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Research Article

Formulation Development and Evaluation of Face Serum containing Kojic acid dipalmitate

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Abstract:
This study aimed to develop and evaluate a face serum formulated with kojic acid dipalmitate to address hyperpigmentation and enhance skin brightness. Kojic acid dipalmitate was selected for its potent skin-lightening properties and superior stability compared to kojic acid. The formulation process involved creating a stable macroemulsion, which was then integrated into a serum base. Comprehensive evaluations were conducted, focusing on various parameters such as pH, viscosity, globule size, zeta potential, spreadability, antioxidant activity, entrapment efficiency, drug content, and in vitro release profile. The development process aimed to achieve a balance of physicochemical properties to ensure the serum's effectiveness and user-friendliness. Key attributes like pH and viscosity were meticulously controlled to maintain compatibility with skin application. The globule size and zeta potential were optimized to ensure stability and proper dispersion of the active ingredient. Spreadability tests confirmed ease of application, while antioxidant activity assessments determined the serum's potential to protect skin from oxidative damage. The serum exhibited promising physico chemical characteristics and stability, demonstrating significant potential for reducing hyperpigmentation. The sustained release properties ensured prolonged efficacy, and stability studies under various storage conditions validated the formulation's robustness. Overall, the face serum containing kojic acid dipalmitate showed potential as an effective skin-lightening agent in cosmetic skincare products, making it a valuable addition to formulations aimed at improving skin tone and reducing hyperpigmentation.

Keywords: Kojic acid dipalmitate, Face serum, hyperpigmentation, skin brightening, macroemulsion, Formulation development

INTRODUCTION:

TRANSDERMAL DRUG DELIVERY SYSTEM

Transdermal drug delivery involves the application of a drug-containing patch to the skin to deliver the drug into the bloodstream at a controlled rate. This method provides systemic effects, differentiating it from topical drug delivery. TDDS offers several advantages, including increased patient compliance and avoidance of first-pass hepatic metabolism. The development of scopolamine patches in the 1980s marked the beginning of its commercial use. Current developments include treatments for conditions such as Parkinson's disease and ADHD, using advanced technologies like liposomes and microemulsions to enhance drug delivery [1].

Advantages of TDDS:

- Avoids first-pass metabolism.
- Provides convenience and ease of application.
- Reduces drug level fluctuation.
- Allows easy medication termination.
- Offers selective drug delivery.
- Avoids gastrointestinal incompatibility.
- Effective for drugs with short half-life and narrow therapeutic window [1].

Disadvantages of TDDS:

- Potential skin irritation and allergic reactions.
- Poor skin drug absorption.
- Limited to drugs with low plasma concentrations.
- Difficulty absorbing larger particles [2,3].

Anatomy and physiology of skin

The skin, covering about $2m^2$ in an adult body, plays a crucial role in topical drug delivery. It contains numerous hair follicles and sweat glands, with a pH ranging from 4 to 6. Understanding the skin's structure is essential for developing effective topical formulations [4].

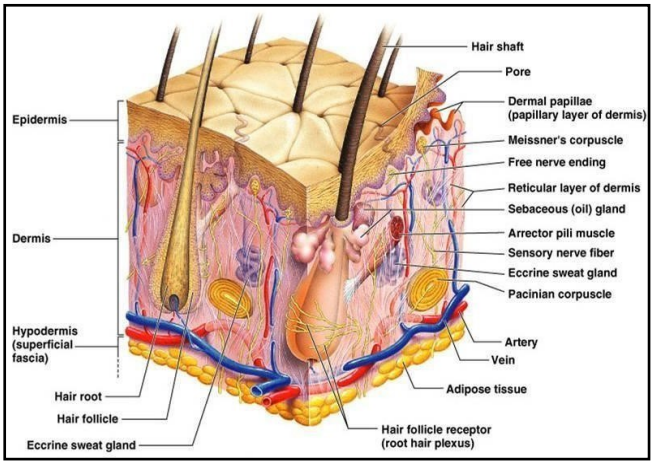


Fig 1: Structure of Skin

Skin colour:

Melanin, a pigment produced by melanocyte cells in the basal layer, determines skin and hair colour. It can be yellow, red, or black, and exposure to the sun increases melanin formation, protecting the skin from the sun [5].

Hyperpigmentation:

Hyperpigmentation is a common skin disorder characterized by darker patches due to overproduction of melanin, a pigment produced by melanocytes [5].

EMULSION

Emulsions are two-phase preparations with one uniformly stacked on top, requiring a physical stabilizing mechanism in pharmaceutical emulsions. This mechanism may consist of polymers, surfactants, or a combination [6].

Emulsions are categorized into two forms: oil-in-water (o/w) and water-in-oil (w/o), and various types are determined by droplet size or distribution technique [7].

A. Macroemulsion:

Macroemulsions, with droplet particles larger than 400nm, are visually opaque but visible under a microscope. Surface-active substances stabilize these thermodynamically unstable emulsions [8].

B. Microemulsion:

Microemulsions, created by combining oil and water with a suitable surfactant, are thermodynamically stable isotropic systems due to their single phase [9].

C. Nanoemulsion:

A Nanoemulsion is a transparent, stable dispersion of two immiscible liquids, maintained by surfactant molecules, with a droplet size less than 100 nm [10].

Skin brightening and skin rejuvenation:

Skin brightening involves inhibiting melanin production, which protects the skin from UV rays. The global market for skin brightening products is growing rapidly, with hyperpigmentation and melasma spots common. Over time, pigmented cells are shed off, resulting in a lighter complexion. A quick method involves deep cleaning, cutin removal, and using skin brightening and nourishing products. Skin moisturizing is crucial for skin whitening, as active ingredients in skin brightening products cannot penetrate cutins. Skin rejuvenation is a multifaceted approach to revitalize and restore skin's appearance, texture, and overall health. Techniques include stimulating collagen production, reducing pigmentation, smoothing wrinkles, and improving skin texture, aiming to promote a more youthful, radiant complexion [5].

Mechanism of skin brightening and rejuvenation:

Skin rejuvenation techniques stimulate collagen production, enhancing skin tone, reducing pigmentation, and improving texture by using lightening agents like kojic acid, vitamin C, and retinol.

Serum:

A cosmetic serum is a specialized skincare product with a lightweight texture and concentrated formulation, designed to address specific skin concerns. It can be water-based or oil-based and contains high concentrations of active ingredients, ensuring deep penetration into the skin. It is essential to apply after cleansing and toning to prepare the skin for the serum's effects, and a moisturizer provides additional hydration.

Serum Effects:

Serums are concentrated solutions that absorb active ingredients in the skin, providing immediate cosmetic effects and psychological satisfaction. They function similarly to moisturizers, rejuvenators, and lifting agents but with faster results when used correctly. Serums are categorized based on their effects, such as lifting, revitalizing, moisturizing, nourishing, anti-inflammatory, and anti-stress. They are suitable for all ages and come in various types, ranging from transparent to semi-transparent, viscous liquids [5].

