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"Preparation and Characterization of a Novel Kojic Acid-Encapsulated Vesicular System for Enhanced Skin Delivery"

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ABSTRACT
The study focused on the development, optimization, and evaluation of a novel vesicular drug delivery system glycerosomesfor the efficient topical delivery of kojic acid, a skin-lightening and antioxidant agent. The physicochemical characterization of kojic acid confirmed its purity and compatibility through analyses of solubility, pH, melting point (157°C), UV absorption (λmax = 262 nm), and FTIR spectra. Five glycerosomal formulations (F1–F5) were prepared and assessed for particle size, zeta potential, entrapment efficiency, and appearance. Among these, Formulation F3 was identified as optimal, exhibiting a particle size of 154.3 nm**,** 94.81% entrapment efficiency**,** and a zeta potential of -45.8 mV, signifying excellent stability and uniformity. In-vitro release studies revealed a controlled drug release over 18 hours, best fitting the Zero-order kinetic model (R^2 = 0.949)**,** indicating sustained and concentration-independent release behavior. Stability evaluation over 90 days under both room and accelerated conditions showed no significant variation in key parameters, confirming formulation stability. Overall, the optimized kojic acid-loaded glycerosomal formulation demonstrated favorable physicochemical properties, sustained drug release, and strong potential for enhanced skin retention. These results establish glycerosomes as a promising nanocarrier platform for improving the topical efficacy, stability, and patient compliance of kojic acid in dermatological and cosmetic applications.

Keywords: Kojic acid, Glycerosomes, Topical delivery, Nanocarrier, Controlled release, Stability, Zero-order kinetics

I. INTRODUCTION

In recent years, vesicular drug delivery systems have emerged as innovative carriers for enhancing the topical and transdermal delivery of therapeutic agents. Among these, glycerosomes represent a novel advancement—modified liposomes containing a high concentration of glycerol—that improve membrane flexibility, stability, and skin penetration**(Witikaet al., 2021).**The incorporation of glycerol into the lipid bilayer enhances vesicle deformability and hydration, facilitating the transport of both hydrophilic and lipophilic drugs across the stratum corneum while maintaining biocompatibility and safety **(Abdallah et al., 2025).**

Among various lipid-based vesicular systems, glycerosomes have gained attention as a promising approach for improving the dermal bioavailability of both hydrophilic and lipophilic drugs. Glycerosomes are bilayer vesicles used for dermal and transdermal drug delivery. These vesicles differ from conventional liposomes in bilayer fluidity, formed by the addition of phospholipids and varying concentrations of glycerol (10-30 % v/v)**(Gupta et al., 2020).** These are so named, as they contain high amount of glycerol. These vesicles deliver the active ingredients to skin with high efficiency. Glycerosomes are found to be more stable and possess greater fluidity then liposomes and hence are predominantly used as topical drug delivery systems**(Mancaet al., 2013).**Glycerol ameliorates the deformability index of liposomal bilayers, thus enhancing skin penetration. Glycerosomes are modified liposomes incorporating glycerol, which enhances membrane flexibility, improves skin hydration, and promotes deeper penetration through the stratum corneum. This distinctive composition makes glycerosomes particularly suitable for the topical delivery of sensitive bioactive agents like kojic acid**(Abdallah et al., 2025).**

The concept of glycerosomes was introduced by Kojic acid offers several additional benefits beyond its depigmenting properties. It exhibits antioxidant activity, which means it shields the skin surface from the detrimental impact of ultraviolet radiation and sunlight by countering free radicals produced as a result of reactive oxygen species**(Ishaqet al., 2023).**Furthermore, kojic acid

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is recognized for its role as a key component in treatments for skin whitening, combating browning of the skin, and serving as an antibacterial agent.17,18 These properties make it a versatile ingredient in skincare formulations. Notably, kojic acid is also extensively utilized in various food products to prevent browning. Moreover, Food and Drug Administration (FDA) has authorized the utilization of kojic acid and other compounds combination in the US for the purpose of dermatological treatment**(Cheng et al., 2019).**

