RESEARCH PAPER

FORMULATION AND EVALUATION OF ELLAGIC ACID NANOETHOSOME LOADED IN-SITU GEL FOR THE MANAGEMENT OF ORAL ULCER IN DIABETIC ANIMAL MODEL

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ABSTRACT

The present study aimed to develop and evaluate an ellagic acid-loaded polymeric in-situ gel formulation for sustained topical drug delivery and enhanced therapeutic efficacy in oral ulcer management. Ellagic acid, a natural polyphenolic compound with antioxidant, anti-inflammatory, antimicrobial, and wound healing properties, suffers from poor aqueous solubility and limited bioavailability, which restrict its pharmaceutical application. To overcome these limitations, a thermoresponsive in-situ gel system was developed using Poloxamer 407, Hydroxypropyl Methylcellulose (HPMC), and Carbopol 934P.

Pre-formulation studies including organoleptic evaluation, solubility analysis, melting point determination, pH measurement, UV spectrophotometric analysis, and Fourier Transform Infrared Spectroscopy (FTIR) were carried out to characterize the drug and assess compatibility with excipients. The formulation was optimized using a Box–Behnken experimental design under response surface methodology by evaluating the effects of formulation variables on viscosity and drug release. The optimized formulation containing 20% Poloxamer 407, 1% HPMC, and 0.35% Carbopol 934P exhibited desirable physicochemical properties, rapid gelation, suitable viscosity, and sustained drug release behavior. The optimized in-situ gel showed a drug content of 98.36% and demonstrated approximately 97.73% drug release within 360 minutes. Drug release kinetics predominantly followed the Higuchi diffusion model, indicating diffusion-controlled release from the polymeric matrix. Scanning Electron Microscopy revealed a porous and interconnected gel network, while FTIR analysis confirmed the absence of chemical interaction between ellagic acid and the polymers. Stability studies conducted according to ICH guidelines demonstrated that the formulation remained physically and chemically stable under both long-term and accelerated storage conditions.

The developed formulation also exhibited concentration-dependent antimicrobial activity against Bacillus subtilis and Pseudomonas aeruginosa. Furthermore, in-vivo anti-ulcer studies in streptozotocin-induced diabetic rats demonstrated significant ulcer healing, improved epithelial regeneration, and reduction in ulcer diameter compared to untreated controls. The 1% ellagic acid gel showed superior therapeutic efficacy compared to the 0.5% formulation. Overall, the developed ellagic acid-loaded in-situ gel formulation demonstrated promising physicochemical characteristics, sustained drug release, antimicrobial activity, and anti-ulcer potential, suggesting its suitability as an effective topical drug delivery system for oral ulcer management.

Keywords: Ellagic acid; In-situ gel; Poloxamer 407; HPMC; Carbopol 934P; Oral ulcer; Sustained drug release; Antimicrobial activity; Anti-ulcer activity; Box–Behnken design; Thermoresponsive gel; Drug delivery system; Diabetic animal model.

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1. INTRODUCTION

Oral ulcers are among the most common lesions affecting the oral mucosa and are characterized by painful inflammation, tissue erosion, and delayed healing, particularly under compromised physiological conditions such as diabetes mellitus. Diabetes-associated oral ulcers exhibit prolonged healing due to impaired angiogenesis, excessive oxidative stress, reduced collagen synthesis, and microbial infection, which collectively interfere with the normal wound healing process. Conventional topical therapies often provide only temporary relief and suffer from limitations such as poor retention time, inadequate drug penetration, and frequent application requirements. Therefore, there is a growing need for novel drug delivery systems capable of improving local drug retention, sustained release, and therapeutic efficacy **(Scully and Porter, 2008; Guo and DiPietro, 2010).**

Ellagic acid is a naturally occurring polyphenolic compound abundantly present in fruits and medicinal plants such as pomegranate, berries, and walnuts. It possesses significant pharmacological properties including antioxidant, anti-inflammatory, antimicrobial, anticancer, and wound healing activities. Several studies have demonstrated that ellagic acid can effectively scavenge free radicals, reduce inflammatory mediators, and accelerate tissue regeneration, making it a promising candidate for the management of oral ulcers and other inflammatory conditions **(Vattem and Shetty, 2005; Sepúlveda et al., 2011).** However, the therapeutic application of ellagic acid is limited by its poor aqueous solubility and low bioavailability, which reduce its effectiveness in conventional dosage forms **(Lei et al., 2003).** To overcome these limitations, the development of polymeric in-situ gel systems has gained considerable attention in pharmaceutical research. In-situ gels are liquid formulations that undergo sol-to-gel transformation upon exposure to physiological stimuli such as temperature, pH, or ionic concentration. These systems combine the advantages of both liquid and semisolid dosage forms by allowing easy administration while forming a gel matrix at the site of application, thereby enhancing residence time and controlled drug release **(Dixit and Maurya, 2012).** Thermoresponsive polymers such as Poloxamer 407 are widely used in in-situ gel formulations due to their ability to undergo gelation at body temperature. Additionally, polymers such as Hydroxypropyl Methylcellulose (HPMC) and Carbopol 934P improve viscosity, mucoadhesion, and structural integrity of the gel system, contributing to prolonged drug retention and sustained therapeutic action **(Schmolka, 1972; Bodmeier et al., 1989).**

The present study was therefore designed to develop and evaluate an ellagic acid-loaded polymeric in-situ gel formulation for sustained topical drug delivery. The formulation was optimized using Box–Behnken experimental design under response surface methodology to investigate the influence of formulation variables on viscosity and drug release behavior. Various pre-formulation, physicochemical, morphological, in-vitro, antimicrobial, and in-vivo evaluations were performed to assess the suitability of the developed formulation for oral ulcer management. The developed in-situ gel system is expected to improve drug retention at the site of application, provide sustained release of ellagic acid, and enhance therapeutic efficacy in diabetic ulcer conditions.

