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2³ FACTORIAL DESIGN OPTIMIZATION OF ELLAGIC ACID-LOADED POLYMERIC NANOPARTICLES FOR SERUM-BASED TOPICAL DELIVERY

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ABSTRACT
This study aimed to develop and optimise Ellagic Acid (EA)-loaded polymeric nanoparticles for topical delivery through a serum formulation, with the goal of enabling efficient incorporation of plant-derived bioactives into topical therapies. A UV spectroscopic calibration curve of EA ($y = 0.1062x - 0.0077$; $R^2 = 0.989$) confirmed linearity for accurate quantification. Using a 2^3 factorial design, the influence of Poly (lactic-co-glycolic acid) (PLGA), Polyvinyl alcohol (PVA), and homogenization cycles on formulation parameters was optimised. The resulting nanoparticles exhibited a particle size of 185 nm, a PDI of 0.490, and an entrapment efficiency of 99.15%, demonstrating strong stability and uniformity. FTIR spectra confirmed characteristic functional groups of EA and PLGA, while XRD analysis revealed a crystalline pattern with retained major EA peaks, indicating successful encapsulation. The serum formulation showed a viscosity of 14,300 cP ± 0.1 and a drug content of 72.2%, ensuring suitable topical consistency. In-vitro studies indicated 97.87% cumulative drug release within 4 hours, confirming improved solubility and diffusion. The developed EA serum demonstrates excellent potential as a safe and stable topical formulation for plant-derived bioactive compounds. This research supports SDG 3 (Good Health and Well-being) by promoting sustainable, plant-derived nanocarriers for advanced dermatological applications.
Keywords: Ellagic Acid, Polymeric Nanoparticles, Topical Delivery, Serum Formulation, In Vitro Permeation, Particle Size.
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INTRODUCTION

In recent years, the demand for effective topical delivery systems has surged, driven by the need to improve therapeutic outcomes of bioactive compounds such as Ellagic Acid (E.A). E.A., a naturally occurring polyphenolic compound found in fruits and vegetables, is well recognised for its antioxidant, anti-inflammatory, and anticancer properties.[1] However, its clinical application is constrained by poor solubility, low bioavailability, and rapid metabolism[2], limiting its effectiveness in conventional topical formulations. To overcome these challenges, polymeric nanoparticles have emerged as a promising strategy.[3] They offer biocompatibility, biodegradability, and controlled release, while enhancing drug loading, protecting E. A from degradation, and improving cellular uptake.[4,5] Serum-based topical formulations provide additional advantages over creams by promoting better spreadability, penetration, and targeted delivery.[6] E.A-loaded nanoparticles also demonstrate skin benefits, including protection against oxidative stress, reduction of inflammation, inhibition of melanogenesis, and stimulation of collagen production.[7–10] Nanoencapsulation ensures sustained release, improved bioavailability, and safer administration, enhancing the overall effectiveness and applicability of E.A.[11] Despite these advances, few studies have translated E.A.-loaded polymeric nanoparticles into optimised serum formulations[12], highlighting a critical gap in developing safe, effective, and innovative delivery platforms. This study aims to address this gap (Fig.-1) by preparing a placebo polymeric nanoparticle formulation, finalising

suitable excipients, optimising the E.A-loaded nanoparticles using Design-Expert® Version 13.0 software (Stat-Ease Inc., USA), and incorporating the nanoparticles into a serum for evaluation of topical delivery performance. The outcomes of this work may contribute to advancing safe therapeutic interventions and fostering innovation in topical drug delivery systems.

