RESEARCH PAPER

Design, Development and Characterization of Nanostructured Lipid Carriers of Kojic Acid for Topical Drug Delivery to Treat Hyperpigmentation

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ABSTRACT

The aim of this study is to develop, characterize and evaluate Kojic acid loaded nanostructured lipid carriers (NLCs) for the treatment of hyperpigmentation like melasma through topical route in gel formulation. Many patients are varied about their hyper pigmentation problems nowadays and want to get rid of it as soon as possible. To increase the effectiveness of the kojic acid gel topically as compared to conventional gel formulations is the objective of the study. The NLC formulation was prepared by using stearic acid (solid lipid) and oleic acid (liquid lipid) along with phospholipon 90H and tween 80 as surfactants. The method used was melt dispersion ultrasonication technique. Characterization of KA-NLC was done for FTIR, drug entrapment efficiency and in vitro drug release. The NLC showed high entrapment efficiency near to 81-85%. The particle size was confirmed using SEM study. Nanostructured Lipid carrier-based gel containing kojic acid was formulated by using the gelling agent Carbopol 934. It has been observed that NLC gel produces the gel with good consistency, homogeneity, spreadability and rheological behaviour. The present study concluded that the NLC-based gel containing kojic acid dissolved in a mixture of solid lipid and liquid lipid in the nanoparticulate form helped us to attain the objective of faster onset yet prolonged action as evident from in vitro release profile.

Key words: Nanostructured Lipid Carrier, Hyperpigmentation, Ultrasonication, Phospholipon

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Introduction:

There is a rising demand for therapies to increase patient compliance, and so topical drug delivery which aims to topical or a systemic delivery are considered whenever possible. The prospective of delivering bioactive molecules all the way through the skin represents an interesting substitute to oral or parenteral preparations. This is so said that Topical drug delivery bypasses the gastrointestinal tract and thus prevents the first-pass effect and also allows self-administration. Moreover, in transdermal delivery drug delivery system the skin is main route which is preferred for the treatment of dermatological disorders and also local anesthesia. Topical drug delivery can potentially reduce the need of systemic administration of drugs, reduces the total drug dose requirement and thus reduces adverse drug effects. Therefore, topical delivery is more useful in the treatment of skin inflammation, photo aging, microbial and fungal infections and also skin cancer. [1]

The oral route is the mostly used conventional method of drug administration. Unfortunately, oral drug delivery systems have many major limits, such as drug degradation in the gastrointestinal track ⁅e.g. enzymes, pH⁆, first pass effect or toxic effects. The methods which can overcome these problems associated with the oral route are transdermal and topical drug delivery systems ⁅TDDS⁆. Use of drugs in the liquid, semisolid or solid form for treating skin diseases is being used from ancient times. Now a day's both topical and transdermal drug delivery systems are post preferred systems for drug targeting to a specific site. [2]

Nanotechnology is a modern and quickly developing trend in topical and transdermal drug delivery which includes numerous forms of nanocarriers such as Liposomes, Nanoemulsion, Nanocrystals, Polymeric Nanoparticles; Lipid based nanocarriers or nanoparticles and Dendrimers. Lipid nanocarriers show various advantages over conventional dosage forms because they are formulated with biodegradable, non-toxic and non-irritant lipids. The small size ⁅nearly 40 to 800 nm⁆ of lipid nanocarriers allows to attach them to the lipid film of stratum corneum

and to increase the number of drug molecules that penetrate into deeper layers of the skin. Beside this they show the occlusion effect which results in increased skin hydration by improving the absorption of the drug. [3]

Still there are different problems which are associated with drug stability even if given topically. stability problem, a new system was developed called as colloidal system from binary mixtures of lipids in which part of the solid lipid is replaced by a liquid lipid or a mixture of liquid lipids, giving rise to the Nanostructured Lipid Carriers (NLC). In this the solid state of the particle is maintained at room and body temperature. The advantages of the Nanostructured lipid carriers over the Solid lipid nanoparticles include a better drug loading capacity because it can be lodged a good quantity of active pharmaceutical ingredient in the imperfections of the particles which avoids the early expulsion of the active ingredient from preparation. Similarly, NLC offer greater stability because they do not allow the recrystallization of solid lipids. And the size remains practically unchanged during storage. [4]

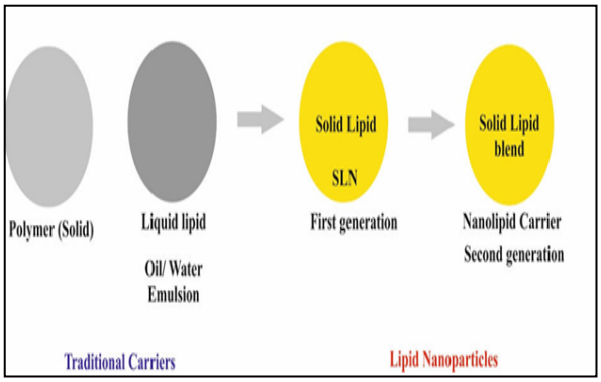

Figure 1: Nanostructured lipid carrier. [5]