2. MATERIALS AND METHODS

2.1 Pre-formulation Studies

Pre-formulation studies of ellagic acid were carried out to evaluate its physicochemical properties including organoleptic characteristics, solubility, melting point, pH, λmax, and
Fourier Transform Infrared (FTIR) spectroscopy. Organoleptic properties such as color, odor, and appearance were visually examined. Solubility studies were performed in different solvents including water, ethanol, methanol, chloroform, and DMSO **(Jouyban and Fakhree, 2012).** The melting point was determined using a digital melting point apparatus **(Young, 2013).** The pH of the drug solution was measured using a calibrated digital pH meter **(Vazquez et al., 2018).** The λmax of ellagic acid was determined using a UV–Visible spectrophotometer by scanning the drug solution in the range of 200–400 nm. A calibration curve was prepared in methanol within the concentration range of 2–14 µg/mL **(Bala et al., 2006).** FTIR spectroscopy was performed to identify characteristic functional groups and evaluate compatibility with excipients **(Saputera, 2021).**

2.2 Formulation and evaluation of Nano Ethosome

The Nano ethosomal system of drug composed 1%, 5% Soya lecithin, 10% propylene glycol, 35% ethyl alcohol, 1% cholesterol, and aqueous phase to 100% w/w. The drug along with the phospholipid and cholesterol were dissolved in ethyl alcohol. The suspension was stirred with a magnetic stirrer at 700 rpm with heating at 30°C for 30 min. After that propylene glycol was added to this solution and the stirring was continued for further 5 min. In a separate vessel, water was heated to 30°C. Once both mixtures reached 30°C, at a rate of 1 ml/min, water was added through a syringe. Stirring was continued for additional 30 min. The final milky suspension was left to cool at room temperature and then stored in refrigerator until further investigation **(El-Shenawy et al.,2019).**

Table 1: Final Composition of Nano ethosomes formulation

S. No	Ingredients	Concentration (%)
1	Ellagic acid	100mg/ml
2	Soya lecithin (Phospholipid)	5.0%
3	Cholesterol	1.0%
4	Ethyl alcohol	35%
5	Propylene Glycol	10.0%
6	Distilled water	10.0ml

2.3 Characterization of Nano Ethosomes

The prepared nano ethosomes were characterized for particle size and zeta potential using a Malvern Zetasizer. Surface morphology was examined using Scanning Electron Microscopy (SEM). Entrapment efficiency was determined by centrifugation followed by UV spectrophotometric analysis of the unentrapped drug.

2.4 Formulation of in situ gelling system

The method of preparation of in situ gel involved slow addition of polymers (Poloxamer 407and HPMC), and methyl paraben were solubilized in required quantity of cold deionized water. An appropriate amount of (1.0%) nano formulation was solubilized in polymer solution with continuous stirring until uniform drug solution obtained. Required quantities of Carbopol 934P were added into the solution with continuous stirring by mechanical stirrer until uniform solution obtained. The final solution was kept overnight in refrigerator at 5°C to completely dissolve polymers in solution, and then addition of small amount of triethanolamine (TEA) was added to adjust the pH.

Experimental design Box behnken design (BBD) was employed for systemic study of joint influence of the effect of independent variables [Poloxamer 407 (X1), HPMC (X2) and Carbopol (X3)] on responses such as Viscosity (cPs) and cumulative percentage release (CPR)

at 120 minute and time for drug release. Based on preliminary trials, three factors were determined as follows: Poloxamer 407 (X1): 15–20% w/v and HPMC (X2): 0.5-1% w/v and Carbopol 934P (X3): 0.2-0.5% w/v. In this design, three factors with two levels were probed to investigate the main effects and interaction of the three factors on two responses [Table]. The design consists of fifteen runs yielding 15 experiments in total. The main purpose of the replication runs was to increase the precision and to minimize experimental error **(Garala et al., 2013, Balu et al., 2020).**