The present study focuses on the development, optimization, and characterization of a kojic acid-loaded glycerosomal formulation designed for effective topical application. The work includes formulation preparation**,** physicochemical characterization (particle size, zeta potential, entrapment efficiency), in-vitro release studies**,** and stability assessment**.** The ultimate objective is to establish a stable and efficient vesicular delivery system capable of enhancing kojic acid's therapeutic efficacy and ensuring prolonged skin retention, thus contributing to improved patient compliance and potential clinical applications in skin depigmentation and antioxidant therapy **(Ayuhastutiet al., 2024).**

II. MATERIAL AND METHODS

2.1 Chemicals

Leadacetatewas obtained from SamuhLaxmiChemicals(Bom) P.Ltd. G KeziIndustries provided the Coppersulphate. OrientMicroAbrasivesLimited provided the Hydrochloricacid whilePanoliIntermediate supplied Conc.Sulphuricacid. Methanol were procured from MeruChemPvt.Ltd, a well-known provider of high-quality laboratory chemicals. Kojic acid was supplied by Sihauli Chemicals Pvt Ltd. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Suvchem**,** Vizagchemical, ManasPetroChem, Pandora Industries, GHCLLimited,AmsFineChemical and DrashtiChemicals.

2.2 Physicochemical Profiling of Kojic Acid

2.2.1 Organoleptic Properties

Organoleptic evaluation refers to the assessment of the drug's physical characteristics that can be perceived by the senses namely color, odor, taste, and appearance **(Clapham, 2022).**

2.2.2 Solubility test

Solubility evaluation is a critical step in pharmaceutical formulation, as it significantly influences a drug's absorption rate, bioavailability, and overall therapeutic performance. To determine the solubility characteristics of the selected drug, an initial qualitative screening was carried out using a variety of commonly employed solvents. In this experiment, 1 mg of thiocolchicoside was precisely measured and added to individual test tubes, each containing 1 ml of different solvents including methanol, ethanol, chloroform, dimethyl sulfoxide (DMSO), distilled water, and ethyl acetate**(Jagtap et al., 2018).**

2.2.3pH Determination

The pH of Kojic acid was evaluated to understand its acid-base characteristics, which are vital for forecasting the drug's stability, solubility, and compatibility with excipients during formulation development. The measurement was carried out using a calibrated digital pH meter to ensure accurate and reproducible results **(Mosangi, 2017).**

2.2.4 Melting Point

The melting point of kojic acid was determined to assess its thermal characteristics and purity, which are essential for formulation development. The compound was analyzed using a Differential Scanning Calorimeter (DSC) under a controlled heating rate **(Tazeshet al., 2021).**

2.2.5 Determination of Lambda max and calibration curve of kojic acid

2.2.5.1 Lambda (λ) max

To identify the wavelength of maximum absorbance (λmax) for kojic acid, a standard stock solution was prepared by dissolving an accurately weighed amount of the drug in methanol. A working solution with a concentration of 100 µg/mL was obtained through appropriate dilution using the same solvent.

Additionally, a calibration curve was constructed by measuring the absorbance of solutions at varying concentrations to establish a linear relationship for accurate drug quantification in formulation studies **(ZaidAlkilaniet al., 2025).**

2.2.5.2 Standard calibration curve analysis

A standard calibration curve for kojic acid was established to quantify its concentration in solution, ensuring accurate dosing in formulation development. A series of standard solutions with known concentrations of kojic acid were prepared

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 6 Nov - Dec 2025, pp: 299-308 www.ijprajournal.com ISSN: 2456-4494

by serial dilution of a stock solution. Each solution's absorbance was measured at the identified λmax using a UV-Vis spectrophotometer. The absorbance values were plotted against the corresponding concentrations to construct the calibration curve. A linear regression analysis was performed to determine the equation of the line and the coefficient of determination (R^2), confirming the linearity and reliability of the calibration curve for subsequent quantitative analyses (**Javaidet al., 2024).**

2.2.6 Preparation of calibration curve

A calibration curve for kojic acid was developed to define the relationship between concentration and absorbance for precise quantification in formulations. A stock solution was prepared by dissolving a measured amount of kojic acid in methanol and then diluted to produce standard solutions ranging from 5 to 25 µg/mL. Absorbance readings were taken at the determined λmax using methanol as a blank. The absorbance values were plotted against concentration to construct the calibration curve. Linear regression analysis provided the equation of the line, used for determining kojic acid concentration in unknown samples. The curve showed excellent linearity with a high R^2 value, confirming its suitability for accurate analytical use **(Zenginet al., 2025).**

2.2.7 Fourier transmission Infra-Red Spectroscopy

Materials: Kojic acid, KBr, FTIR spectrometer, mortar and pestle, analytical balance, desiccator.