Advantages of NLC's

- NLC's are biodegradability and shows greater drug protection.
- NLCs can be used for the extended release of the drug.
- NLCs are used for the entrapment of both hydrophobic as well as hydrophilic drugs.
- Due to the nano size of lipid particles, the drug penetration into the mucosa is increased.
- NLCs are considered as safest type of drug delivery carriers. [5]

Disadvantages of NLC's

- Cytotoxic effects are observed due to the nature of matrix and concentration.
- Some surfactants show irritative and sensitizing actions.
- Clinical and preclinical studies are not performed in the preparation of NLC's. [5]

Types of NLC's are summarized as follows:

A) Type I NLC's: This is considered as imperfect type. Substitution of a fraction of solid lipid by liquid lipid/oil causes formation of imperfect crystal lattice/matrix. This incident shows availability of more space for accommodation of drug and allows high drug loading. Formation of imperfect crystal core gives more space for drug incorporation, avoiding formation of highly structured or ordered matrix which would have excluded drug out of the core.

B) Type II NLC's: This is also known as amorphous/unstructured type. Here the solid lipids which remain in α polymorph after solidification and storage are used along with liquid lipids which tend to form amorphous core. This is favourable over type I NLC's as no crystallization occurs and drug remains embedded in amorphous matrix. The β polymorph of solid lipids builds up crystalline structured matrix.

C) Type III NLC's: This is multiple types and developed from the concept of w/o/w emulsion. It is basically oil-in-solid or fat-in-water type NLC, which can be developed only by phase separation technique. In order to improve drug loading capacity and stability of the drug the approach is used in formulation of NLCs where drug shows higher solubility in oil. Tiny droplets of oil are dispersed uniformly in solid lipid matrix and this system is dispersed in the aqueous medium. [7]

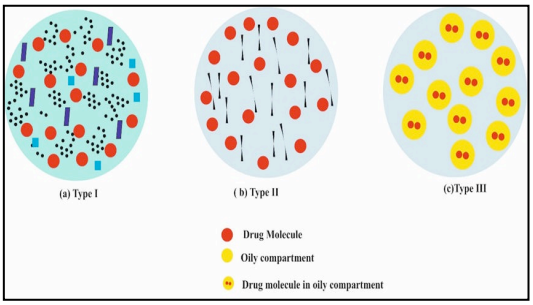

Figure: 2 Drug molecule, oily compartment and drug molecule in oily compartment model of NLC's [7]

Objective of Topical Preparation

Formulating an efficient and effective topical preparation that are directly act on the site of action and shows desired action these preparations may be used for; Tran epidermal water loss (TEWL) Bioactive penetration into the stratum corneum can be enhanced by occlusion caused by the product, which enhances hydration of the stratum corneum due to the inhibition of water evaporation. Application of NLC's based product on skin reduces water loss from skin compared to the untreated control. This is because the small sizes of particles in NLC's are having larger surface area, which give greater adhesion. They form a uniform packed layer on the skin surface, which prevent water evaporation from the skin.[8]

Increase of skin occlusion

The occlusion effect was reported for lipid nanoparticles. By using extremely small lipid particles, which are produced from highly crystalline and low melting point lipids, the highest occlusion will be reached. Particles smaller than 400 nm containing at least 35% lipid of high crystallinity have been most effective. Comparing NLC with different oil content showed that an increase in oil content leads to a decrease of the occlusive factor. Enhancement of skin permeation and drug targeting. The stratum corneum in healthy skin has typically a water content of 20% and provides relatively an effective barrier against percutaneous absorption of exogenous substances skin hydration after applying NLC leads to a decrease of corneocytes packing and an increase in the size of the corneocytes gaps. This will facilitate the percutaneous absorption and drug penetration to the deeper skin layers. [9]

Enhancement of ultraviolet (UV)blocking activity

Some side effects of organic UV blockers were reported due to the penetration of these compounds into the skin causing skin irritation and allergic reaction. This penetration can be reduced by incorporating these compounds in lipid nanoparticles; furthermore, a significant increase in sun protection factor (SPF) up to about 50 was reported after the encapsulation of titanium dioxide into NLC. Encapsulation of inorganic sunscreens into NLC is a potential approach to gain well tolerable sunscreens with high SPF. [10]

Modulation of drug release

The basic principles of drug release from lipid nanocarriers are explained below; release of drug is inversely proportional to the partition coefficient of the drug. As particle size of drug decreases the surface area increases which ultimately increases the drug release. Slow release of the drug could be accomplished when the drug is equally dispersed in the lipid matrix. Drug release from lipid nanocarriers occurs either by diffusion or by lipid particle degradation in the body. In some cases, there might be desirable controlled and fast release going beyond diffusion and degradation. Preferably, this release is triggered by an impulse when the particles are administered. NLCs accommodate the drug because of their highly unordered lipid structures. By applying the trigger impulse to the matrix to convert into a more ordered structure, such a desired burst drug release can be initiated. NLCs of certain structures can be triggered by applying the particles to the skin by incorporated it in cream. Increase in temperature and water evaporation leads to an increase in drug release rate. [10]

❖ Materials and Methods

- **Material**

Kojic acid, Stearic acid, Oleic acid, Phospholipon 90 H, Carbopol 940 P was purchased from Himedia and other reagents used were of analytical grade.