Different Types of Serums and their Features

Table 1: Types of serum and their features

Types	Technology	Features
Transparent Or semi - transparent Lotion type.	Solubilization, Micro emulation, Liposomes, Disc like capsule.	In general, it includes more humectants than lotion. The texture may be altered by selecting a humectant and a water-soluble polymer and varying their combinations. This is the most common type of serum preparation.
Emulsion type	O/W type W/O type W/O/W type	As this type contains a large amount of emollient, it is ideal for formulations that include substantial quantities of UV absorbers and oily ingredients. The water-in-oil (w/o)

		type is particularly suitable for preparations that need to be water-repellent.
Oil type		In this type, the texture is adjusted using solid or semi-solid oils, as well as animal fats or plant oils in varying proportions. However, because the texture is not as favourable as other formulations, this type is becoming less common in the market.
Two agents mix together type	In addition to above, spray dry, freeze-dry, Microcapsule technology is used.	To prevent instability in pharmaceutical agents and preparations, or to avoid visual changes, two agents are often combined in either liquid-liquid or liquid-powder formulations.
Others	Lotion with powder type much alcohol type.	Serum for the T-zone, which secretes a lot of sebum, includes powder that absorbs sebum, boosts the longevity of makeup and has a germicidal impact that helps prepare acne.

Materials and Methods:

Materials

Kojic acid dipalmitate was gift sample from Cummins Pharmachem Pvt. Ltd. of Ahmedabad. Licorice Extract were purchased from aethon international, Mumbai. Isopropyl Myristate (Loba Chemie Pvt. Ltd, Mumbai.) Carbopol 940 (Research- Lab Fine Chem Industry,

Mumbai), Tween 80 (Research- Lab Fine Chem Industry, Mumbai), Glycerine (Thomas Baker, Mumbai), Vitamin E (Healing Pharma India Pvt Ltd), Olive oil (Thomas Baker, Mumbai), Disodium EDTA (Thomas Baker, Mumbai), Sodium Benzoate (Thomas Baker, Mumbai) Rose oil Research- (Lab Fine Chem Industry, Mumbai) all of the experiments used double distilled water. All other chemicals used in the formulation of serum were procured and used in this investigation.

Methods

The formulation design was done using Design Expert e stat software version (7.0)

Design of experiment (DoE)

For the present work 3^2 full factorial designs was selected. In this design ,2 Factors were evaluated each at 3 levels and experimental trials were performed at all 9 possible combinations. The two independent variables selected were Carbopol 940(X1) and Speed of homogenizer (X2). And their interaction on the response i.e. Viscosity (Y1), Globule size (Y2).

Statistical analysis: An ANOVA (Design Expert Version 07) was used to compare results. The ANOVA test was used to compare the stability results. Data shown, a significant difference of at least 0.05 was taken into consideration.

Optimization Study: All experiments were performed in triplicates. Every data point is expressed as a mean ± standard deviation (SD), and an ANOVA was used to compare the groups; a value of p.

Table 2: Experimental Design as per 3^2 Full Factorial Designs

Formulation Code	Coded values			
	X1	**%**	**X2**	**RPM**
F1	-1	0.5	0	5500
F2	+1	1	+1	8000
F3	+1	1	0	5500
F4	0	0.75	-1	3000
F5	+1	1	-1	3000
F6	-1	0.5	+1	8000
F7	0	0.75	+1	8000

F8	0	0.75	0	5500
F9	-1	0.5	-1	3000

Preformulation study:

Preformulation studies were conducted to determine the solubility and compatibility of kojic acid dipalmitate with the selected excipients. Solubility analysis involved determining the solubility of kojic acid dipalmitate in various solvents. Compatibility studies were performed using Fourier Transform Infrared (FTIR) spectroscopy to assess any potential interactions between kojic acid dipalmitate and the excipient [11,12,13,14].

Drug Characterization:

A small quantity of drug powder was taken on butter paper and observed in well illuminated place.

- Colour: A little amount of kojic acid dipalmitate was taken in butter paper and examined under well lighted area.
- Odour: Small amount of kojic acid dipalmitate sample was smelled to get the odour.
- Appearance: A pinch of kojic acid dipalmitate was taken between two fingers and appearance was observed.

Determination of melting point:

The melting point of kojic acid dipamitate was determined using an open capillary technique, using Econazole delivered through a flame-sealed glass capillary.

UV -Visible spectrophotometric analysis:

The UV spectrum of Kojic Acid Dipalmitate was obtained using a Shimadzu 1800 spectrophotometer, with a calibration curve prepared by analysing stock solutions with varying strengths.

FT-IR of Kojic acid dipalmitate:

The FTIR spectrum of Kojic acid dipalmitate was obtained using a Fourier transform infrared spectrophotometer in the 4000 to 400 cm^{-1} wave number range. The analysis was conducted using the ATR method, which allows direct measurement of the powder sample using a high-refractive-index prism [14,15,16].

Drug Excipient compatibility study:

Compatibility study of drug, excipient, and polymers was done by preparing physical mixture of drug and excipients, polymer in ratio of 1:1 was placed in vials for one month at 40º C after 1 month the physical mixture was checked for gas formation, colour change and liquefaction [17].

Table no. 3: Drug Excipient compatibility

Sr. No.	Sample	Ratio
1	Kojic acid dipalmitate: Isopropyl myristate	1:1
2	Kojic acid dipalmitate: Tween 80	1:1
3	Kojic acid dipalmitate: Carbopol 940	1:1
4	Kojic acid dipalmitate: Olive oil	1:1
5	Kojic acid dipalmitate: Glycerine	1:1

Formulation of Face serum by using Mechanical homogenisation method:

Phase I: Initially, kojic acid dipalmitate was dissolved in isopropyl myristate. Carbopol was dispersed in 80% of the water along with disodium EDTA and left to hydrate overnight. Triethanolamine and sodium benzoate were then added to achieve the desired consistency. The mixture of kojic acid dipalmitate in isopropyl myristate was incorporated and stirred at 500 rpm using a magnetic stirrer.
Phase II: In this phase, measured quantities of Tween 80, olive oil, and perfume were added.
Phase III: Vitamin E, glycerine, and liquorice extract were combined. Phase III was first added

to Phase I, followed by the addition of Phase II. The entire process was conducted on a magnetic stirrer at 500 rpm. To ensure proper mixing and a stable emulsion, a homogenizer was used, with the rotation speed adjusted according to the batch requirements.

Table no. 4: Batch Wise Formulation

Sr. No.	Name of Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	**Kojic acid Dipalmitate**	0.6 gm	0.6 gm	0.6 gm	0.6 gm	0.6 gm	0.6 gm	0.6 gm	0.6 gm	0.6 gm
2.	**Isopropyl Myristate**	1.5ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml
3.	**Liquorice extract**	0.9ml	0.9m l	0.9 ml	0.9m l	0.9m l	0.9m l	0.9m l	0.9m l	0.9m l
4.	**Carbopol 940**	0.3gm	0.2g m	0.3g m	0.3g m	0.2g m	0.1g m	0.1g m	0.3g m	0.3g m
5.	**Tween 80**	0.72 ml	0.72 ml	0.72 ml	0.72 ml	0.72 ml	0.72 ml	0.72 ml	0.72 ml	0.72 ml
6.	**Olive oil**	0.9ml	0.9m l	0.9 ml	0.9m l	0.9m l	0.9m l	0.9m l	0.9m l	0.9m l
7.	**Vitamin E**	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
8.	**Glycerin**	1.5ml	1.5m l	1.5 ml	1.5m l	1.5m l	1.5m l	1.5m l	1.5m l	1.5m l
9.	**Disodium EDTA**	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm
10.	**Triethanolamine (TEA)**	0.003 ml	0.00 3 ml	0.00 3 ml	0.00 3 ml	0.00 3 ml	0.00 3 ml	0.00 3 ml	0.00 3 ml	0.00 3 ml
11.	**Sodium benzoate**	0.06 gm	0.06 gm	0.06 gm	0.06 gm	0.06 gm	0.06 gm	0.06 gm	0.06 gm	0.06 gm
12.	**Rose oil (Perfume)**	0.1ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1m l	0.1m l	0.1m l	0.1m l

13.	**Purified Water**	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml	q.s upto 30ml

Evaluation study of Face serum:

Physical Evaluation:

Visual observations were utilized to evaluate the physical characteristics of the manufactured solid dispersion, including colour, odour, and appearance [18].