Procedure: About 1–2 mg of kojic acid was finely ground, mixed with KBr, and compressed into a transparent pellet. The pellet was scanned from 4000–400 cm^{-1} at 2 cm^{-1} resolution with 32 scans.

Analysis: The obtained FTIR spectrum was examined for characteristic functional group peaks and compared with reference data to confirm the compound's identity and purity. FTIR analysis thus verified kojic acid's structural integrity and suitability for formulation use.

2.3 Preparation of drug loaded Glycerosomes formulation by thin film hydration process.

The thin film hydration method was employed to prepare Kojic acid-loaded glycerosomes (GMs). In each formulation (GS1–GS5), 3.0% (300 mg) Kojic acid, 40 mg cholesterol, and varying phospholipid concentrations (20–100 mg) were accurately weighed and dissolved in a 1:1 solvent mixture of methanol (15 ml) and chloroform (15 ml). The solution was transferred to a round-bottom flask and stirred mechanically at 40°C for one hour to achieve uniform mixing. Solvent removal was carried out using a rotary evaporator under reduced pressure, forming a thin lipid film on the flask wall. The film was kept under vacuum overnight to remove residual solvents.

Hydration of the dried film was performed with 10 ml phosphate buffer saline (PBS, pH 6.8) containing 10% glycerol at 40°C, followed by one hour of mechanical stirring to promote vesicle formation. The resulting multilamellar vesicle suspension was sonicated for half a cycle to reduce particle size and ensure uniformity. Unentrapped Kojic acid was removed by centrifugation at 1500 rpm for 10 minutes at 4°C. The purified glycerosomal formulations were then lyophilized and stored for subsequent characterization and analysis **(Firoznezhadet al., 2022).**

Table 1: Composition of Glycerosomes Formulation

Formulation code	Kojic acid Drug (%)	Phospholipid (mg)	Glycerol (%)	Cholesterol (mg)	Methanol (ml)	Chloroform (ml)	PBS 6.8 (ml)
GS 1	3.0	20	10.0	40.0	15	15	10
GS 2	3.0	30	10.0	40.0	15	15	10
GS 3	3.0	40	10.0	40.0	15	15	10
GS 4	3.0	50	10.0	40.0	15	15	10
GS 5	3.0	100	10.0	40.0	15	15	10

International Journal of Pharmaceutical Research and Applications
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2.4 Evaluation parameter of Drug loaded Glycerosome

2.4.1 Physical Appearance

The drug-loaded glycerosome formulation was visually examined to evaluate its physical characteristics, including clarity, color, homogeneity, and any signs of phase separation **(Elerakyet al., 2023).**

2.4.2 Particle Size determination

Particle size is a pivotal characteristic in vesicular drug delivery systems like glycerosomes, influencing parameters such as drug release behavior, formulation stability, and permeation through the skin. The DLS analysis yielded the average hydrodynamic diameter of the vesicles along with the polydispersity index (PDI), which serves as an indicator of the vesicle size distribution uniformity **(Anweret al., 2025).**

2.4.3 Zeta potential determination

Zeta potential, a critical parameter for assessing the surface charge and stability of vesicular drug delivery systems like glycerosomes, was measured using a Malvern Zetasizer**(Gupta et al., 2020).**

2.4.4 Scanning Electron Microscopic (SEM) Analysis

The surface morphology and structural features of the drug-loaded glycerosomes were investigated using Scanning Electron Microscopy (SEM)**(Mohammed et al., 2021).**