- **Methodology:**

1. Pre-formulation Studies

Pre-formulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rationale development of dosage forms. Pre-formulation studies

yield necessary knowledge to develop suitable formulation. It gives information needed to define nature of drug substance and provide a dosage form. [11]

a. Melting Point

The melting point of the pure drug was measured adopting the capillary test. The substance was contended in a modest quantity in a side sealed capillary tube attached to the thermometer at its mercury bulb. Thermometer was placed into the Thieles tube containing liquid paraffin in such a way that the upper, open end of the capillary tube remained above the oil layer. The side arm of the Thieles tube was then heated with a burner until the solid drug melts, and the melting temperature was measured. [11]

b. Saturation Solubility

The saturation solubility studies were performed according to method given by Higuchi and Connors in triplicate. The solubility was determined by dissolving excess quantity of drug in the 10 ml vials containing water. The vials were subjected to agitation on rotary shaker for 6 hrs and allowed to stand for equilibrations for 24hrs. The samples were filtered after 24hrs using Whatman filter paper, diluted with distilled water and analysed by UV Spectrophotometer at 268 nm. [12]

2. Ultrasonication method (NLC's Preparation)

Methanol-dissolved kojic acid and Phospholipon 90 H were combined with an acetone solution containing a mixture of stearic acid and oleic acid. The mixture was then homogenised at 15000 rpm and ultrasonically processed for 10 minutes at 70°C to create a pre-emulsion. The mixture was then added dropwise to Tween 80. To further avoid lipid crystallisation, this pre-emulsion was ultrasonically treated for 15 min. The resulting o/w emulsion was then cooled to room temperature while being constantly stirred, and the lipid was recrystallized to create a nanostructured lipid carrier (NLC). After being lyophilized, the resultant NLC dispersions were employed for additional characterisation research. [12]

3. Composition of NLC's of Kojic Acid:

Table 1: Composition of Nanostructured lipid carrier dispersion

Ingredients	F1	F2	F3
Kojic acid	40 mg	40 mg	40 mg
Oleic acid	50 mg	75 mg	100 mg
Stearic acid	200 mg	200 mg	200 mg
Phospholipon G	0.030 mg	0.030 mg	0.030 mg
Tween 80	2 ml	2 ml	2 ml

4. Characterization of Prepared Nanostructured lipid carrier (NLC'S)

a. Fourier Transforms-Infra Red (FT-IR) of Kojic Acid NLC's:

FT- IR spectrum studies assist to confirm the identity of the drug and to detect the interaction of the drug with polymers. FT- IR spectral measurement for pure kojic acid, lipids, phospholipon H, physical mixture and kojic acid loaded NLC dispersion were carried out in order to find out the incompatibility study. [12]

b. Particle Size , Drug content and Entrapment Efficiency:

A volume of 2 ml of each kojic acid loaded sample was centrifuged at 12500 rpm for 45 min to separate the lipid and aqueous phase. The supernatant was then diluted with methanol filtered through 40 µm filter paper and the drug content was determined by the UV- VIS spectrophotometer. The entrapment efficiency of NLC was calculated as

$$\% \text{ Entrapment Efficiency (EE)} = \frac{(Wka - Ws) \times 100}{Wka}$$

$$\text{Drug Loading (DL)} = \frac{(Wka-Ws)}{(Wka-Ws+Wl)} \times 100$$

Where EE is entrapment efficiency, DL is drug loading, Wka stands for the mass of kojic acid added to the formulation, and Ws - analysed weight of the drug in supernatant and W l - weight of lipid added. [13]

c. In vitro release study of Nanostructured lipid carrier (NLC)

The *in vitro* release studies were performed using Franz diffusion cell (FDC) to evaluate the kojic acid release profile from each formulation. Dialysis membrane was mounted on the Franz diffusion cell. Phosphate buffer of saline (PBS) pH 7.4 was used as the receptor medium (12 ml) being stirred at 700 rpm. NLC dispersion (equivalent

to 1 mg of kojic acid) was placed in the donor compartment. During the experiments the solution in the receptor side was maintained at 37 ± 0.5ºCat predetermined time intervals, 1 ml of the samples were withdrawn from the receiver compartment and replaced by the same volume of freshly prepared PBS (pH 7.4) to maintain the sink condition. The samples were analysed by the UV spectrophotometer at specific wavelength. [14]

d. Scanning Electron Microscope (SEM)

Scanner electron microscopy images of the Nano lipid carrier formulations F1's particle sizes were taken. Following are the steps for SEM sample preparation. The sample was moved to a glass slide that was 20 mm by 20 mm in size, and it was mounted on an aluminium stub using double-sided carbon tape. A drop of the solution was put to the glass slide, where it at room temperature slowly evaporated. The fully dried sample was coated with gold using an HITACHI evaporator and a sputter coating equipment at 10 Pascal vacuum for 10 seconds. The desired magnification was used to acquire the image in SEM mode.[15]

e. Stability Studies

The factors influencing the lipid nanoparticles' chemical and physical stability were assessed. According to ICH recommendations, the first condition was carried out for 6 months at 25°C, 2°C, and 60°C with 5 percent RH. Every month, measurements of the pH, viscosity, and medication release were made. [16]

5. Preparation and evaluation of kojic acid loaded NLC gel

Based on evaluation criteria such particle size, entrapment effectiveness, and in vitro release, the best NLC formulation for the topical delivery of kojic acid was chosen. It was discovered that out of the other formulations, F 1 is the most suited. As a gelling agent, Carbopol was dissolved in the NLC dispersion at a speed of 1200 rpm using a mechanical stirrer. Triethanolamine was used to neutralise the dispersion. To release trapped air, the gel was left to stand overnight.