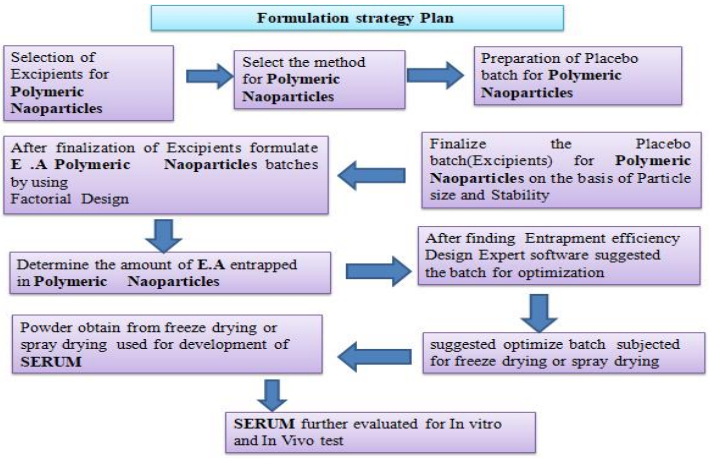

Fig.-1: Formulation Strategy Step by Step

EXPERIMENTAL

Material

Poly (lactic-co-glycolic acid) PLGA, purchased from biodegradable and biocompatible copolymer, Evonik, India, polyvinyl alcohol (PVA), Chitosan 90%, Eudragit® RL, Poloxamer188, Acetone,Dimethyl sulfoxide (DMSO), Ascorbic Acid, Hyaluronic Acid, Sodium Benzoate, Citric Acid, Xanthan Gum, Glycerin Rose oil and water purchased from S D Research Lab Fine Chemicals, India and Ellagic Acid purchased from yucca enterpriseswadala (e), Mumbai, India.

Characterisation of a Drug by UV-Visible Spectrophotometer

A UV-visible spectrophotometer was used to measure the absorbance of Ellagic acid (E.A) solutions at a λmax value of 254 nm.[13] A 100 ppm stock solution was prepared by dissolving 10 mg of E.A in 100 ml of methanol. Aliquots of this stock solution were diluted with methanol to create concentrations of 2, 4, 6, 8, and 10 ppm. The UV absorbance of these solutions was measured to establish a standard curve for E.A.

Fourier Transform Infrared Spectroscopy (FTIR)

The sample was prepared by mixing the formulation with KBr (30:70 ratio), triturating with a glass mortar and pestle, and forming a pellet. The pellet was placed in the sample holder, and the spectrum was scanned 40 times to record the peaks.

X-ray Diffraction (XRD)

The crystallinity of the encapsulated formulation was assessed using XRD with a diffractometer. (Bruker D2 PHASER Benchtop XRD). Scanning was done up to a 2θ range between 2 and 90° using a Ni-filtered. The XRD patterns of the SF were compared with EA.

Particle Size Determination

Particle Size was determined using Malvern Zetasizer ZS. Particle size is one of the most crucial parameters for Polymeric nanoparticles. Particle size is measured by the principle of the Laser Diffraction Technique. Large-sized particles scatter light at smaller angles and vice versa.

Entrapment Efficiency

The entrapment efficiency of E. A in nanoparticles was determined by measuring the amount of unencapsulated drug in the supernatant after centrifugation. Spectrophotometric analysis was used to quantify the free drug, and the percentage of drug entrapped (EE% %) was calculated based on the difference between the total drug amount and the amount of free drug.[14]

Viscosity Determination
Viscosity of the serum formulations was measured using a Brookfield viscometer with a T-96 spindle at 37°C. The average viscosity in centipoises (cP) was determined from five readings taken at 6 rpm and 3 rpm. [14]

Determination of Drug Content (DC)
The DC percentage was calculated using UV spectrophotometry, where the absorbance of the SF was compared to that of pure ellagic acid, using the formula-

DC% = (Absorbance of formulation / Absorbance of standard) × 100."

***In-vitro* Study**
In vitro diffusion studies were conducted using Franz diffusion cells with dialysis membranes (2.4 nm pore size, Analab Fine Chemicals). The receptor compartment had 25 mL phosphate buffer (pH 7.4) at 37°C, stirred at 100 rpm. One mL formulation was placed in the donor compartment, and samples were withdrawn at intervals (1-8 hours) for UV analysis.[15]

Formulation
Preparation of EA Polymeric Nanoparticles
The formulation process involved combining an organic phase (PLGA and EA in DMSO) with an aqueous phase (PVA and Tween 80 in water) via sonication, creating an oil-in-water emulsion. High-pressure homogenization at 10,000 bar further reduced droplet size to nanodroplets. Each formulation was prepared in triplicate for reproducibility.