2.4.5 Entrapment efficiency

The entrapment efficiency of the drug within the glycerosomes was determined to evaluate the formulation's ability to encapsulate the active compound effectively. This parameter is crucial for understanding the formulation's loading capacity and predicting its therapeutic efficacy. Entrapment efficiency (%) was then computed using the formula: **(Moolakkadathet al., 2020).**

Entrapment Efficiency (%) = (Total drug −Free drug/Total drug) ×100

2.5 In Vitro Drug Release Study of kojic acid-Loaded Glycerosomes

a) Method:

The dialysis bag diffusion method was employed, where a measured amount of glycerosomal formulation was sealed in a dialysis membrane and immersed in phosphate buffer (pH 6.8) under continuous stirring at physiological temperature.

b) Sampling:

Samples were collected at specific intervals, and equal volumes of fresh buffer were added to maintain sink conditions.

c) Data Analysis:

Cumulative drug release (%) was calculated and plotted against time to obtain the release profile.

d) Kinetic Models:

Drug release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine release kinetics and mechanism.

e) Purpose:

To identify the best-fitting kinetic model and elucidate the mechanism of Kojic acid release from glycerosomes.

2.6 Stability Testing of Glycerosome-Loaded Formulation of kojic acid

- **Purpose:**

To establish suitable storage conditions, shelf life, and retest periods for the formulation.

- **Study Design:**

Accelerated stability testing of the optimized kojic acid-loaded glycerosome was performed following ICH guidelines.

- **Storage Conditions:**

Samples were stored in airtight containers at:
25 ± 2°C / 60 ± 5% RH
40 ± 2°C / 70 ± 5% RH

- **Duration:**

The study lasted three months, with evaluations at 30, 45, 60, and 90 days.

- **Parameters Assessed:**

Physical appearance, particle size, and drug entrapment efficiency were monitored.

- **Evaluation:**

Results were compared to initial data (day 0) to identify any significant variations and assess formulation stability.

International Journal of Pharmaceutical Research and Applications
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- **Outcome:**

The study provided insights into formulation stability, shelf life, and suitability for further pharmaceutical development **(Gurramet al., 2021).**

III. RESULTS AND DISCUSSION

3.1 Physicochemical Profiling of Kojic Acid

3.1.1 Organoleptic properties

Table 2: Organoleptic properties of Kojic Acid

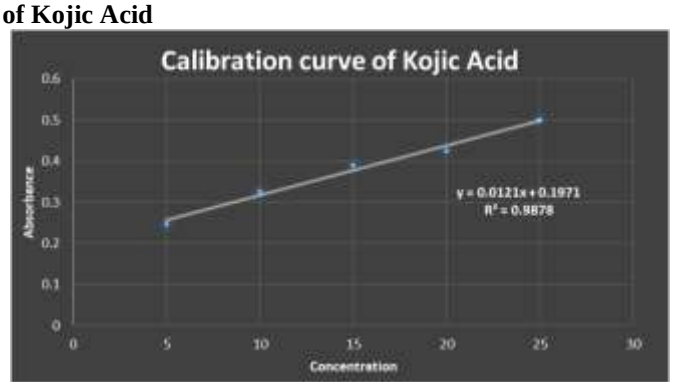
Drug	Organoleptic properties	Observation
Kojic Acid	Color	White to off-white
	Odor	Odorless or slight odor
	Appearance	Crystalline powder
	State	Solid