6. Evaluation parameters of Nanostructured Lipid carrier topical gel

a. Consistency and clarity

The gel formulations were prepared and then examined visually with unaided eyes. We looked at the gel compositions' uniformity and clarity.

b. pH determination

A digital pH metre was used to determine the gel formulation's pH. Using distilled water, the gel compositions were diluted in a ratio of 1:25. The pH metre was calibrated using standard buffer solutions of pH 4, 7, and 10. To determine the mean pH value, the gel composition was tested three times. To allow the pH values to stabilise, the diluted gel was in contact with the pH electrode for 10 minutes. Between each sample, the electrode was carefully cleaned. [17]

c. Drug content uniformity

Gel samples were precisely weighed in a fixed amount (40 mg) into a 10 ml volumetric flask. The sample was subjected to sonication for roughly 10 minutes to accomplish complete drug extraction after the appropriate dilution with phosphate buffered saline of pH 7.4. The stock solutions were then filtered and diluted further after being prepared up to 10 ml in volume. In a UV-visible spectrophotometer, the solutions' absorbance was measured at a given wavelength. [18]

d. Rheology

The Brook field LDV prime I viscometer model was used to calculate viscosity. The dial reading was recorded at 100 rpm for 60 seconds with spindle number CP 52 at a temperature of 30º C while the gel sample was obtained in a beaker. The gel's viscosity was measured and tabulated. [18]

e. Spreadability

In terms of patient compliance, spreadability is one of the crucial properties for topical formulation. It was calculated by sandwiching one gramme of gel between the two glass slides that held the weights. A 100 gramme weight was pulled on the top slide. The spreadability of the gel is related to the time in seconds needed for the top slide to move 100 cm. [19]

f. Permeability study

The permeation and percentage release studies of KA loaded NLC gel were carried out beyond cellulose acetate using Franz diffusion cells and examine using UV-visible spectrophotometer (Shimadzu 1650PC, Japan) at lmax ¼ 306 nm. The Franz diffusion cells contain two important section that were the donor and receptor medium where a cellulose acetate laminate (13 mm) was deposited in between them. Both divisions were secure tightly to escape leaking of the sample. The region during the diffusion of gel through the media was 0.64 cm2. The kojic

acid loaded NLC gel was placed on top of the donor's division although the receptor division accommodate phosphate-buffered saline (PBS) (5 mL) among pH 7.4 was maintained at 37℃ and stirred at speed 600 rpm. Every hour, 3 mL was taken out from the receptor division and further 3 mL of PBS was replaced towards the equal division. The total volume of 5 mL PBS was maintained inner side the receptor division between the process. A total of nine samples were composed at time t ¼ 0, 1, 2, 3, 4, 5, 6, 7 and 8 h. The 8 h confirmation manifest kojic acid as a 30% w/v gel among 0.5% methylcellulose required to be applied 2 to 4 times daily (or around 4 to 8 h) during topical application. The method was performed in triplicate and samples were examine using a UV-visible spectrophotometer among respect to the percentage of cumulative kojic acid ester disclosed in the receptor division. [20]

g. Assay

Apparatus

The chromatographic system composed of a Constametric II pump, an ISS-100 autosampler, an LC3 adaptable wavelength detector set at 244 nm and 1.28 a.u.f.s. and a column oven or column block heater to manage the analytical column at 35°C. Injections of 20 were assembled towards the system and chromatograms were estimate by manual peak-height measurements or choice, by data system. Detachment was carried out using a 125 x 5 mm i.d. column, slurry packed among Zorbax NH* phase; a 300 x 5 mm i.d. precolumn packed with Porasil was used through the pump and autosampler to preserve the analytical column. The mobile phase flow-rate was 1.0 ml min. [22]

Chemicals and reagents

l-Chlorobutane was HPLC grade, tetrahydrofuran was equilibrated Laboratory Reagent grade or HPLC grade and all additional solvents were Laboratory Reagent grade. Triphenylamine was at least 98% pure. The mobile phase was l-Chlorobutane-tetrahydrofuran-glacial acetic acid-methanol (97.4:2.0:0.5:0.1, v/v). The acetylating mixture was newly formulated (daily) pyridine-acetic anhydride (1:1, v/v). An inner standard, used to assistance assess, was attached earlier to derivatisation. The inner standard solution was about 5 mg ml-’ solution of triphenylamine in tetrahydrofuran. (The emergence of additional small, yet non-impede, peaks usually discern in the final chromatogram can be reduced or avoided by the use of HPLC grade tetrahydrofuran and high-quality pyridine and acetic anhydride.) [23]