2.4.1 Composition of in situ gel

Table 2: Composition of In situ gel formulation

S. No	Formulation code	Poloxamer 407 (%)	HPMC (%)	Carbopol 934P (%)	Nanoethosomes (ml)	Triethanolamine (Drop)	Distilled Water (ml)
1	ISGS 1	17.5	0.75	0.35	1.0	1-2	10.0
2	ISGS 2	20	0.75	0.5	1.0	1-2	10.0
3	ISGS 3	20	0.75	0.2	1.0	1-2	10.0
4	ISGS 4	15	0.75	0.2	1.0	1-2	10.0
5	ISGS 5	17.5	1	0.2	1.0	1-2	10.0
6	ISGS 6	20	0.5	0.35	1.0	1-2	10.0
7	ISGS 7	17.5	0.75	0.35	1.0	1-2	10.0
8	ISGS 8	15	1	0.35	1.0	1-2	10.0
9	ISGS 9	17.5	0.5	0.2	1.0	1-2	10.0
10	ISGS 10	15	0.75	0.5	1.0	1-2	10.0
11	ISGS 11	15	0.5	0.35	1.0	1-2	10.0
12	ISGS 12	17.5	0.75	0.35	1.0	1-2	10.0
13	ISGS 13	17.5	1	0.5	1.0	1-2	10.0
14	ISGS 14	17.5	0.5	0.5	1.0	1-2	10.0
15	ISGS 15	20	1	0.35	1.0	1-2	10.0

2.4.2 Experimental Design and Optimization

A Box–Behnken Design (BBD) was employed for optimization of the formulation using Design Expert® software. Poloxamer 407 (X1), HPMC (X2), and Carbopol 934P (X3) were selected as independent variables, while viscosity and percentage drug release were selected as dependent responses. Fifteen experimental runs were generated and evaluated statistically to determine the optimized formulation **(Balu et al., 2020).**

2.4.3 Evaluation of In Situ Gel

The prepared formulations were evaluated for physical appearance, pH, viscosity, gelation capacity, gel strength, drug content, SEM analysis, and in-vitro drug release. Viscosity was measured using a Brookfield Digital Viscometer **(Dabhi et al., 2010).** Drug content and in- vitro release studies were carried out using UV–Visible spectrophotometry at 256 nm **(Garala et al., 2013).** In-vitro drug release was performed using a Franz diffusion cell apparatus with phosphate buffer pH 6.8 as receptor medium **(Rahman et al., 2015).** Release kinetics were analyzed using Zero-order, First-order, Higuchi, and Korsmeyer Peppas models **(Dash et al., 2010; Kumar et al., 2019).**

2.3.4 Stability Study

The optimized formulation was subjected to stability studies according to International Council for Harmonisation (ICH) guidelines under long-term and accelerated storage conditions for

three months. The formulations were evaluated periodically for physical appearance, pH, viscosity, and drug release **(Balu et al., 2020).**

2.3.5 In-vitro Antimicrobial Study

Antimicrobial activity of the optimized formulation was evaluated using the agar well diffusion method against *Bacillus subtilis* and *Pseudomonas aeruginosa*. The zones of inhibition were measured after incubation at 37°C for 24 h **(Balouiri et al., 2016).** Minimum inhibitory concentration (MIC) was determined using the broth dilution method **(Parvekar et al., 2020).**

2.3.6 In-vivo Anti-ulcer Activity

The anti-ulcer activity study was conducted after obtaining approval from the Institutional Animal Ethics Committee (CPCSEA), Adina Institute of Pharmacy (Approval Number: **1652/PO/Re/S/11/CCSEA**). Wistar albino rats weighing 180–250 g were selected for the experiment. The animals were maintained under controlled conditions at a temperature of 22 ± 1°C with a 12-hour light/dark cycle. They were provided with standard pellet diet and water ad libitum.

The anti-ulcer activity was evaluated in streptozotocin-induced diabetic Wistar rats using an acetic acid-induced oral ulcer model. Animals were divided into control, standard, and treatment groups. Ulcer diameter, body weight, and epithelial thickness were evaluated at predetermined intervals. Histopathological examination was performed using hematoxylin and eosin staining to assess re-epithelialization and tissue healing **(Cavalcante et al., 2011; Novianty et al., 2011).**

3. RESULT AND DISCUSSION

3.1 pre-formulation study of drug

3.1.1 Solubility study

Table 3: Solubility study of Ellagic acid

Drug	Solvents	Observation
Ellagic acid	Water	Poorly soluble
	Ethanol	Sparingly soluble
	Methanol	Freely soluble
	Chloroform	Slightly Soluble
	DMSO	Freely Soluble

3.1.2 Melting point, pH and Lambda max analysis

Table 4: Melting point, pH and Lambda max of Ellagic acid

Sr. No.	Drugs	Observed Melting point	Reference Melting point	Observed pH	Reference pH	UV absorption maxima (Lambda max)
1	Ellagic acid	354.23 °C	350°C to 364°C	5.98 Ph	5.6 to 6.76 pH	256.0 nm