3.1.2pH and Melting point determination

Table 3: pH and Melting point determination

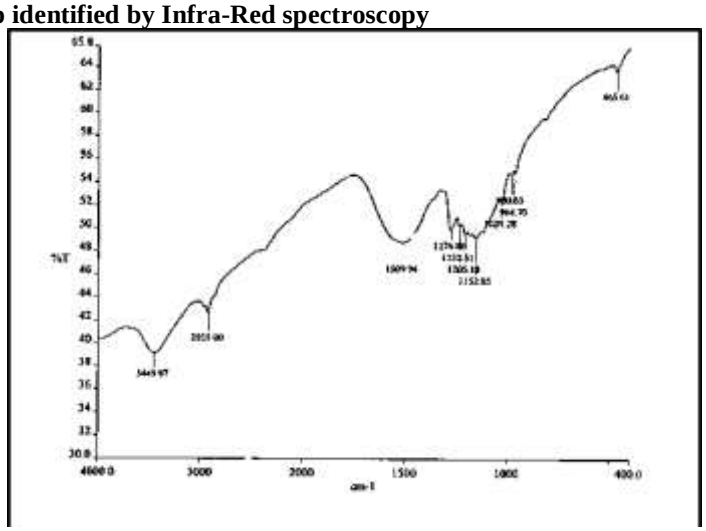
Drugs	Observed (pH)	Observed (Melting point)	Reference (Melting point)
Kojic Acid	4.7	157 °C	152 to 158 °C

3.1.3 Calibration curve of Kojic Acid



Graph 1: Calibration curve of Kojic Acid

3.1.4 Functional group identified by Infra-Red spectroscopy



Graph 2: FTIR study of Kojic Acid

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 6 Nov - Dec 2025, pp: 299-308 www.ijprajournal.com ISSN: 2456-4494

Table 4: Interpretation of IR spectrum ofKojic Acid

Peak obtained	Reference peak	Functional group	Name of functional group
3449.87	3500- 3400	N-H stretching	primary amine
2921.00	3000-2840	C-H=Stretching	Alkene
1509.94	1620-1610	C=O Stretching	α, β-unsaturated ketone
1205.18	1225-1200	C-N stretching	Amine
1152.85	1205-1124	C-O stretching	Tertiary alcohol
1029.28	1050-1040	CO-O-CO stretching	anhydride
980.63	980-960	C=C bending	alkene

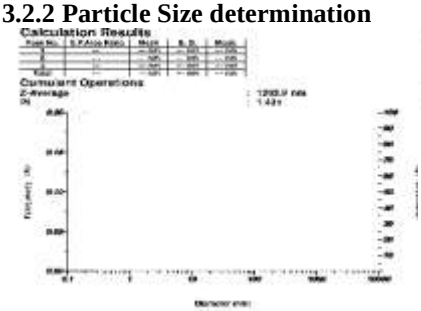
3.2 Characterization of optimized formulation

3.2.1 Physical appearance of drug loaded glycerosome formulation

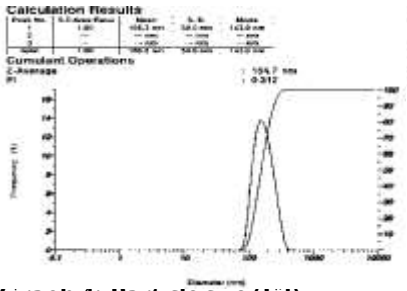
Table 5: Physical appearance of glycerosome formulation

Physical appearance	Observation
Color	off-white
Clarity	Slightly translucent
Consistency	Smooth and uniform dispersion
Odor	Odorless
Phase Separation	No phase separation observed

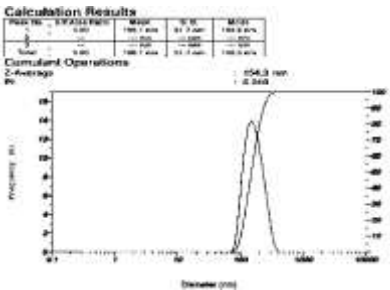
3.2.2 Particle Size determination



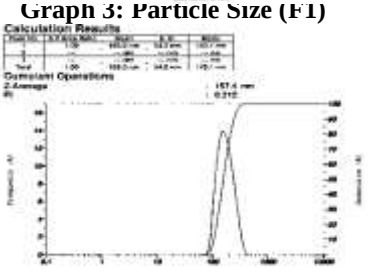
Graph 3: Particle Size (F1)



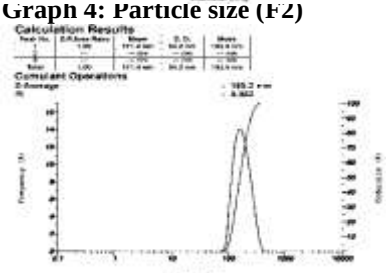
Graph 4: Particle size (F2)