Standard procedure

Approximately 30 mg of (I) of defined dominance was correctly weighed towards a 100 ml volumetric flask, dissolved and diluted to volume among tetrahydrofuran and mixed well. Towards an appropriate vial were moved 2 ml of solution, 2 ml of internal standard solution and 3 ml of acetylating mixture. The vial was sealed using a cap except a metal liner and the contents were mixed assorted and heated in a water-bath at 60°C for 25-30 min. The solution was cooled to room temperature and then 1 ml was transferred to additional vial and evaporated to dryness below a fume hood using a condensation of dry nitrogen till the smell of pyridine or acetic anhydride was absent. The residue was dissolved in 5 ml of 1-chlorobutane and assorted well. [24]

Sample procedure

Around 1 g of gel was precisely weighed towards a 100-ml volumetric flask around 30 ml of tetrahydrofuran was attached and the mixture deposited in an ultrasonic bath till absolute solution was achieved. For the standard solution, the solution was diluted to volume among tetrahydrofuran, assorted well and then treated. [24]

❖ Result and Discussion

1) Pre-formulation study of Kojic acid

a. Melting Point

The melting point of the pure drug was measured adopting the capillary test. The substance was contended in a modest quantity in a side sealed capillary tube attached to the thermometer at its mercury bulb. Thermometer was placed into the Thieles tube containing liquid paraffin in such a way that the upper, open end of the capillary tube remained above the oil layer. The side arm of the Thieles tube was then heated with a burner until the solid drug melts, and the melting temperature was measured. The observed results are shown in the table.

Table:2 Melting Point of Kojic Acid

Name	Standard	Obtained

Kojic acid	152 ^{0}C	150 ^{0}C

b. Solubility

The saturation solubility of Kojic acid was determined according to the method given by Higuchi and Conners in distilled water. The solubility of Kojic acid was 0.097±0.032mg/ml indicating the poor aqueous solubility of drug. The results are similar to the previously reported.

c. Maximum Absorption (λ-max) and Calibration Curve of Kojic Acid:

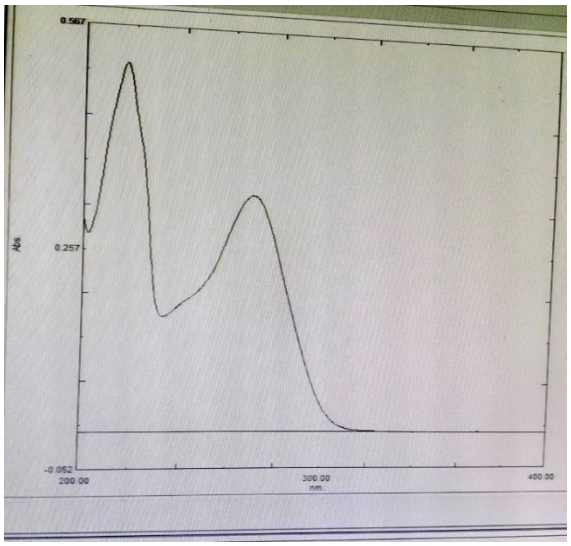

Fig.3: UV spectra of Kojic acid

Table:3 Maximum Absorption (λ-max) of Kojic Acid

Name of Drug	Solvent	Reported	Observed
Kojic acid	Methanol	270 nm	268 nm

Calibration Curve:

An aliquot of standard stock solution was further diluted with methanol to get solutions of concentration within range 5-45 µg/ml. The absorbance was measured at 282 nm against methanol as blank. All measurements were repeated three times for each concentration.

Table:4 Calibration Curve Absorbance of Kojic Acid

Sr. no	**Concentration**	**Absorbance**
1	5	0.073
2	10	0.195
3	15	0.276
4	30	0.561
5	45	0.834

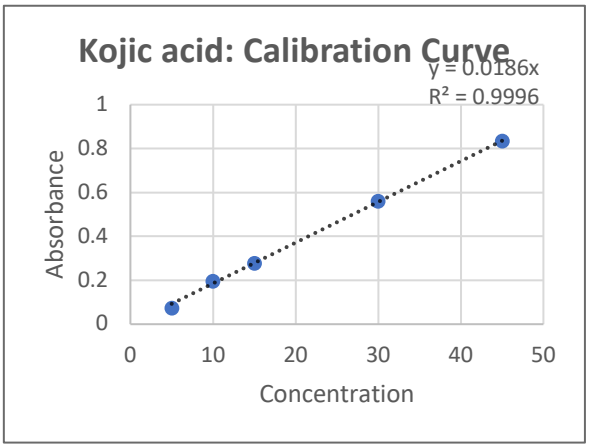

Fig.4: Calibration curve of Kojic acid

d. FTIR spectra

FTIR spectrum of Kojic acid was shown in following Fig.5, revealed characteristic peaks representing the presence of functional groups claim by its chemical structure. From this we can consider that the Kojic acid was of pure quality.

