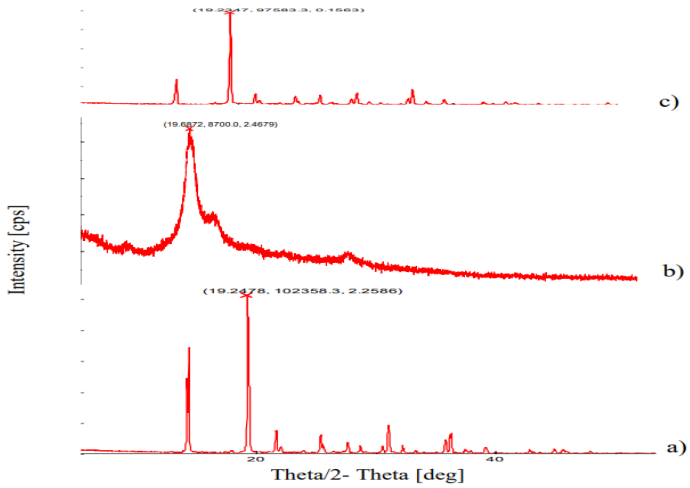

Figure 5: XRD images of a) pure kojic acid b) PVA & xanthan gum mixture and c) physical mixture of Kojic acid

pH measurement:
The pH value of the optimized formulation was 5.62, showing its suitability to be used topically as stated by Martínez et al, who reported that the pH values should be in the range of 4.0–7.0. [13]

In vitro release study
The cumulative drug release of the formulation was carried out by the procedure mentioned earlier. The release studies are carried out for the formulation for about 12 h. The release rate of kojic acid from nanoemulsion at the end of the analysis was obtained 87.67 %, As per diffusion guidelines diffusion release of formulation was good. The release profile of kojic acid can be observed in Figure 6. The results reveal that the release of kojic acid took place slowly and progressively showed sustained release, thereby proving the modified release character of nanoemulsion.

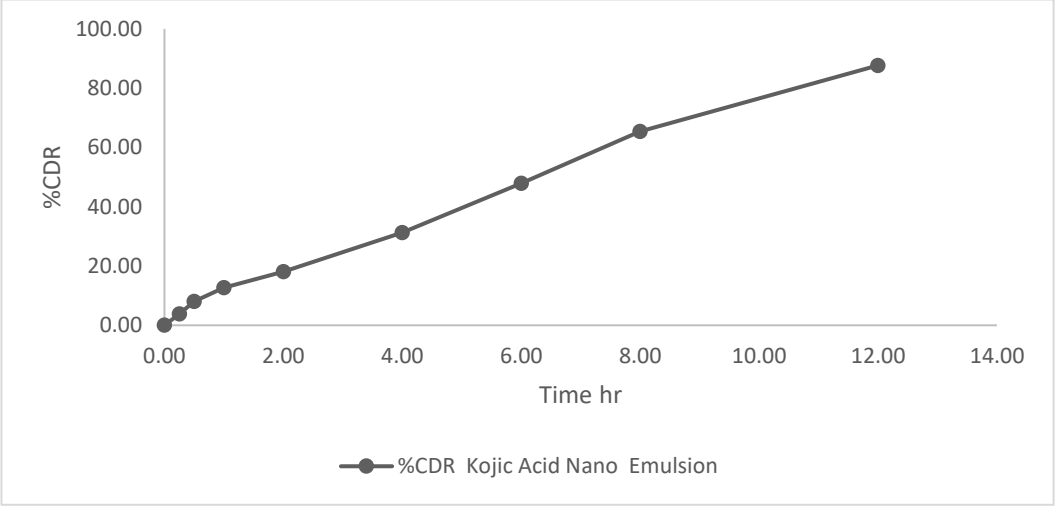

Figure 6: Drug release study of kojic acid nanoemulsion

***Ex -vivo* permeation study**
Further, the skin permeation study revealed the actual situation and shown in below figure 7. The amount of the kojic acid permeated at the end of 8 h from the kojic acid nanoemulsion on to the pig skin (used in the ex-vivo study) was 81.24 %. Thus, it can be concluded that the kojic acid nanoemulsion provided sustained release as well as better retention of a drug on the skin surface.