Graph 5: Particle size (F3)

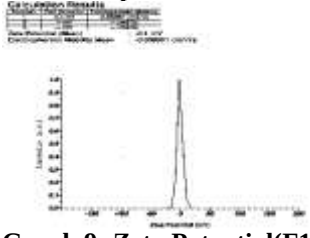


Graph 6: Particle size (F4)



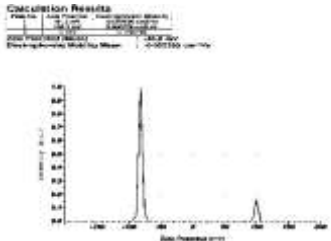
Graph 7: Particle size (F5)

3.2.3 Zeta potential determination



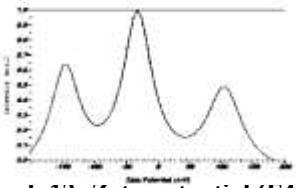
Graph 9: Zeta Potential(F1)

Graph 10: Zeta potential (F2)

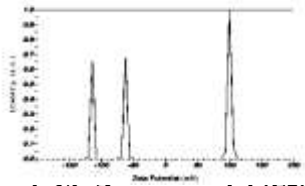


Graph 11: Zeta potential(F3)

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 6 Nov - Dec 2025, pp: 299-308 www.ijprajournal.com ISSN: 2456-4494



Graph 12: Zeta potential (F4)



Graph 13: Zeta potential (F5)

3.2.4 Entrapment efficacy, Particle size and zeta potential determination

Table 6: Entrapment efficacy,Particle size and zeta potential

Formulations (F1-F5)	Entrapment efficacy (%)	Particle size (nm)	Zeta potential
F1	89.78	1283.9 nm	-0.1mV
F2	82.65	154.7 nm	-0.3 mV
F3	**94.81**	**154.3 nm**	**-45.8** mV
F4	78.34	157.4 nm	-35.5 mV
F5	73.91	165.2 nm	-8.5 mV

3.2.5 Scanning electron microscope (SEM)

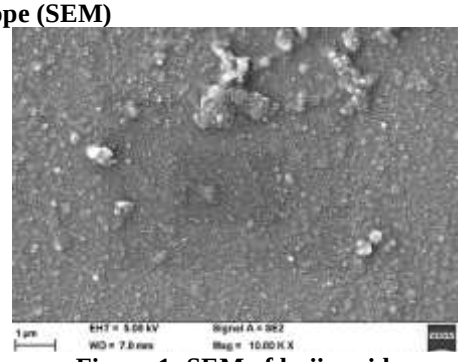


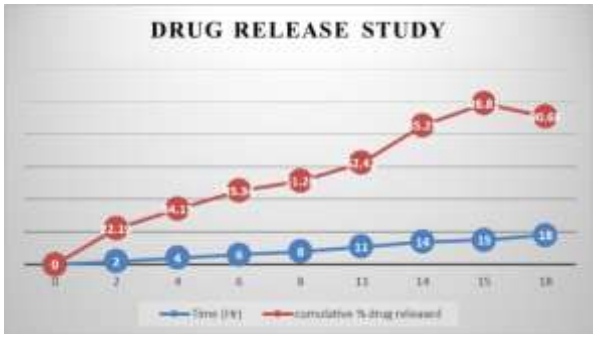
Figure 1: SEM of kojic acid

3.3 In-vitro drug release

Table 7: In-vitro drug release studies

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
2	22.19	77.81	1.414	1.891	0.301	1.346
4	34.15	65.85	2.000	1.819	0.602	1.533
6	45.34	54.66	2.449	1.738	0.778	1.656
8	51.28	48.72	2.828	1.688	0.903	1.710
11	62.42	37.58	3.317	1.575	1.041	1.795
14	85.29	14.71	3.742	1.168	1.146	1.931
15	98.81	1.19	3.873	0.076	1.176	1.995
18	90.68	9.32	4.243	0.969	1.255	1.958