After Feel:

The serum provides a moisturizing, soothing, and cooling effect to the skin, leaving no residue due to its faster absorption rate [19,20].

pH of serum:

pH of formulations was measured using a calibrated digital pH meter, recording values in triplicate and calculating the average mean to determine the pH range [21].

Determination of viscosity:

The viscosity of formulated batches was measured using a Brookfield Viscometer. The formulation was soaked in a beaker for 30 minutes at 254°C, then the spindle was lowered into the centre and rotated at 60 rpm for 10 minutes. The viscosity reading was recorded [18,19].

Globule size determination and PDI:

The formulation's droplet size distribution was analysed using a Malvern size analyser, and dynamic light scattering measurements were taken at 25°C with a 90° scattering angle. The sample was diluted with distilled water, filtered, and analysed for droplet size and polydispersity index [18,19].

Zeta potential measurement:

Zeta potential is a crucial indicator of formulation stability, indicating electronic repulsion between particles. The optimized batch's zeta potential was assessed using a zetasizer. A 1ml sample was dispersed in double-distilled water, ultrasonicated for 5 minutes, and then measured for size and zeta potential [22].

Refractive index:

The refractive index is a crucial characteristic of a substance that determines how light passes through it. It is essential in understanding how light interacts with cosmetic serums and affects skin appearance. To measure the refractive index, take a small amount of serum, remove air bubbles, and place a few drops onto the prism of the refractometer. Adjust the focus until the "critical angle" is clearly visible. The refractive index value on the scale represents the serum sample's refractive index.

Redispersion test:

The microcentrifugation method was used to conduct a redispersion test on formulations, which was then agitated and observed for redispersion, indicating the formulation's quality.

Spreadability test:

The slides were placed on a platform, with the lower slide securely held by clamps, and the upper slide detachable due to a 20-gram weight attached. The time it took for the upper slide to separate was recorded **[5,18,25]**.

$$S=M.\ L/T$$

Determination of anti-oxidant property:

The DPPH method was used to analyse substances' ability to scavenge free radicals, by adding 0.1Mm DPPH to samples in methanol, incubating in dark, and folding [26].

Entrapment efficiency:

Entrapment efficiency measures the drug's percentage within a formulation. It's determined by centrifuging unentrapped drug, collecting the supernatant, dilution with methanol, and analysing it using a UV-visible spectrophotometer at 215 nm.

EE% = Total amount of drug – amount of the free drug / Total amount of drug × 100

Drug content:

To measure the drug content, 10 mg of the formulation was placed in a 10ml flask, filled with methanol, shaken for 2 hours, and then filtered. The absorbance of the filtered solution was measured using a spectrophotometer at 215 nm, ensuring proper mixing and proper mixing.

In-vitro Drug Release Study:

A Franz diffusion cell was used to evaluate drug release patterns from serum. The cell had two compartments: the donor compartment, open to the atmosphere, and the receptor compartment for sampling, and used phosphate buffer solution at pH 6.8 as the diffusion medium.
The drug-containing formulation was placed in a donor compartment over a soaked cellophane membrane, secured using a clamp, and placed on a magnetic stirrer. The receptor compartment, containing 25 ml of PBS, was placed on a thermostatically controlled stirrer. Samples were withdrawn at predetermined intervals, diluted with solvent, and analysed for drug diffusion using a UV Spectrophotometer. The sink condition was maintained by replenishing the receptor phase with equal volume of phosphate buffer.

Release kinetics of selected formulation:

The study analysed drug release kinetics and mechanism by fitting cumulative release data to Zero order and Higuchi models.

Microbial examination of the product:

Clean the work area thoroughly with disinfectant, ensuring equipment is dry. Prepare a product dilution by adding 1g/ml to the first test tube and shaking. Transfer 1ml to the second tube and prepare additional dilutions.

Total Microbial Count Method

- Weigh nutrient agar and add 50ml of water in an autoclave.
- Autoclave the flask at 121°C for 15 minutes.
- Dilute the product at 45°C.
- Mix 20ml of nutrient agar medium in a petri dish.
- Inoculate serum onto agar plates using streak plate method.
- Set plates in an incubator at 37°C for 24 hours.
- Remove plates and compare with control for microbial growth.

Skin irritation study on animals:

Methods:

The study aimed to meet OECD guidelines for testing chemicals on Albino rabbits with acute dermal irritation.

Study designs:

Fur was removed 24 hours before testing, and healthy, undamaged animals were used. A 0.5 ml test sample was applied to the animal's skin, with untreated skin sections serving as the control. A gauze patch was placed over the patch, secured with non-irritating tape. The gauze patch was applied evenly and made excellent contact with the skin, ensuring the sample was evenly distributed and effective.

Method of standards to follow in experiments:

The study involved animals receiving three test patches in succession, with the first patch removed three minutes later. The animals were then exposed to a second patch, a third patch, and a fourth patch. The remaining test sample was removed without affecting the epidermis or

reaction. The animals were monitored for up to three days to determine the reversibility of the effects. The test site was inspected immediately.

The study involves testing New Zealand White Rabbits (Oryctolagus cuniculus) for erythema and edema. The animals weigh 2.588-2.812kg, are female, and will be housed for 5 days. The animals are 8 to 12 weeks old at treatment.
A 0.5ml dose of the sample is applied to the test site. The animals' responses are evaluated at various intervals, including 0, 60, 120, 240, 24, 48, and 72 hours. The test will continue for seven and fourteen days if no edema or erythema is present, and the test place was checked immediately after patch removal in one animal.

Irritation study on humans (Patch test):

A study involved removing human forearm hairs 24 hours before a test, and administering a 0.5ml test sample to untreated skin regions. A small patch was placed with the sample, secured with non-irritating tape. Five participants underwent the skin irritation test, with two patches applied sequentially. The response was assessed after 24 hours, with the initial patch removed after an hour.

Accelerated stability of serum:

Pharmaceutical or cosmetic product formulation requires thorough stability studies to ensure product safety, especially under accelerated conditions. A three-month accelerated stability study was conducted for the prepared formulation, storing samples under varying temperatures and humidity levels. Monthly withdrawals were conducted for analysis of various parameters, ensuring the stability of the products under ICH guidelines [23,24].

RESULTS AND DISCUSSION:

UV -Visible and IR Analysis:

The UV spectrum of Kojic acid dipalmitate was collected using an 1800 series Spectrophotometer, Shimadzu Corporation Kyoco from Japan, and the IR spectra were recorded using potassium bromide (KBr) as a blank, IR affinity spectra with a resolution of 4

cm spanning a 400–4000 cm range were obtained. The Kojic acid dipalmitate spectrum's peaks and the primary peaks of the infrared spectrum were contrasted.

Table no. 5: Calibration curve of kojic acid dipalmitate

Sr. No.	Concentration	Absorbance
1	2	0.274
2	4	0.107
3	6	0.169
4	8	0.241
5	10	0.281

Table 5 displays absorbance values at 2, 4, 6, 8, and 10 ppm, while Figures 2 and 3 display UV spectrophotometry and Infrared spectra of kojic acid dipalmitate.