3.1.3 Calibration curve of Ellagic acid

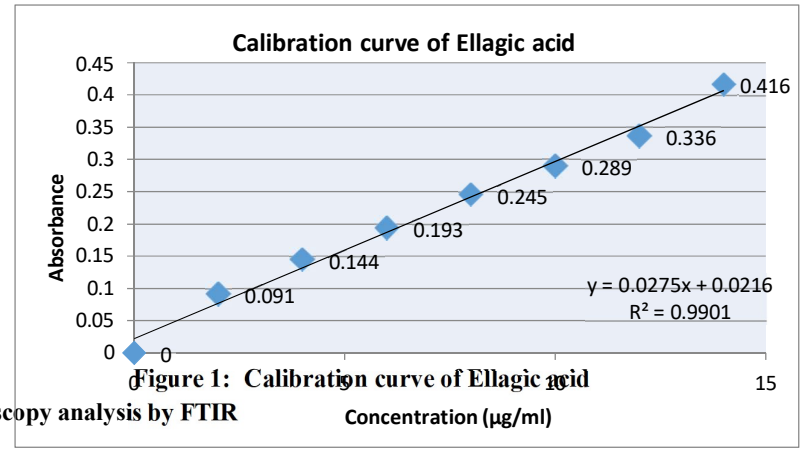


Figure 1: Calibration curve of Ellagic acid

3.1.4 Spectroscopy analysis by FTIR

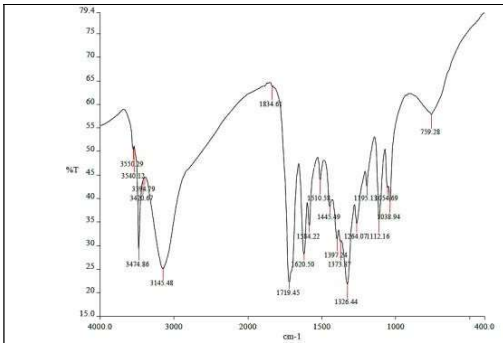


Figure 2: Ellagic acid

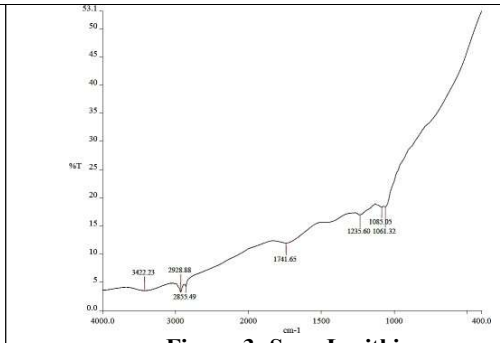


Figure 3: Soya Lecithin

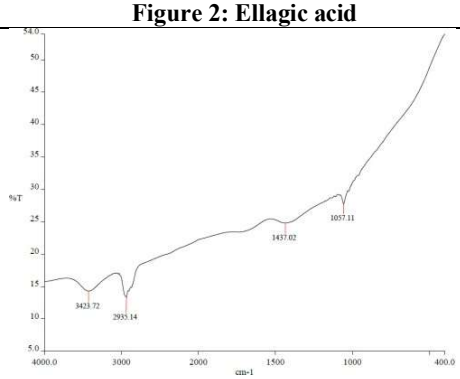


Figure 4: Cholesterol

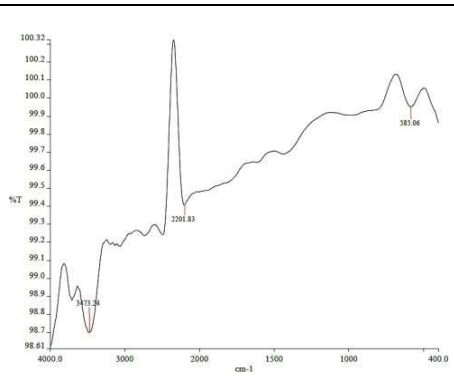


Figure 5:HPMC FTIR

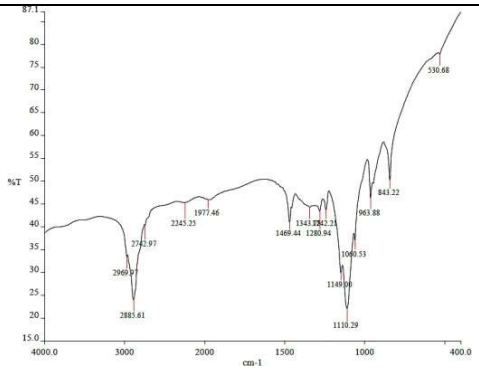


Figure 6: Poloxamer FTIR

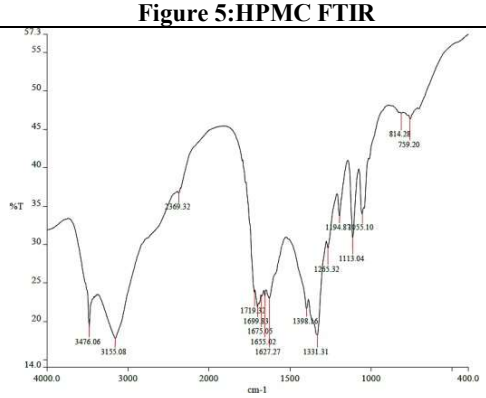
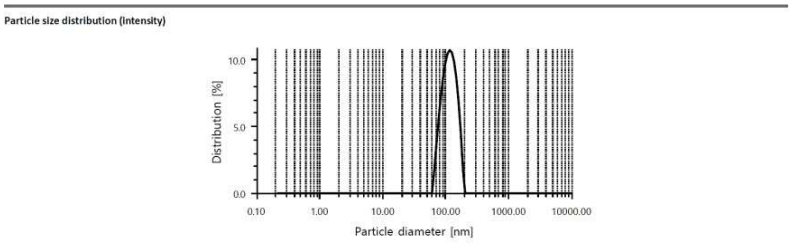