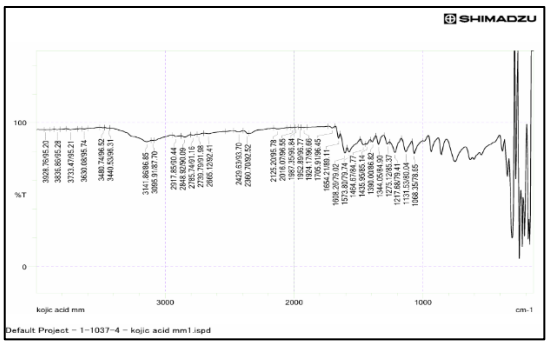

Fig.5: FTIR spectra of kojic acid

After interpretation of FT-IR Spectrum of kojic acid, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of kojic acid were found within the reference range and confirming its identity.

e. DSC of Kojic Acid Drug:

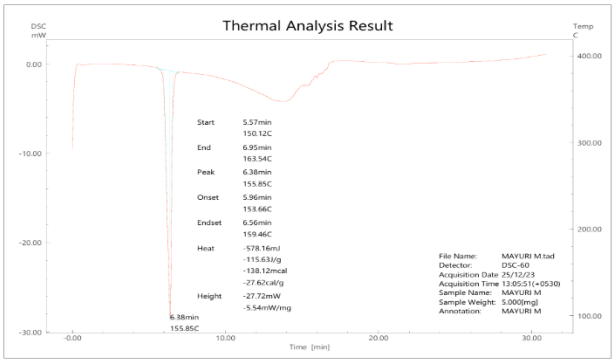

Fig.6: DSC of kojic acid

4. Characterization of Prepared Nanostructured lipid carrier (NLC'S)

a. Fourier Transforms-Infra Red (FT-IR) of Kojic Acid NLC's:

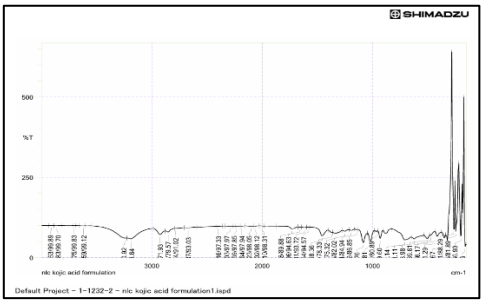

Fig.7: FTIR spectra of kojic acid

b. Particle Size, Drug Content and Entrapment Efficiency

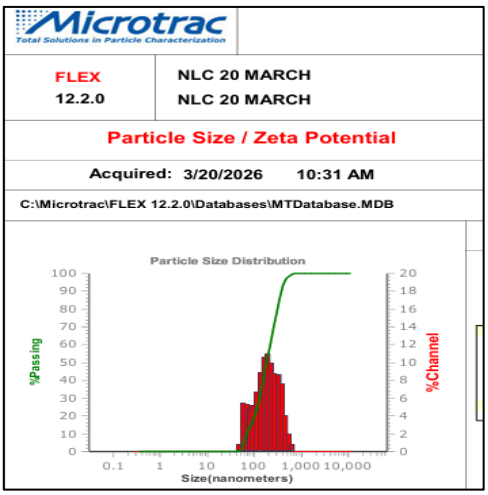

Fig.8: Particle Size (Zeta Sizer) of kojic acid

Table 5: Shows the mean particle size, drug content and entrapment efficiency

Batches	Mean particle size	Drug content	% Entrapment Efficiency
F1	205.7 ± 2.59	16.87 ± 1.43	85.76
F2	287.76 ± 4.98	18.65 ± 1.23	83.09
F3	192.12 ± 3.87	20.45 ± 1.67	81.12

5. Evaluation of NLC based Gel of Kojic Acid:

- **pH, drug content, viscosity and spreadability:**

Table 6: Shows the parameters of Kojic acid loaded NLC gel and observation

Parameters	Observation
Homogeneity	Good
pH	4.88 ± 0.65
Drug content	91.55%
Viscosity	7988cP
Spreadability	21.98 gm cm/sec

- **In-Vivo drug release study:**

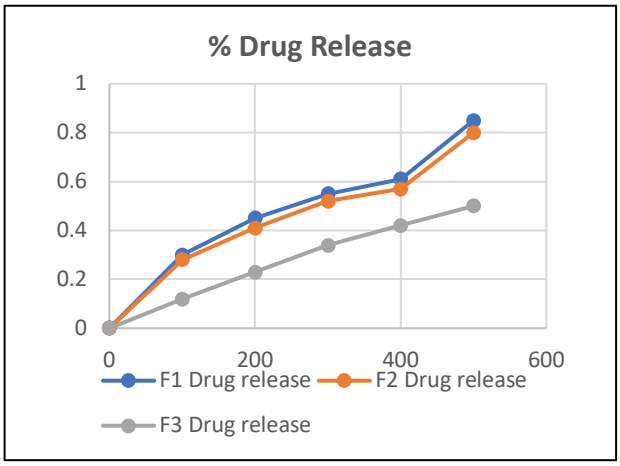

Fig. 9: % Cumulative drug release studies of all formulation F1-F3

- **SEM results for NLC's:**

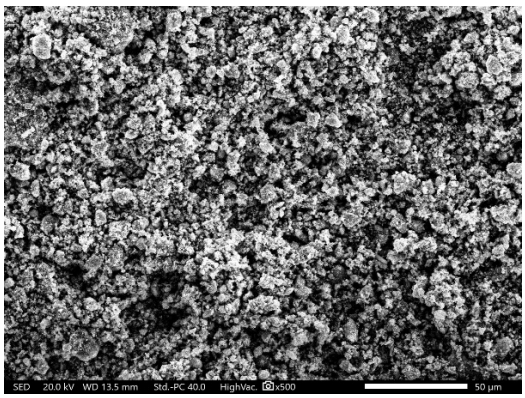

Figure 10: SEM images of NLC formulations. Shows the spherical in shape.