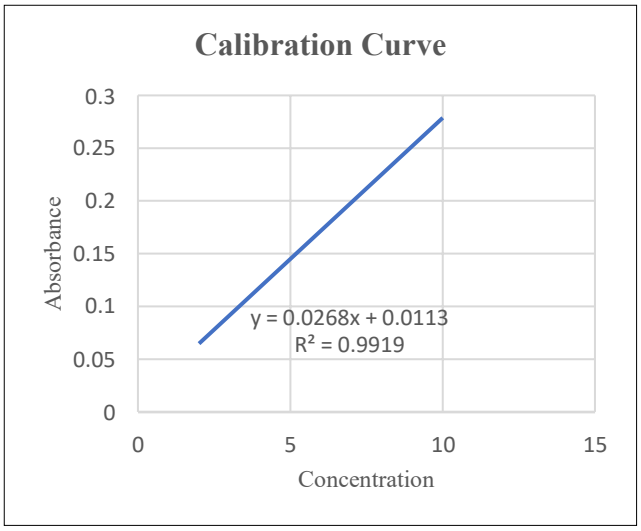


Fig. 2: Calibration curve of Kojic acid dipalmitate

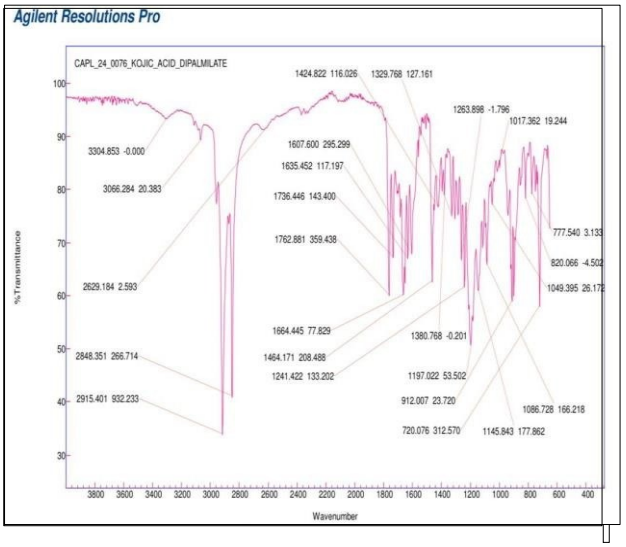


Fig. 3: Representative IR Spectrum of Kojic acid dipalmitate in methanol.

Result of evaluation parameter of face serum:

The physical evaluation of prepared face serum involved Visual observation, pH measurements, and spreading time.

Table 6: Physical Characteristics of Formulation batches

Batches	Colour	Odour	Appearance	pH	Viscosity	Spreadability	Drug content (%)
F1	Translucent white	Characteristic	Smooth	5.5 ± 0.005	1536	7.1±0.015	96.30±0.0288
F2	Translucent white	Characteristic	Smooth	5.49 ± 0.005	2888	9.2±0.041	92.84±0.0230
F3	Translucent white	Characteristic	Smooth	4.89 ± 0.005	2551	8.2±0.230	88.37±0.0680
F4	Translucent white	Characteristic	Smooth	4.99 ± 0.015	1786	9.7±0.404	92.42±0.0404
F5	Translucent white	Characteristic	Smooth	4.97 ± 0.040	2065	9.3±0.404	93.91±0.0472
F6	Translucent white	Characteristic	Smooth	4.80 ± 0.0.61	1855	8.1±0.255	90.55±0.3146
F7	Translucent white	Characteristic	Viscous	4.73± 0.035	2298	8.2±0.251	97.64±0.2927
F8	Translucent white	Characteristic	Smooth	5.01 ± 0.024	1992	6.5±0.057	98.53±0.0378
F9	Translucent white	Characteristic	Viscous	5.07 ± 0.457	1482	6.3±0.057	92.79±0.0404

Note: (All values are expressed as mean ± SD, n=3)

Globule size determination and PDI:

Smaller globule sizes improve product stability, skin absorption, effectiveness, shelf life, and prevent ingredient degradation by reducing aggregation and separation.

Table 7: Globule size and PDI

Formulation code	Particle Size	PDI
F1	260.6	0.54
F2	251.4	0.39
F3	328.5	0.52
F4	354.1	0.63
F5	329.6	0.59
F6	225.8	0.46
F7	185.6	0.42
F8	274.5	0.40
F9	304.8	0.80

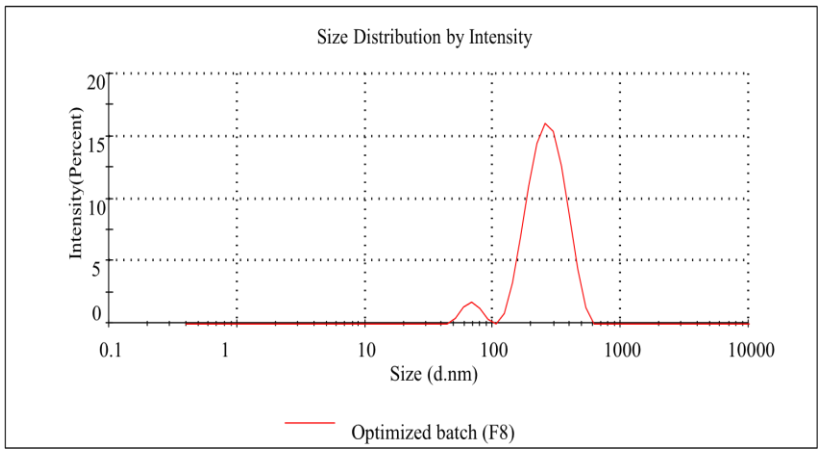


Fig 4: Graph of globule size for serum of optimized batch(F8)

The serum samples from batches F1 to F9 had particle sizes ranging from 225.8nm to 354.1 with the optimized batch (F8) having a particle size of **274.5nm**.

Zeta potential of serum:

Zeta potential measurement reveals particle charges and stability, evaluating surface properties and particle modification. Serum's zeta potential was found to be **-10.87mV**.

Measured data	Zeta Run Time – 30Sec
Zeta potential = -10.87(mV)	
Polarity – Negative (Automatic)	
Mobility – -8.437e-005 (cm^2/Vs)	
Conductivity- 0.5370 (mS/cm)	

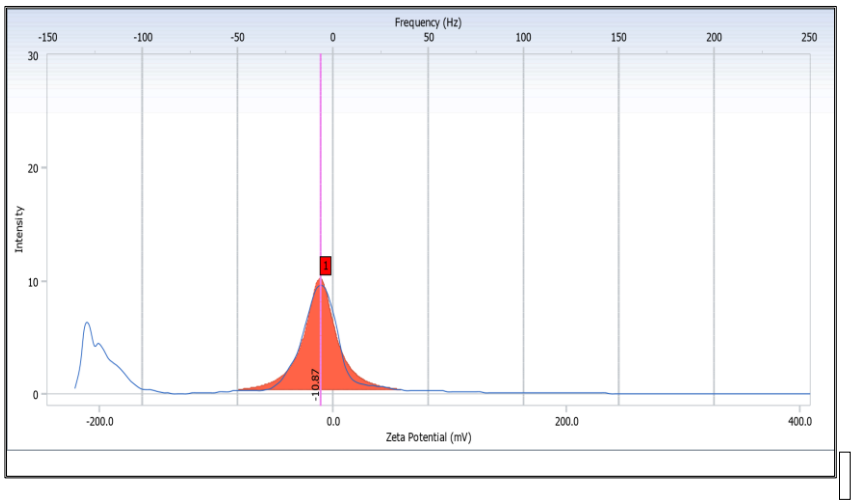


Fig 5: Graph of zeta potential for serum

Refractive index:

The refractive index was found to be 1.334 ± 0.002. The serum was thermodynamically stable but also chemically stable and remained isotropic.