Factorial Design Experiments
This study employed a 2^3 randomised reduced factorial design to examine the impact of polymer quantity (X1), surfactant concentration (X2), and high-pressure homogeniser (HPH) cycles (X3) on entrapment efficiency and particle size. The relationship between variables and responses was described using a quadratic equation[16], and Design-Expert software generated and evaluated the statistical design. ANOVA assessed model adequacy, and eight formulations were prepared with results expressed as mean ± SD, considering $p < 0.05$ as statistically significant. And the assigned formulation codes are given in Table-1.

Table-1: DOE Code Batches

Batch No	PLGA (mg)	PVA (gm)	HPH Cycle	EA Quantity(mg)	DMSO (mL)	Tween 80
B1	200 (+1)	1(-1)	12(+1)	50	5	4- 5 drops or Q.S
B2	100(-1)	3(+1)	12(+1)	50	5	
B3	100(-1)	3(+1)	8(-1)	50	5	
B4	200(+1)	1(-1)	8(-1)	50	5	
B5	200 (+1)	3(+1)	8(-1)	50	5	
B6	100(-1)	1(-1)	8(-1)	50	5	
B7	200 (+1)	3(+1)	12(+1)	50	5	
B8	100(-1)	1(-1)	12(+1)	50	5	

Quantity of distilled water sufficient for 100 Ml

Suggested Batch by DOE
Based on the factorial design, the optimised batch of 500 mL of E. A polymeric nanoparticle suggested by DOE consisted of 150 mg PLGA, 1.4 mg PVA, and 12 HPH Cycles, with a desirability of 1. This optimised batch was then spray-dried using a Labultima LU222 device with parameters set at an inlet temperature of 163.5°C, feed flow rate of 4 mL/min, and outlet temperature of 69.9°C. The dried nanoparticles were stored in a desiccator and later incorporated into a serum formulation (SF) for further evaluation.

Preparation of Serum Formulation (SF)
A serum formulation was prepared by trituration of Xanthan gum (0.1 g) and Hyaluronic Acid (1gm) with rose water, followed by the addition of a water phase containing citric acid (0.5 g), sodium benzoate (0.5

g), and spray-dried E.A nanoparticle (69mg, equivalent to 50mg of drug). Glycerin (0.3ml) and rose oil (1ml) were also incorporated. Ascorbic Acid (2gm) was included in the formulation. The mixture was stirred until a consistent serum was formed, with the final volume adjusted with rose water. The serum was then evaluated using various parameters.[15]

RESULTS AND DISCUSSION

UV Spectroscopic Analysis

A calibration curve was constructed, yielding a linear equation y = 0.1062x - 0.0077 with R^2 = 0.989, indicating a strong linear relationship between absorbance and concentration, enabling accurate quantification of E.A. as shown in Fig.-2.

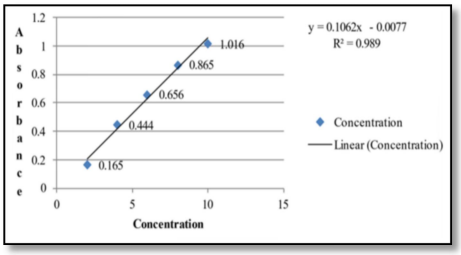

Fig.-2: Calibration Curve of E.A at 254 nm

Optimise EA Polymeric Nano-particle

The polymeric nanoparticles showed a particle size of 185 nm and a PDI of 0.490, indicating the particle size falls within the desired range, and the PDI suggests a relatively broad size distribution Fig.-3.

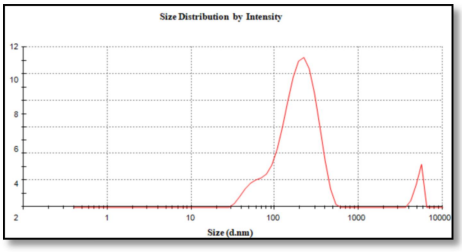

Fig.-3: Particle Size Distribution of EA Polymeric Nanoparticles (Zetasizer)

Statistics Analysis of Factorial Design Batches

Response 1: Entrapment efficiency showed a significant ANOVA model (F-value = 20.59, p = 0.0068, R^2 = 0.9392) as shown in Table-1 and 2, with significant factors including PVA (B), HPH cycle (C), and their interaction (BC). The polynomial equation was EE% = 98.12 + 0.2550B - 1.05C + 0.3188BC. Entrapment efficiency increased with higher PLGA and surfactant concentrations but decreased with more HPH cycles, ranging from 96 ± 0.21% to 99.15 ± 0.23%. Response surface plots visualised the relationships between variables, Fig.-4A.