Figure 7: Mixture FTIR with drug

3.2 Characterization of nano formulation

3.2.1 Particle size Distribution



Result			
Hydrodynamic diameter	159.02 nm	Mean intensity	314.1 kcounts/s
Polydispersity index	16.6 %	Absolute intensity	6300.1 kcounts/s
Diffusion coefficient	4.9 µm²/s	Intercept g1²	0.4096
Transmittance	82.3 %	Baseline	1.059

Figure 8: Particle size of optimized formulation

3.2.2 Zeta potential determination

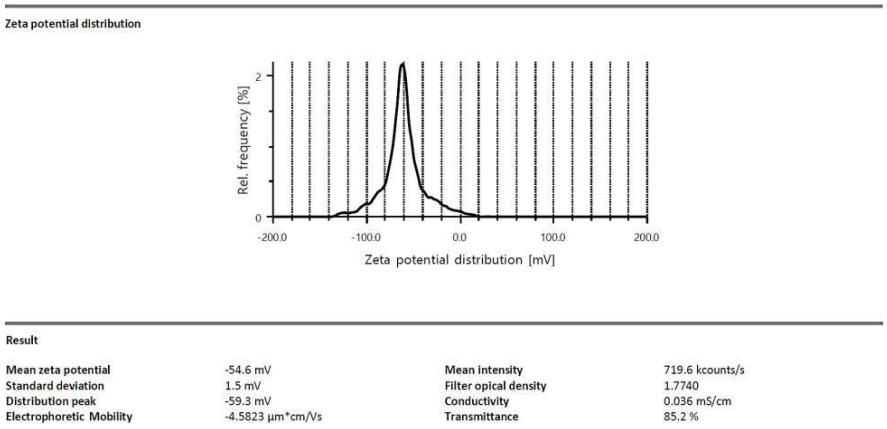


Figure 9: Zeta potential

Table 5: Particle size, Zeta potential and Entrapment efficacy

S. No	Formulation	Particle size	Entrapment efficacy	Zeta potential
1.	Nano formulation	159.02 nm	98.34%	-54.6 mV

3.2.3 SEM analysis

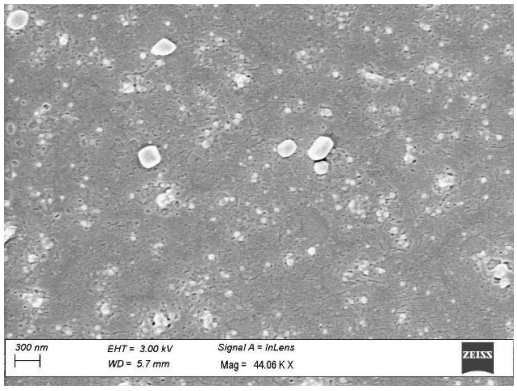


Figure 10: SEM analysis of optimized formulation

3.3 Build information of software Table 6: Build Information

File Version	12.0.1.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	15
Design Model	Quadratic	**Blocks**	No Blocks

3.4 Formulation trials as per Box-Behnken design Table 7: Formulation trials

S. No	Formulation code	Factor 1: Poloxamer 407 (%)	Factor 2: HPMC (%)	Factor 3: Carbopol 934P (%)	Response 1: Viscosity (cPs)	Response 2: % drug release of drug (%)
1	ISGS 1	17.5	0.75	0.35	2686.98	89.5
2	ISGS 2	20	0.75	0.5	3015.25	97.56
3	ISGS 3	20	0.75	0.2	2255.96	95.77
4	ISGS 4	15	0.75	0.2	2369.32	81.29
5	ISGS 5	17.5	1	0.2	2468.2	88.52
6	ISGS 6	20	0.5	0.35	2487.35	96.23
7	ISGS 7	17.5	0.75	0.35	2764.8	91.48
8	ISGS 8	15	1	0.35	2623.11	82.32
9	ISGS 9	17.5	0.5	0.2	2201.63	90.67
10	ISGS 10	15	0.75	0.5	3023.17	81.22
11	ISGS 11	15	0.5	0.35	2856.02	82.53
12	ISGS 12	17.5	0.75	0.35	2745.86	90.55
13	ISGS 13	17.5	1	0.5	3166.86	89.63
14	ISGS 14	17.5	0.5	0.5	2985.12	90.85
15	ISGS 15	20	1	0.35	2881.38	97.82

3.5 Fit Summary

Table 8 Response 1: Viscosity

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
2FI	0.0024	0.3757	0.9677	0.9223	Suggested

3.5.1 ANOVA for 2FI model Table 9: Response 1: Viscosity

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	1.204E+06	2.006E+05	70.82	< 0.0001	Significant

3.5.2 Fit Statistics

Table 10: Regression coefficient (R^2) value

Std. Dev.	**53.23**	**R^2**	0.9815
Mean	2702.07	**Adjusted R^2**	0.9677
C.V. %	1.97	**Predicted R^2**	0.9223
		Adeq Precision	24.0975

3.5.3 Final equation in term of coded factor

Viscosity (R1) = +2702.07 Intercept -28.96 AX1+76.18 BX2 +361.91CX3 +156.74 ABX1X2+26.36AX1X3-21.21BCX2X3 OR 2702.07 Intercept -28.96 Poloxamer 407 Concentration+76.18 HPMC Concentration +361.91 Carbopol 934P+ 156.74 Poloxamer and HPMC Concentration +26.36 Poloxamer and Carbopol 934P -21.21 HPMC and Carbopol 934P