Table 8: Refractive Index of Optimized batch

Sr no.	Refractive Index
1	1.331 ± 0.001
2	1.352 ±0.002
3	1.334 ±0.001
Avg	**1.334 ± 0.001**

Note: (All values are expressed as mean ± SD, n=3)

Antioxidant property of Serum:

DPPH (2,2-diphenyl -1-picrylhydrazyl) assay: Topical antioxidants, such as kojic acid dipalmitate and liquorice extract, prevent oxidative damage to skin cells, preventing wrinkles and cell rejuvenation. The combination of ingredients works synergistically, resulting in a more potent antioxidant effect. Serum has greater antioxidant activity than ascorbic acid.

Table 9: Antioxidant Activity of serum compared with Ascorbic Acid

Sr.no	Concentration	Ascorbic acid	%RSA of Serum
1	20	27.14	44.28 ± 0.265.
2	40	38.57	52.85 ±0.3626.
3	60	54.14	61.42 ± 0.099.
4	80	67.71	74.28 ±0.588.
5	100	83.08	92.85 ±0.3896

Note: (All values are expressed as mean ± SD, n=3)

Entrapment efficiency:

The entrapment efficiency ranged from 84.3-93%, with the optimized batch achieving 90%. High polymer concentrations entrap more drugs but decrease drug release, affecting the selected batch.

Table 10: Entrapment efficiency of formulation F1- F9

Sr no	Formulation code	Entrapment Efficiency (%)
1	F1	92.2
2	F2	94.4
3	F3	93.2
4	F4	87.1
5	F5	84.3
6	F6	88.2
7	F7	85.5
8	F8	90.0
9	F9	93.0

In vitro- Drug Release Studies:

The formulation underwent In-Vitro drug release for 8 hours, with the optimized batch's drug release and cumulative release details listed in Table 11.

Table 11: Cumulative Drug Release of formulations

Sr. No	**Time (hr)**	**% Cumulative Drug Release (%)**
0	0	0
1	1	9.67

2	2	14.24
3	3	19.03
4	4	25.80
5	5	32.91
6	6	40.58
7	7	48.29
8	8	53.63

Drug Release kinetic:

The study analysed drug release kinetics, revealing results for zero order and Higuchi model kinetics, as depicted in the Figure 6.

a) Comparative evaluation of zero order kinetic model:

Table 12: % CDR and Time

Time	% CDR
0	0
1	9.67
2	14.24
3	19.03
4	25.80
5	32.91
6	40.58
7	48.29
8	53.63

The graph plotted between cumulative amounts of drug release v/s time

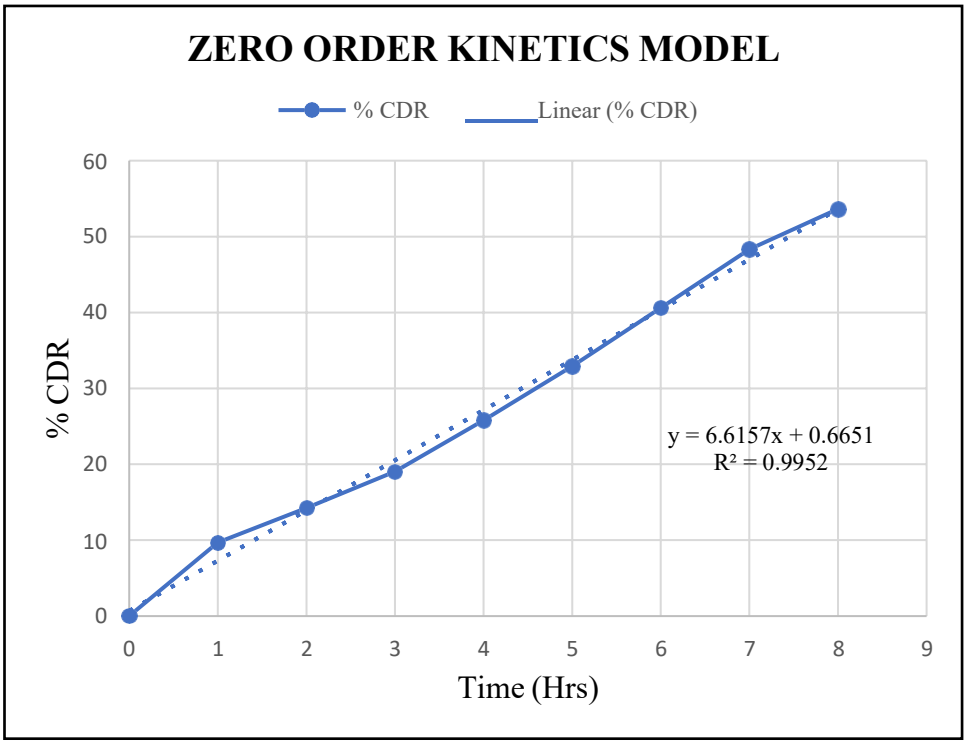


Fig 6: Model graph for zero order release kinetics

b) Comparative evaluation of Higuchi model

Table 13: Square root of time and % CDR

Time	% CDR
0	0
1	9.67
1.4142	14.27
1.732	19.03
2	25.80
2.236	32.91
2.4494	40.58
2.6457	48.29

2.8284	53.63

The graph plotted between cumulative amounts of drug release v/s time

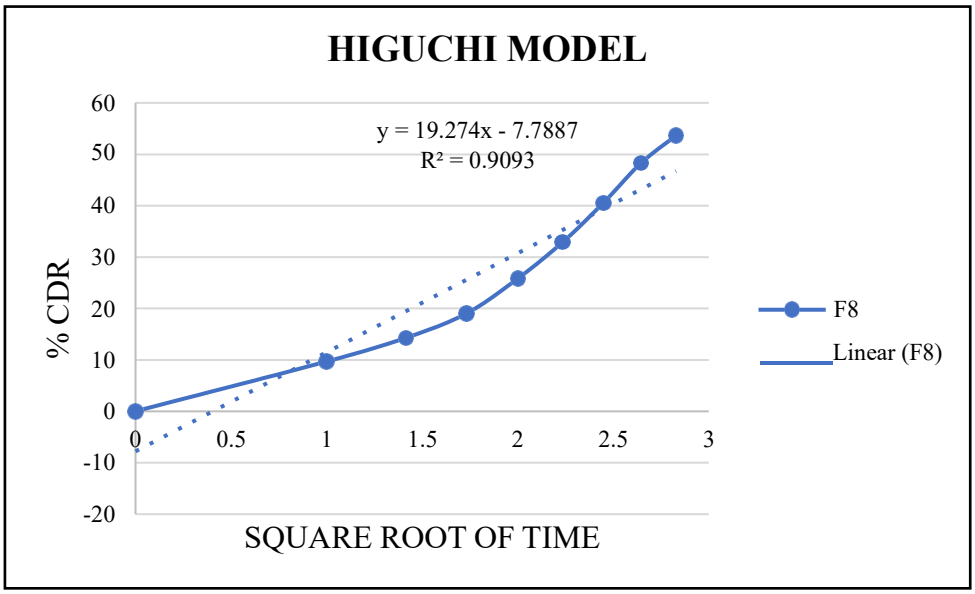


Fig 7: Model graph for Higuchi release kinetics

Microbial stability study:

The optimized batch formulation was found to be microbially stable, free from microbes, as evidenced by the absence of a zone of inhibition when inoculated in agar.