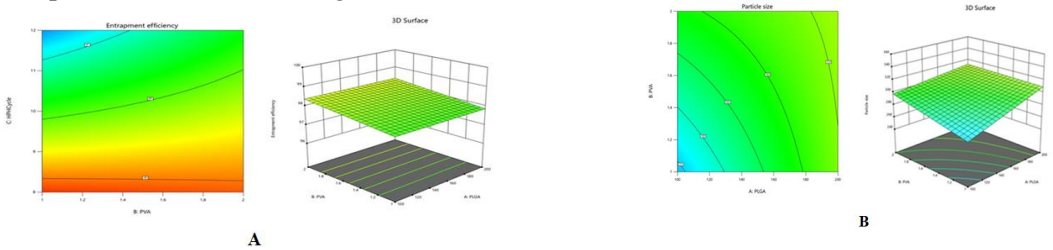

Fig.-4: (A.) 3-Dimensional Response Surface Plots and Contour Plot for the EE% of Polymeric Nanoparticle of EA and (B) for the Particle Size of Polymeric Nanoparticle of EA

Response 2: Particle size showed a significant ANOVA model (F-value = 21.71, p = 0.0150) with a strong fit (R^2 = 0.9666) as shown in Tables-5 and 6. Significant factors included PLGA (A), PVA (B), HPH cycle (C), and the interaction between PLGA and PVA (AB). The polynomial equation was Particle

Size = 296.81 + 13.94A + 8.31B - 22.13C - 6.56AB. Particle size ranged from 250 nm to 345.7 nm, increasing with higher polymer and surfactant concentrations but decreasing with more HPH cycles. Response surface plots visualised the relationships between variables, Fig.-4 B.

Table-2: Factorial Design Variables with Their Responses

Code	B1	B2	B3	B4	B5	B6	B7	B8
EE%	97	98.81	99.15	99.06	98.8	99.4	98.78	96
Particle Size (nm)	277.8	310.7	345.7	341.3	337.4	286.1	295	250

Table-3: Response 1 Entrapment Efficiency ANOVA Analysis

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.57	3	3.19	20.59	0.0068	significant
B-PVA	2.08	1	2.08	13.44	0.0215	
C-HPH Cycle	7.01	1	7.01	45.23	0.0025	
BC	3.25	1	3.25	20.99	0.0102	
Residual	0.6195	4	0.1549			
Cor Total	10.19	7				

Table-4: Values for EE% Fit Statistics

Std.Dev	0.3935	R^2	0.9392
Mean	98.38	Adjusted R^2	0.8936
C.V.%	0.400	Predicated R^2	0.7567
		Adeq Precision	9.8104

Table-5: Response 2 Particle Size ANOVA Analysis

Source	Sum of Squares	df	Mean Square	F-value	p-value	Source
Model	7940.50	4	1985.13	21.71	0.0150	significant
A-PLGA	1243.22	1	1243.22	13.59	0.0346	
B-PVA	2211.12	1	2211.12	24.18	0.0161	
C-HPH Cycle	3916.13	1	3916.13	42.82	0.0073	
AB	1378.12	1	1378.12	15.07	0.0303	
Residual	274.37	3	91.46			
Cor Total	8214.88	7				

Factor coding is coded.
The sum of squares is Type III - Partial.