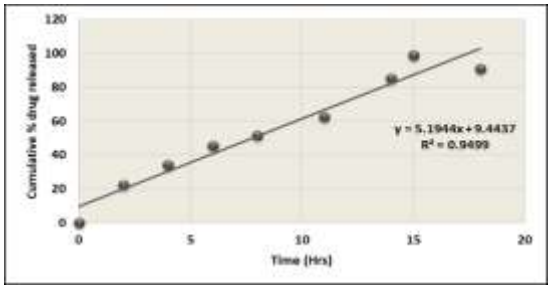
International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 6 Nov - Dec 2025, pp: 299-308 www.ijprajournal.com ISSN: 2456-4494



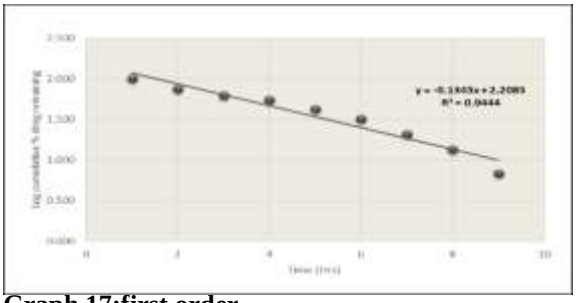
Graph 15: drug release study of kojic acid

Table 8: Release kinetic models

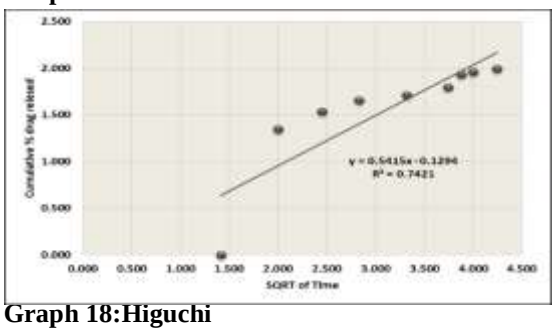
Formulation	Model	Kinetic parameter values
Glycerosome formulation	Zero Order	$R^2 = 0.949$
	First Order	$R^2 = 0.944$
	Higuchi	$R^2 = 0.742$
	Korsmeyerpeppas	$R^2 = 0.809$



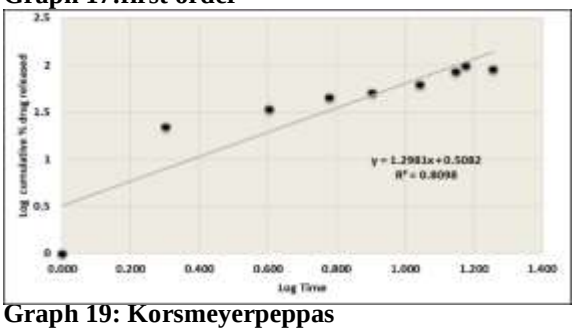
Graph16:Zero order



Graph 17:first order



Graph 18:Higuchi



Graph 19: Korsmeyerpeppas

3.4Stability study

Table 9: Stability Study of optimized formulation (Glycerosomes)

Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH		40^0C±2 ^{0}C and 70 ±5% RH	
	Particle size	Entrapment efficacy (%)	Particle size	Entrapment efficacy (%)
0	154.3 nm	94.81%	154.3 nm	94.81%
30	154.10 nm	94.79%	154.27nm	94.76%
45	154.20 nm	94.65%	154.36 nm	94.62%
60	154.30 nm	94.96%	154.89 nm	94.59%
90	154.45 nm	94.85%	154.62 nm	94.40%

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 6 Nov - Dec 2025, pp: 299-308 www.ijprajournal.com ISSN: 2456-4494

IV. CONCLUSION

The study successfully formulated and optimized a kojic acid-loaded glycerosomal system (F3) with ideal characteristics for topical drug delivery. The optimized formulation demonstrated small and uniform particle size, high drug entrapment, excellent stability, and a sustained drug release profile. The use of glycerosomes significantly enhanced the delivery potential of kojic acid by ensuring prolonged skin retention, stability, and efficient drug loading. These findings suggest that glycerosomes are a promising nanocarrier platform for improving the therapeutic efficacy and patient compliance of kojic acid in dermatological and cosmetic applications.

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