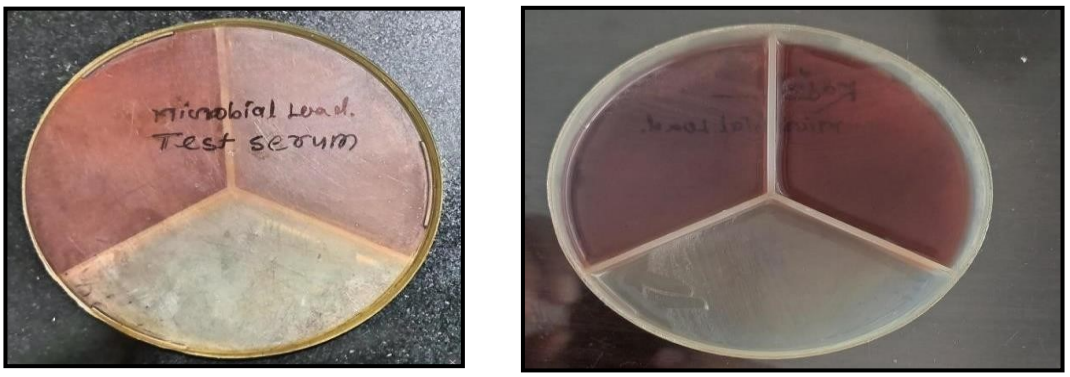
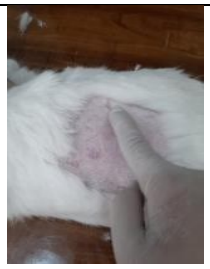
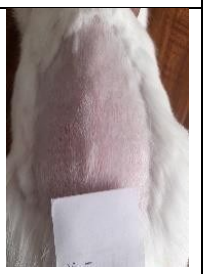
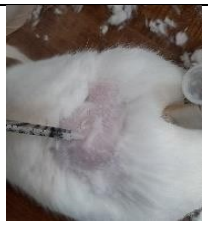
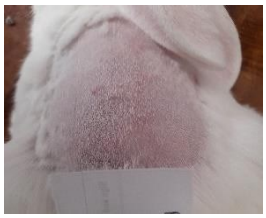
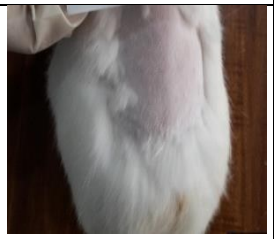


Fig 8 : Microbial stability of serum

Skin Irritation Study:

The study found no dermal irritation in treated rabbits, and the primary skin irritation index of the test material was 0.00, indicating that the substance was non-irritant to rabbit skin.

Time / **No. of Animal**	**0Min**	**60 mins**	**120mins**	**240 mins**
1				
2				
3				

Time / **No. of Animal**	**24hrs +10mins**	**48 hrs+5 mins**	**72hrs+7mins**
1			

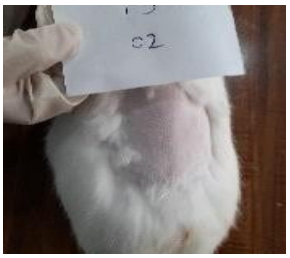
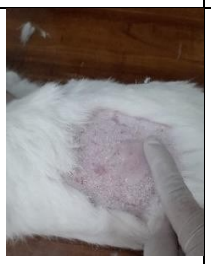
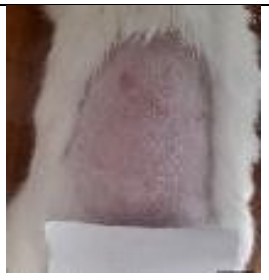
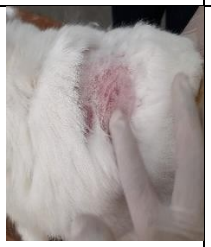
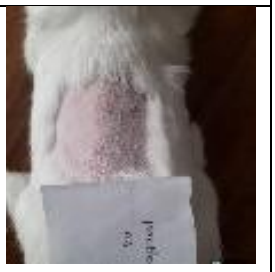
2			
3			

Fig 9: Skin Irritation Study of Serum (Test)

Time / N.O. A	0 Min	120 mins	180 mins	240 mins
1				
2				
3				

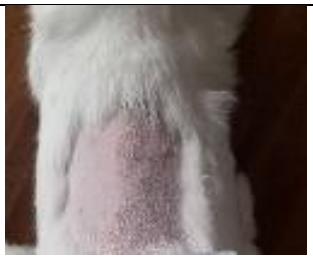
Time N.O. A	24hrs +10mins	48 hrs+5 mins	72hrs+7mins
1			
2			
3			

Fig. 10: Skin Irritation study of Control

Table no. 14: Results of skin Irritation study of Test group

			Test Group				
Time **No. of Animal**	**0min**	**60mins**	**120mins**	**240mins**	**24hrs+10min**	**48hrs+5min**	**72hrs+7mins**
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0

6	0	0	0	0	0	0	0

Table no 15: Results of skin Irritation study of Control Group

Control Group							
Time / **No. of Animal**	**0min**	**60mins**	**120mins**	**240mins**	**24hrs+10min**	**48hrs+5min**	**72hrs+7mins**
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0

Table no.16: Results of Edema and Erythema

Sr. No	Group	Grade of Edema and Erythema
1	Control	0
2	Formulation	0

Irritation study on humans:

During a seven-day observation period, the research observed no dermal irritation in the treated forearm skin of all five patients. The test material's main skin irritation index was 0.00, suggesting that it was non-irritating.

Optimization of prepared formulation:

The study utilized Design Expert 7.0 software to analyse the impact of independent variables on responses, developing experimental designs for nine batches, suggesting suitable models for ANOVA, and generating mathematical models.

Table no. 17: The layout of Actual Design

Runs	Factor1	Factor 2	Response 1	Response 2
	A: Speed of rotation (RPM)	B: Carbopol 940 (mg)	Particle size (nm)	Viscosity (cP)
1	5500	0.5	260.6	1536
2	8000	1	251.4	2888
3	5500	1	328.5	2551
4	3000	0.75	354.1	1786
5	3000	1	395.9	2065
6	8000	0.5	225.8	1855
7	8000	0.75	185.6	2298
8	5500	0.75	295.7	1992
9	3000	0.5	304.8	1482

Results for Particle size:

Fit Summary: The Design-Expert software applied a fit summary to the data, suggesting "Linear vs Mean" based on the input.

Table no.18: Fit summary table for Particle size

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	752498.4	1	752498.4			
Linear vs Mean	31290.19	2	15645.1	29.93218	0.0008	Suggested

2FI vs Linear	1072.563	1	1072.563	2.598833	0.1679	
Quadratic vs 2FI	664.3578	2	332.1789	0.712225	0.5583	
Cubic vs Quadratic	1103.922	2	551.9608	1.869362	0.4594	Aliased
Residual	295.2669	1	295.2669			
Total	786924.7	9	87436.08			

1. ANOVA for Particle size:

The analysis of variance (ANOVA) was used to identify significant and insignificant factors, with the results for particle size being as follows.