Table-6: Fit Statistics

Std.Dev	9.56	R^2	0.9666
Mean	305.13	Adjusted R^2	0.9221
C.V.%	3.13	Predicated R^2	0.7625
		Adeq Precision	13.7226

Serum Formulation (SF)

FTIR

The FTIR spectra of the formulation showed characteristic peaks consistent with E.A., including O-H stretching (3319.166 cm^{-1}), C-H stretching (2910.237 cm^{-1}), and aromatic ring vibrations. A strong peak at 1776.44 cm^{-1} indicated the presence of PLGA, confirming successful formulation. These peaks collectively confirmed the molecular structure and functional groups in the formulation, as shown in Fig.-5.

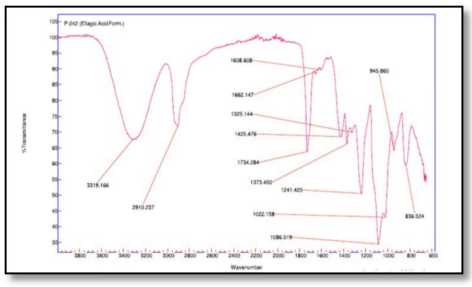

Fig.-5: FTIR of SF

XRD

The XRD analysis of the SF revealed a crystalline nature with sharp peaks, retaining major peaks characteristic of pure E. A (10.37°, 17.08-17.71°, 27.71°) with slight shifts or reduced intensity, indicating successful encapsulation and preservation of crystalline structure. As seen in Fig.-6.

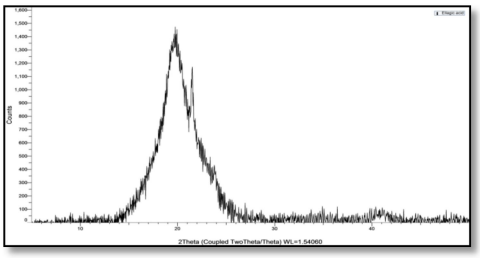

Fig.-6: XRD Graph of SF

Viscosity and Drug Content of SF

The SF showed a viscosity of 14,300 cP ± 0.1 and a drug content of 72.2%, indicating suitable consistency and satisfactory loading of ellagic acid for topical application.

***In-vitro* Study**

According to the in-vitro release profiles of the serum formulation, it was found that the formulation swelled and split due to the penetration of the diffusing media into the matrix, leading to the release of the drug. The optimised ellagic acid serum formulation showed enhanced solubility and permeation, resulting in up to 97.875% cumulative drug release at the end of 4 hours Fig.-7.

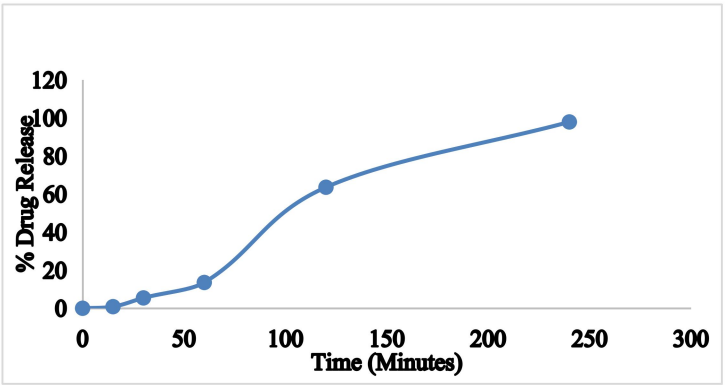

Fig.-7: *In-vitro* Release Profiles of Serum Formulation Containing EA

CONCLUSION

In this study, Ellagic Acid-loaded polymeric nanoparticles were successfully developed, optimised, and incorporated into a topical serum formulation. The nanoparticles demonstrated desirable particle size, polydispersity index, and high entrapment efficiency, while the serum formulation exhibited suitable viscosity, drug content, and sustained in-vitro release. These findings confirm the potential of the developed formulation as a safe and effective topical therapy. Based on the results, it is recommended that

future studies explore in vivo efficacy and stability studies to further validate the therapeutic potential and commercial applicability of the formulation. This work contributes to SDG 3 (Good Health and Well-being) by promoting the development of safe and effective therapeutic interventions through innovative, sustainable topical drug-delivery systems utilising plant-derived bioactive compounds.

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CONFLICT OF INTERESTS

The authors confirm that they have no competing interests related to this work.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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