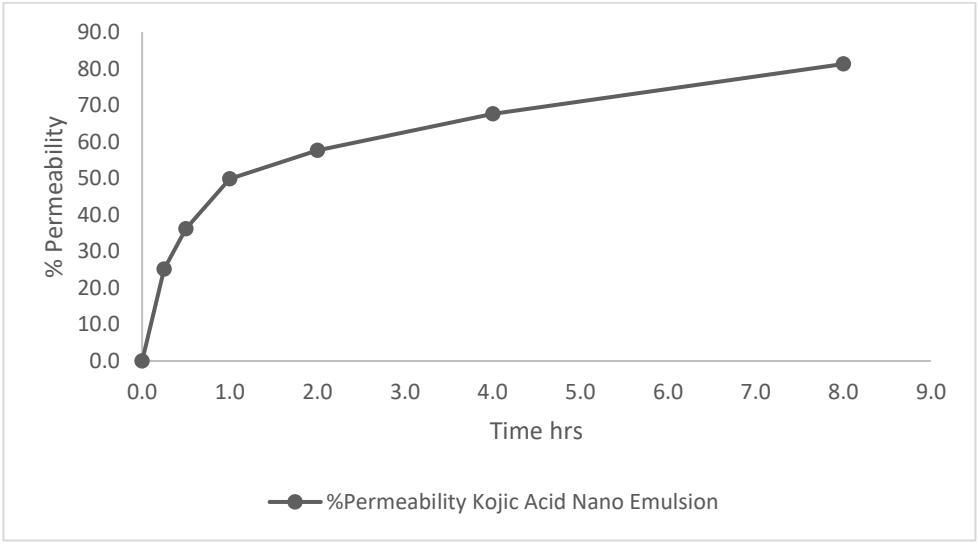

Figure 7: Drug permeation study of kojic acid nanoemulsion

Formulation Stability Study:
The novel system was evaluated for its appearance, microscopical properties, release, and pH for a period of 3 months. The results observed are mentioned below in Table 1& 2. Stability studies conducted as per ICH guidelines suggest that the optimized nano-emulsion was stable over a period of 3 months. In the 25°C storing temperature the

nanoemulsion was able to maintain the nanosized droplets, pH and drug release over time. The nanoemulsion was affected by Ostwald ripening during storage at 45°C where the droplet size became large. The pH and drug release nanoemulsion also decreased. The nanoemulsion displayed good stability and homogeneity against phase separation at 25℃ temperature during 3month of storage but at 40°C formulation showed slight yellowish color in the formulation.

Table 1: stability data of kojic acid loaded nanoemulsion at 25 °C/ 60 ± 5% RH

Parameters	**1 week sample**	**3month sample**
Average Particle diameter	180nm	184nm
Appearance	Smooth, shiny homogenous	Smooth, shiny homogenous
pH	5.5 ± 1.35	5.5 ± 1.28
Drug release	87.44 ± 2.01	83.12 ± 1.22

Table 2: stability data of kojic acid loaded nanoemulsion at 40 ºC / 75 ± 5% RH

Parameters	**1 week sample**	**3month sample**
Average Particle diameter	180nm	191nm
Appearance	Smooth, shiny homogenous	Smooth, slight yellowish homogenous
pH	5.5 ± 1.35	4.8 ± 1.28
Drug release	87.44 ± 2.01	78.12 ± 1.22

CONCLUSION

In this study, formulation kojic acid containing a nanoemulsion was successfully developed. The physicochemical properties (particle size 184nm, PDI 0.201, pH 5.62) and a stability study for 90 days at 2 different conditions of 25 °C and at 40 °C showed the physical properties for cosmeceutical applications. Incorporation of kojic acid in nanoemulsion provided an improvement in its penetrability. In-vitro release and ex-vivo permeation studies revealed that kojic acid nanoemulsion showed slow and progressive sustained release of kojic acid and sufficient skin retention thus, delivering drugs at an effective concentration. This research indicates that an optimized Kojic acid loaded O/W nanoemulsion is a promising approach to facilitate better penetration of the water-soluble skin lightening agents for topical delivery systems.

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ABBREVIATIONS

KA: kojic acid. ICH: International Council on Harmonisation

CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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