Table no.19: ANOVA table for Particle size

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	31290.19	2	15645.1	29.93218	0.0008	significant
A-Speed of rotation	25610.67	1	25610.67	48.9983	0.0004	
B-Carbopol 940	5679.527	1	5679.527	10.86606	0.0165	
Residual	3136.109	6	522.6848			
Cor Total	34426.3	8				

The model's significant F-value of 29.93 indicates its significance, with a 0.08% chance of noise. Significant model terms A and B are indicated by a "Prob > F" value less than 0.0500.

3. Fit Statistics for Particle size

Table no.20: Fit statistics for Particle size

Std. Dev.	22.86	R-Squared	0.9089
Mean	289.16	Adj R-Squared	0.8785
C.V. %	7.91	Pred R-Squared	0.7665
PRESS	8039.84	Adeq Precision	14.561

The model's "Pred R-Squared" and "Adj R-Squared" values are in agreement, indicating an adequate signal to noise ratio of 14.561, useful for design space navigation.

4. Final Equation in Terms of coded Factors for Particle size:

Table no. 21: Final equation in terms of coded factors

Particle size	=
+289.16	
-65.33	* A
-30.77	* B

The equation in terms of coded factors can predict the response for specific levels of each factor.
The equation in terms of coded factors can predict the response for specific levels of each factor.

5. Graphical Presentation:

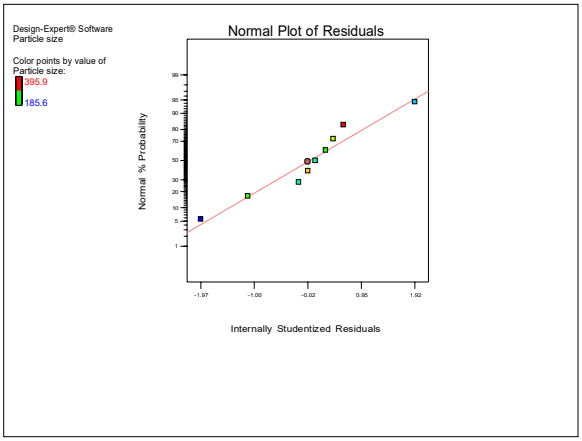


Fig.11: Normal % Probability plot of

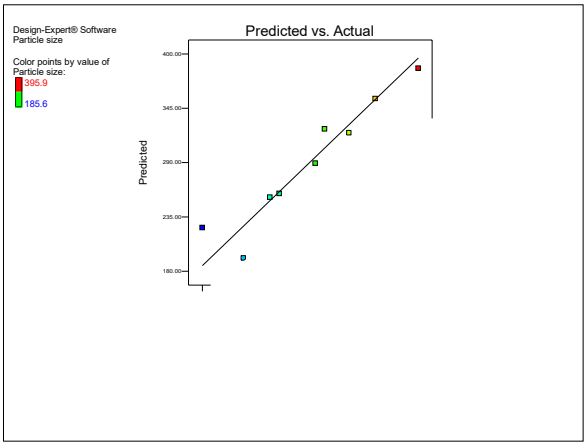


Fig.12: Predicted Vs Actual plot for Particle size

Particle size

6. Model Graphs for Particle size:

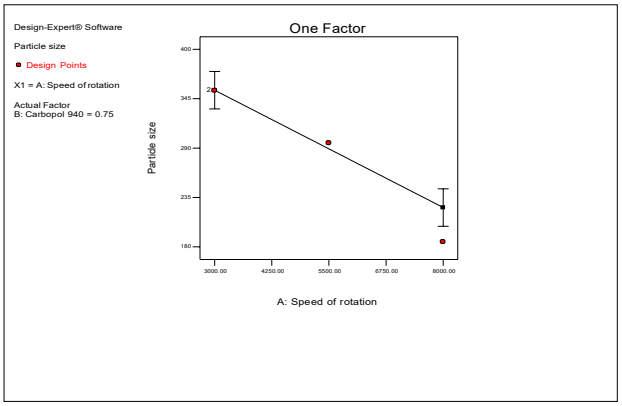


Fig.13: Effect of Speed of rotation on particle size

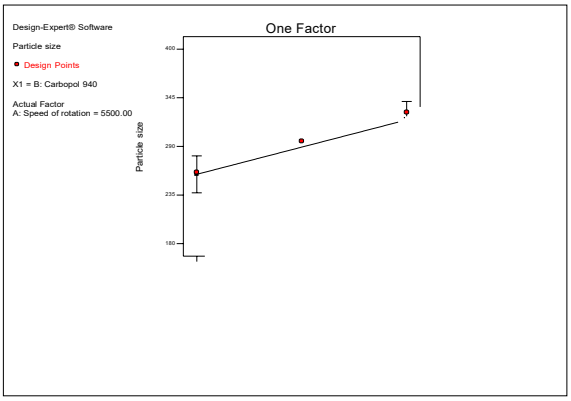


Fig.14: Effect of Carbopol 940 concentration on Particle

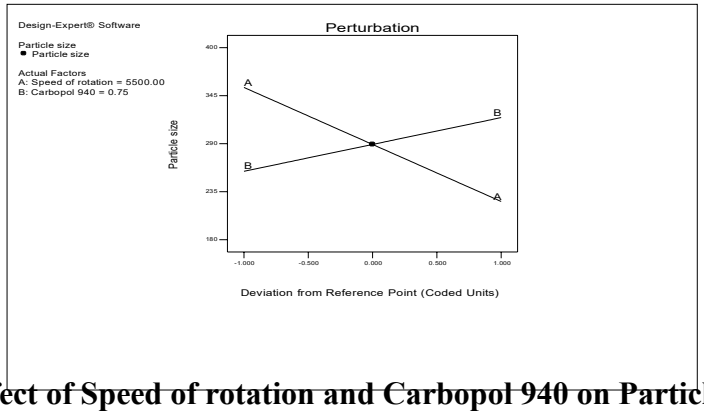


Fig.15: Effect of Speed of rotation and Carbopol 940 on Particle size

The particle size is influenced by the speed of rotation and the concentration of Carbopol 940, with increased rotation speed causing a decrease in particle size.

Results for Viscosity:

1. **Fit Summary:** The data was entered into Design-Expert software, and a fit summary was applied, followed by the software suggesting "2FI vs Linear".

Table no.22: Fit summary table for Viscosity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	37834801	1	37834801			
Linear vs Mean	1639904	2	819952.1	67.41749	< 0.0001	
2FI vs Linear	50625	1	50625	11.32609	0.0200	Suggested
Quadratic vs 2FI	5404.5	2	2702.25	0.478434	0.6602	
Cubic vs Quadratic	16748.33	2	8374.167	42.72534	0.1076	Aliased
Residual	196	1	196			
Total	39547679	9	4394187			

2. ANOVA for Viscosity:

The analysis of variance (ANOVA) was used to identify significant and insignificant factors, with the results for Viscosity being as follows.

Table no.23: ANOVA table for Viscosity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1690529	3	563509.7	126.0714	< 0.0001	significant
A-Speed of rotation	486210.7	1	486210.7	108.7776	0.0001	
B-Carbopol 940	1153694	1	1153694	258.1105	< 0.0001	
AB	50625	1	50625	11.32609	0.0200	
Residual	22348.83	5	4469.767			
Cor Total	1712878	8				

The Model F-value of 126.07 indicates significant model terms, with a 0.01% chance of noise. Values of "Prob > F" less than 0.0500 indicate significant terms, including A, B, and AB.