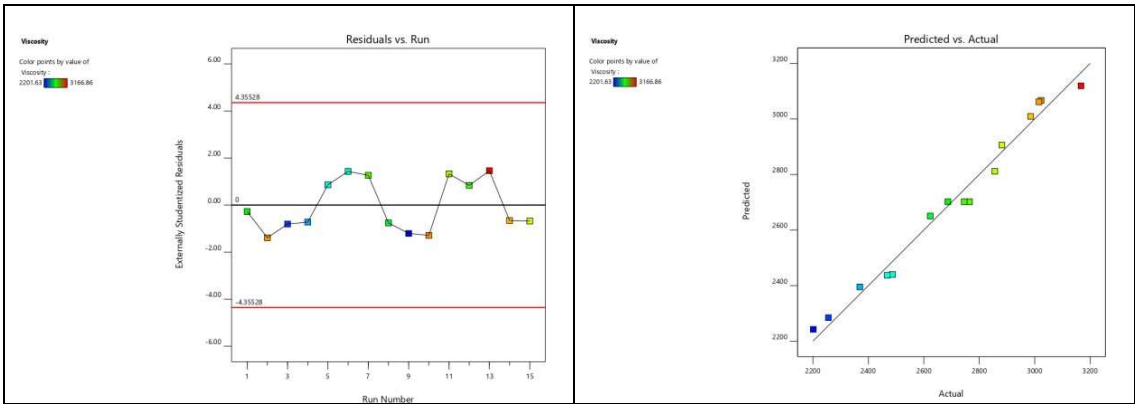


Figure 11: Two-dimensional response surface plots (predicted vs actual and residual vs. run of viscosity) revealing relative effects of independent variables on dependent variable viscosity of drug loaded formulation

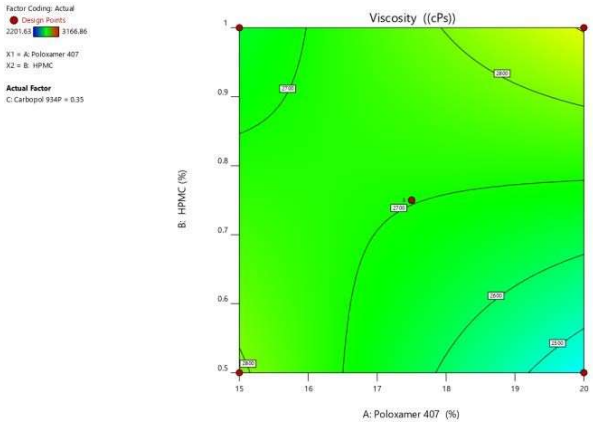


Figure 12: Two-dimensional (2D) contour plots for the effect of Poloxamer 407 and HPMC Concentration on viscosity

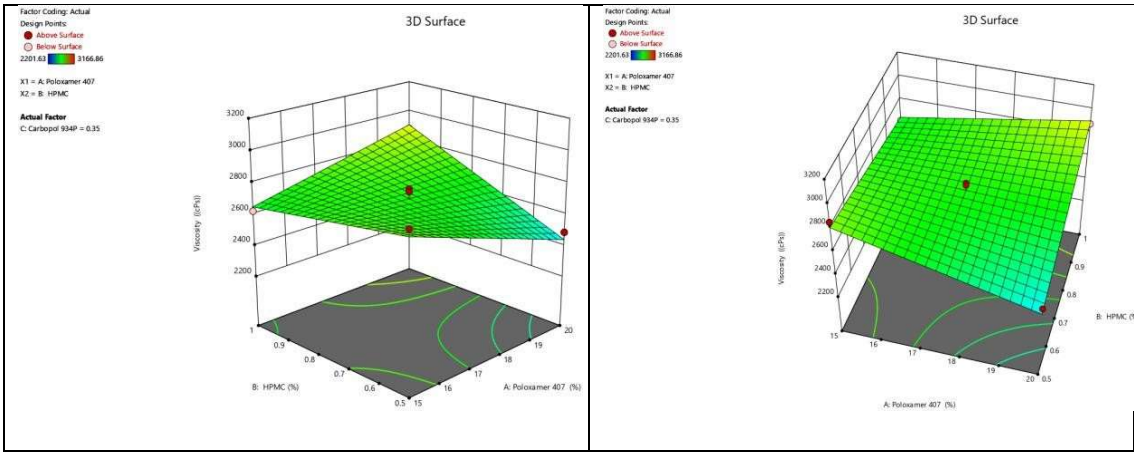


Figure 13: Three-dimensional (3D) Response surface plot showing combined effect of Poloxamer 407 and HPMC on viscosity of in situ gel formulation

3.6 Effect of formulation variables on % drug release study of in situ gel formulation Table 11: Response 2: Drug release study (Fit Summary)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.5740**	**0.9688**	**0.9556**	**Suggested**

3.6.1 ANOVA for Linear model of Drug release study

Table 12: Response 2: Drug release study (ANOVA Linear model)

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	451.93	150.64	145.83	< 0.0001	Significant

3.6.2 Fit Statistics

Table 13: Statics R2 Value

Std. Dev.	1.02	R^2	0.9755
Mean	89.73	**Adjusted R^2**	0.9688
C.V. %	1.13	**Predicted R^2**	0.9556
		Adeq Precision	30.0233

3.6.3 Final Equation in Terms of Coded Factors

% Drug release study (R2) = +89.73 Intercept +7.50 AX1-0.2488 BX2+0.3762 CX3 OR +89.73 Intercept +7.50A- Poloxamer 407 concentration -0.2488 B- HPMC concentration 0.3762 C- Carbopol 934P concentration.