- **Drug Entrapment Efficiency:**

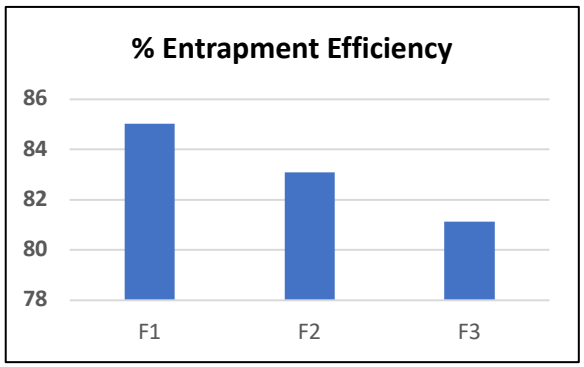

Figure 11: Entrapment Efficiency of all three formulations.

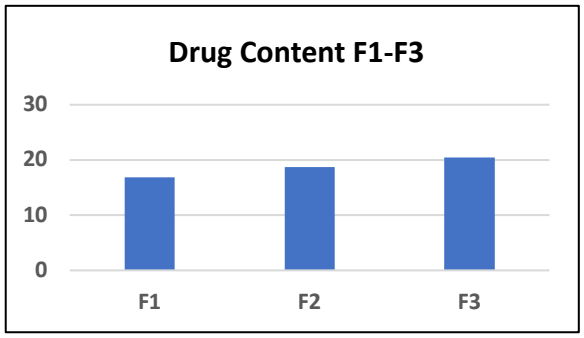

Figure 12: Drug content of all three formulations

Permeability study

Permeation studies goal is to attain superior skin penetration by estimating the relationship through the skin, active ingredient and formulation. In this study, cellulose acetate laminate was used in Franz cell diffusion to excite the skin alternatively animal skin or human cadaver skin to carry out the in -vitro permeation study. The results were used to find the concentration of kojic acid ester in the receptor cells of the Franz diffusion cell and determined the quantity of kojic acid ester transferred between the cellulose acetate laminate (in fig.13) indicate the percentage of cumulative permeation of KAS. The permeability of the kojic acid ester was notably enhanced and the liberation of the active matter enhanced from 4.94% at 1 h to 59.64% at 8 h. Based on the release study, it appears that kojic acid ester released rapidly specifically at the first 4 h and this is because of the condense droplet size of the gel that has an elevated surface region, that's why assemble the commencing release rapid. Fig. 14 illustrate a permeation outline of KA gel. The permeation rate and permeation coefficient, Kp are manifest in Table 6. Kojic acid ester based-gel indicate the elevated permeation rate of kojic acid ester at 4659.50 mg cm2 h^{-1} among Kp value of 0.48 cm h^{-1}. With the exception of the small droplet size of suspension, the surfactant used in the system was accomplished to accommodation among the barricade function of the cellulose acetate laminate consequently, promote the transit of kojic acid ester. [26,27]

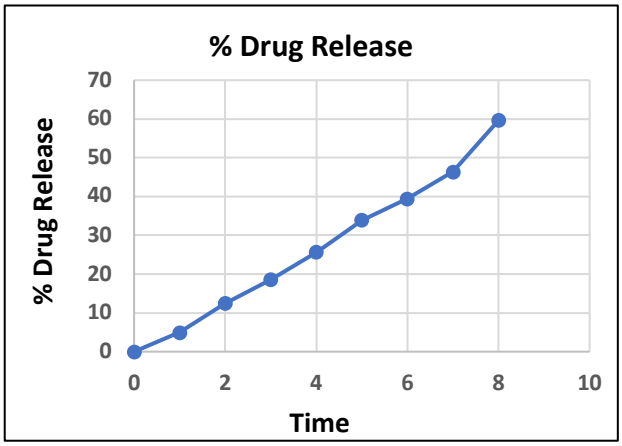

Fig. 13: In-vitro permeation study for kojic acid-based gel

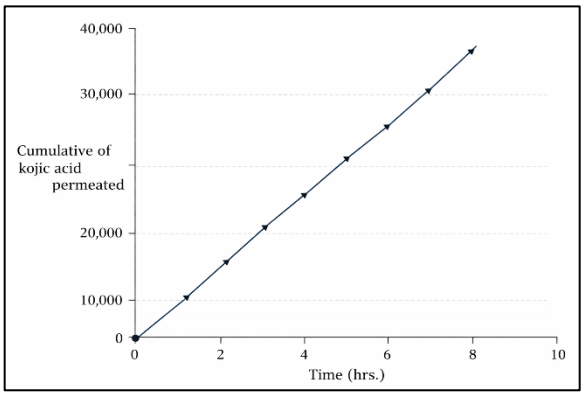

Fig. 14: Cumulative kojic acid ester permeation from gel between cellulose acetate membrane