3. Fit Statistics for Viscosity:

Table no.24: Fit statistics for Viscosity

Std. Dev.	66.86	R-Squared	0.9870
Mean	2050.33	Adj R-Squared	0.9791
C.V. %	3.26	Pred R-Squared	0.9375
PRESS	107005.2	Adeq Precision	32.450

The

"Pred R-Squared" of 0.9375 is in reasonable agreement with the "Adj R-Squared" of 0.9791. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable ratio of 32.450 indicates an adequate signal. This model can be used to navigate the design space.

4. Final Equation in Terms of coded Factors for Viscosity:

Table no.25: Final equation in terms of coded factors

Viscosity	=
+2050.33	
+284.67	* A
+438.50	* B
+112.50	*A*B

The "Pred R-Squared" and "Adj R-Squared" values are in agreement, and an "Adeq Precision" ratio of 32.450 indicates an adequate signal, allowing for effective navigation in the design space.

5. Graphical Presentation:

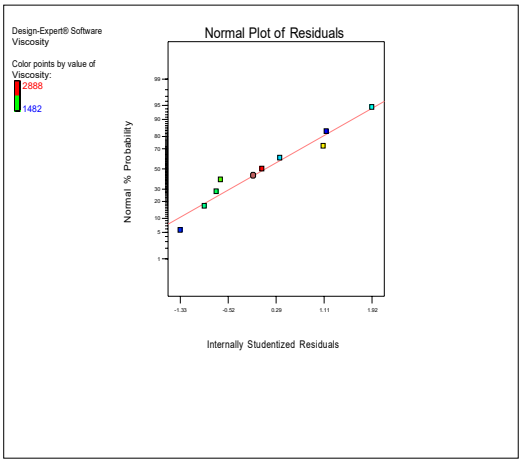


Fig.16: Normal % Probability plot of Viscosity

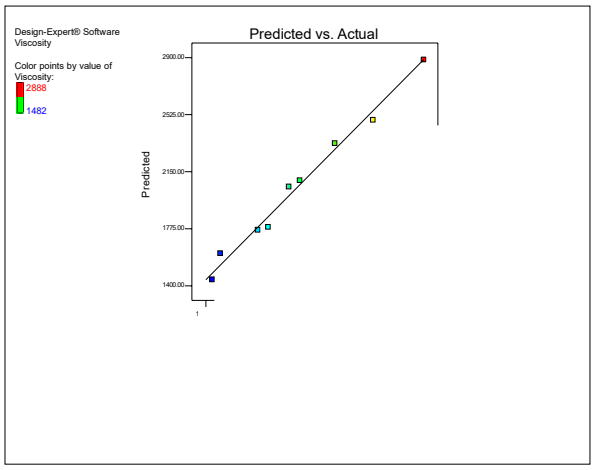


Fig.17: Predicted Vs Actual plot for Viscosity

6. Model Graphs for Viscosity:

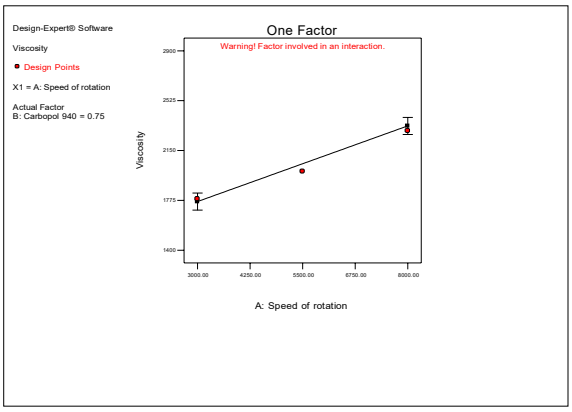


Fig.18: Effect of Speed of rotation on concentration Viscosity

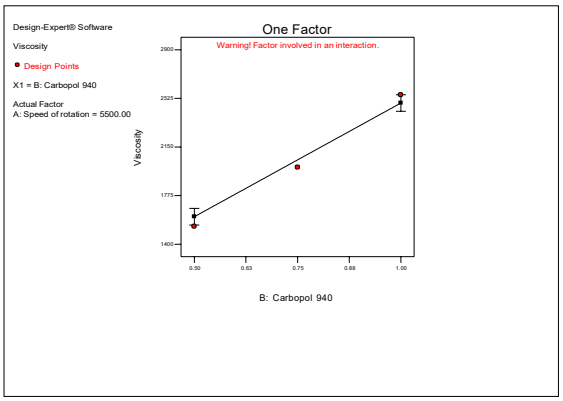


Fig.19: Effect of Carbopol 940 on Viscosity

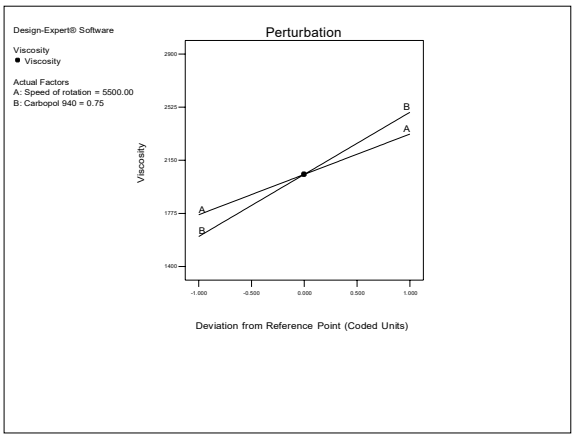


Fig.20: Effect of Speed of rotation and Carbopol 940 on Viscosity

Speed of rotation and Carbopol 940 concentration are having impact on Viscosity. As the speed of rotation increases the Viscosity get increases and as the concentration of Carbopol 940 increases Viscosity also get increases.

Stability study:

The optimized F8 formulation underwent a stability study at room temperature and accelerated for three months. The serum was found to be transparent white with a smooth and opaque appearance, homogenous, and similar to its formulation day. The study revealed slight increases in pH and viscosity, but also slight decreases in spreadability and viscosity. The optimized batch F8 also showed stability at room temperature.

Table 26: Stability Study data for F8 formulation at Accelerated condition (40±2ºC, 75 % RH ± 5% RH)

Parameter					**Observation**					
	Initial	**After 1 Month**			**After 2Month**			**After 3 Month**		
		RT	**At 3º5ºC**	**40±2º C & 75±RH**	**RT**	**At 3º5ºC**	**40±2º C & 75±RH**	**RT**	**At 3º5ºC**	**40±2º C & 75±RH**

Colour	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odour	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
pH	5.0	4.9	4.8	5.0	5.0	4.9	5.0	5.0	4.9	4.9
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Phase separation	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Viscosity (cP)m	1992	1999	1992	1890	1989	1991	1850	1992	1989	1890
Spreadability	6.6cm	6.5cm	6.4cm	6.5cm	6.5cm	6.7cm	6.5cm	6.5cm	6.7cm	6.8cm
Particle size (nm)	274.5	274.5	279.6	273.1	276.1	280.2	264.2	274.2	273.6	264.6

***Note:** **NC** -No Change

CONCLUSION:

The serum was successfully prepared and evaluated, with the compatibility study showing identical results before and after one month. The globule size was 274.5nm for topical penetration, and the PDI and zeta potential were 0.408 and -10.87. After three months, the serum was found to be milky white, smooth, opaque, and homogeneous. The combination of order enhanced antioxidant activity, with the presence of liquorice extract and kojic acid resulting in enhanced antioxidant activity compared to individual ingredients or a control group. The skin irritation study showed no signs of irritation or adverse reaction on rabbit skin. The successful formulation makes it suitable for commercialization, validating the serum's

efficacy and establishing a strong foundation for further product development, potentially leading to a successful and marketable skincare product.

DECLARTION OF COMPITING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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