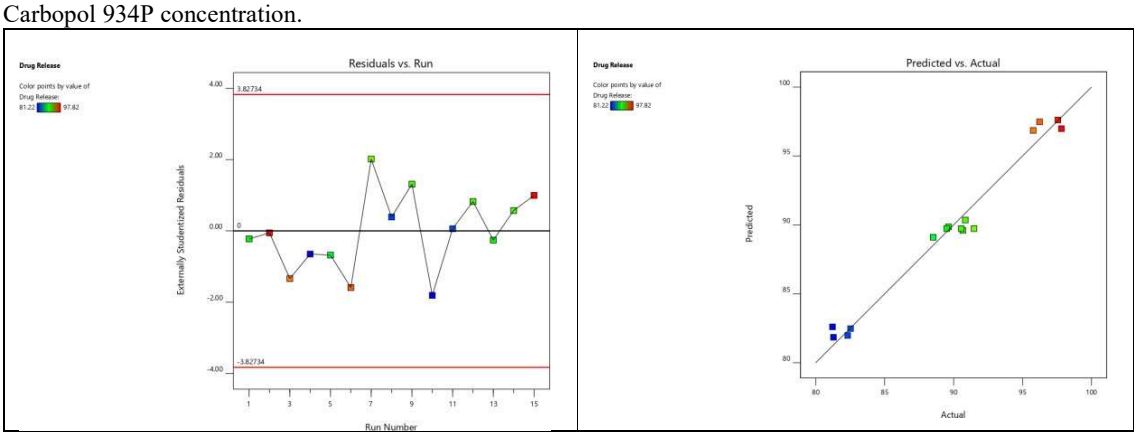


Figure 14: Two-dimensional response surface plots (predicted and actual and residual vs. run of % Drug release study) revealing relative effects of independent variables on dependent variable of drug loaded formulation

3.6.4 Predicted and observed result of % drug release study and (Viscosity R^1) Table 14: Predicted and observed result

S. No	Formulation code	Observed result of % Drug release	Predicted result of % Drug release	Actual Value of viscosity	Predicted Value of viscosity
1	ISGS 1	89.50	89.73	2686.98	2702.07
2	ISGS 2	97.56	97.61	3015.25	3061.38
3	ISGS 3	95.77	96.86	2255.96	2284.84
4	ISGS 4	81.29	81.85	2369.32	2395.48
5	ISGS 5	88.52	89.10	2468.20	2437.54
6	ISGS 6	96.23	97.48	2487.35	2440.19
7	ISGS 7	91.48	89.73	2764.80	2702.07
8	ISGS 8	82.32	81.98	2623.11	2650.47
9	ISGS 9	90.67	89.60	2201.63	2242.77
10	ISGS 10	81.22	82.60	3023.17	3066.58
11	ISGS 11	82.53	82.48	2856.02	2811.58
12	ISGS 12	90.55	89.73	2745.86	2702.07
13	ISGS 13	89.63	89.86	3166.86	3118.95
14	ISGS 14	90.85	90.35	2985.12	3009.01
15	ISGS 15	97.82	96.98	2881.38	2906.02

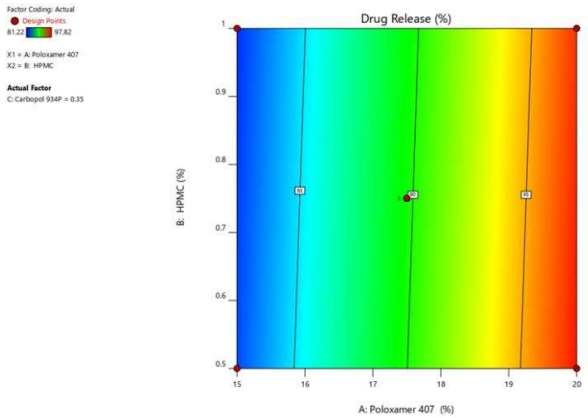


Figure 15: Two-dimensional (2D) contour plots for the effect of Poloxamer 407 and HPMC Concentration on drug release study