Table 7: The kojic acid-based gel permeation parameters

Sample	Flux at 8 hrs J, ug cm^{-2} h^{-1}	Permeated amount at 8 hrs. (%)	Permeation coefficient K_p (cm h^{-1})
Kojic acid-based formulation	4783.43	63.01	0.45

Table 8: The regression coefficient of the five different kinetic models for kojic acid-based gel

Kinetic model	Regression coefficient
Zero order	0.9423
First order	0.8198

Higuchi	0.9276
Hixson-Crowell	0.8965
Korsmeyer-Peppas	0.9899

Chromatography

Identical 20 injections of all standard and sample solutions were assembled and averaged information was used to determined outcomes. Peak heights for (I) and internal standard were persistent and the peak-height proportion Q/internal standard determined. Preference a data system assessing peak heights or region was deposit to carry out the equal function. Sample assays were determined in the accepted approach.

In Fig. 15 a distinctive chromatogram is manifest. Linearity of reaction was characterized using four standard solutions above the range 100-250% of anticipated concentration and manifest to be sustainable by an association coefficient (r) of substantial than 0.999. Improvement of (I) from gel blanks, spiked at 100, 120 and 140% of theory, were in the range 98-104% among mean improvement of 102.4% during the gel. No impede peaks were discern in preparation blanks or from sample solvent. The replicability of the process was estimated by three dissimilar interpreter (in two dissimilar laboratories) who every assayed three weights of the same gel batches utilizing dissimilar apparatus on several occasions. Gel assays a RSD of 2.4%. Mean assays were 2.94% for gel, subsequently, contrast upon a label claim of 3 %. Ultimate assay solutions were manifest to be steady for at least four days when stored in the dark, however disclosure in a light cabinet decomposed the acetate derivative of (I). For one day an accelerated degradation study was carry out, dominating (I) to heat (lOS'C), light (in a light cabinet) and 1 M sodium hydroxide or 1 M hydrochloric acid. The consequent samples (behind neutralisation during the last two provocation) were assayed by the beyond approach. Narrowly the alkali therapy gave remarkable breakdown however no additional peaks were discerned. The process has, although, disclosed light degradation of (I) acetate as various additional peaks escape through the two want peaks on the chromatogram and is acceptable during safety observing. The method was additionally appeal to gel samples stored at room temperature. The outcomes differentiate encouraging among the label claim of 4.00% (m/m) and are specified. It was initiate imperative to permit a 20-min inspection time during gel to permit elution of formulation excipient(s). The approach has been manifesting to be exact, correct and adequately uneven during routine use, however recently narrowly restricted information for various batches have been assembled. [28,29]

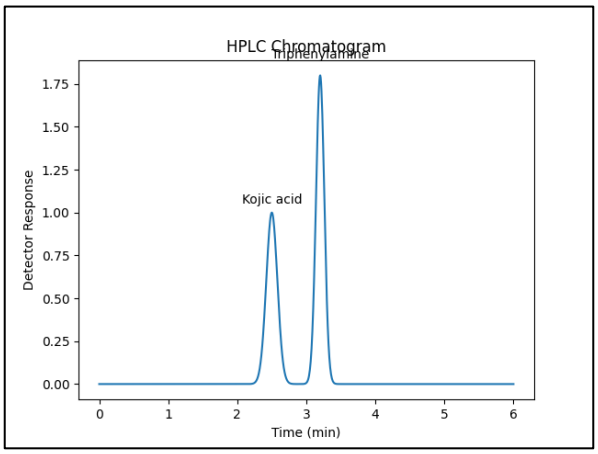

Fig.15: Chromatogram of Kojic acid

Summary and Conclusion

The Kojic acid loaded NLCs were formulated using different concentrations of solid lipid and liquid lipid by the melt dispersion ultrasonication technique as shown in Table 1. Out of three formulations (F1) seems to exhibit good physical stability indicated by high entrapment efficiency value as shown in the Figure-11 and Table 5. The drug release profile from the F1 displayed a biphasic drug release pattern with burst release at the initial stage followed by sustained release as shown in the Figure-13. These results indicated that the F1 is a suitable carrier of kojic acid with improved drug loading capacity and sustained drug release properties. The Scanning Electron microscope (SEM) shows the particles of the F1 formulations were round and spherical in shape as shown in Figure-10. Therefore, F1 was selected and the nanostructured based gel containing kojic acid was formulated by using the gelling agent Carbopol 934. The drug content of the gel was clearly shown the Figure-12. It has been

observed that NLC gel produces the gel with good consistency, homogeneity, spreadability and rheological behavior as shown the Table 5. It was found that NLC gel showed a biphasic release pattern and provided a fast release initially for skin saturation followed by a slow and prolonged release profile to maintain the skin concentration. The present study concluded that the NLC-based gel containing kojic acid dissolved in a mixture of solid lipid and liquid lipid in the nanoparticulate form helped us to attain the objective of faster onset yet prolonged action as evident from *in vitro* release profile.

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