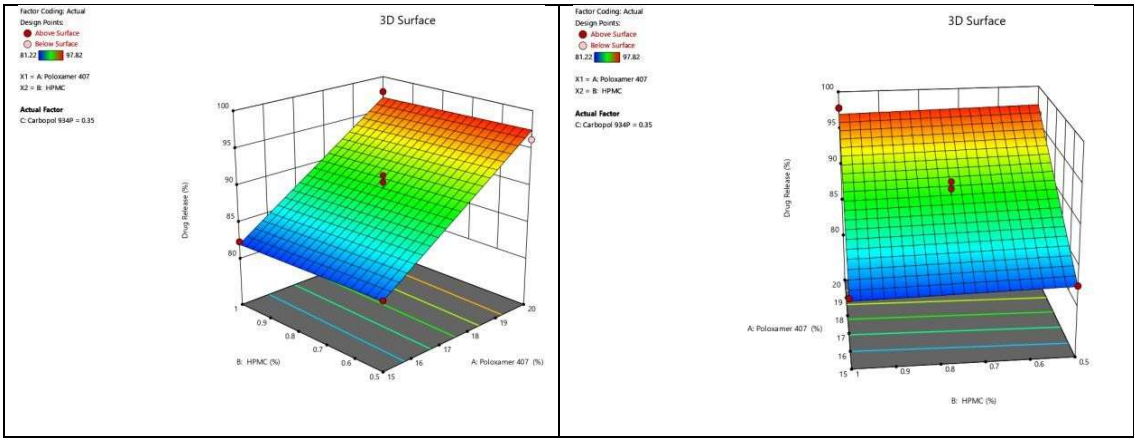


Figure 16:- Three-dimensional (3D) Response surface plot showing combined effect of Poloxamer 407 and HPMC on drug release of in situ gel formulation

3.6.5 Limits

Table 15: Limit of Variables

Name	Goal	Lower Limit	Upper Limit	Importance
A: Poloxamer 407	is in range	15	20	3
B: HPMC	is in range	0.5	1	3
C: Carbopol 934P	is in range	0.2	0.5	3
Viscosity	none	2201.63	3166.86	3
Drug Release	none	81.22	97.82	3

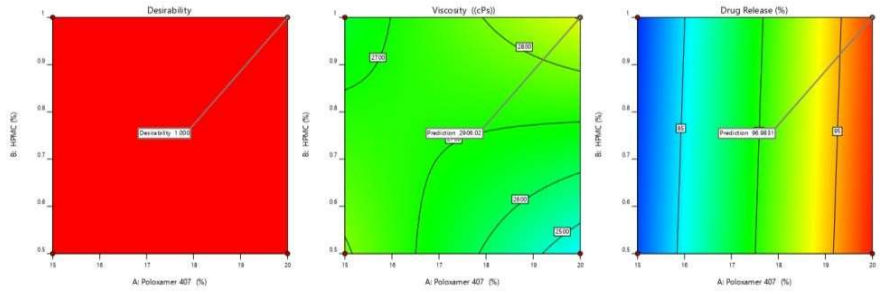


Figure 17: Response surface plot showing prediction data for optimization

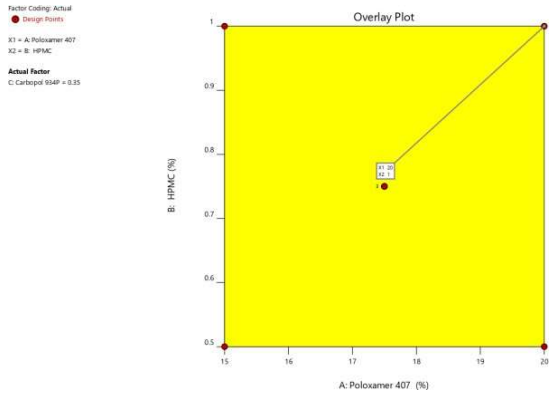


Figure 18: Overlay plots of optimization formulation

3.6.6 Optimized formulation

Table 16: Optimized formula by DOE

S. No	Poloxamer 407 concentration	HPMC concentration	Carbopol 934P	Viscosity	Drug Release	Desirability	
1	**20.000**	**1.000**	**0.350**	**2906.021**	**96.983**	**1.000**	**Selected**
2	17.500	0.500	0.500	3009.007	90.354	1.000	
3	15.000	0.750	0.500	3066.579	82.603	1.000	

3.7 Final Composition of Optimized formulations

Table 17: Final Composition of Optimized formulations

S. No	Formulation code	Poloxamer 407 (%)	HPMC (%)	Carbopol 934P (%)	Ellagic acid (%)	Triethanolamine (Drop)	Distilled Water (ml)	Methyl paraben (%)
1.	ISGS	20.000	1.000	0.350	1.0	1-2	10.0	0.02

3.8 Evaluation parameter optimized formulation

3.8.1 Physical appearance result of drug-loaded formulation Table 18: Physical appearance

Formulation	Color	Odor	Appearance
In situ gel formulation	Off-white	Characteristic	Smooth and uniform

3.8.2 pH determination

Table 19: pH, Drug content and Viscosity of in Situ gel formulation

S. No	Formulation	Observed	Drug content	Predicted viscosity result (cps)	Actual Results (cps)
1.	Gel	6.38 pH	98.36 %	2906.021	2952.28

3.8.3 The *in-vitro* gelation study of in situ gel formulation Table 20: gelation study of in situ gel formulation

S. No.	Parameter Evaluated	Observation / Result	Interpretation
1	Appearance before gelation	Clear, free-flowing liquid	Suitable for administration
2	Gelation Time (sec)	8–15 seconds	Rapid sol-to-gel transition
3	Gelation Capacity	+++	Immediate gel formation and remained stable for extended period
4	Gel Strength (sec)*	45–65 seconds	Adequate mechanical strength
5	Physical Stability of Gel	No immediate erosion	Good structural integrity
6	Viscosity (Post-gelation)	Significant increase	Confirms gel network formation

3.8.4 SEM analysis of optimized formulation

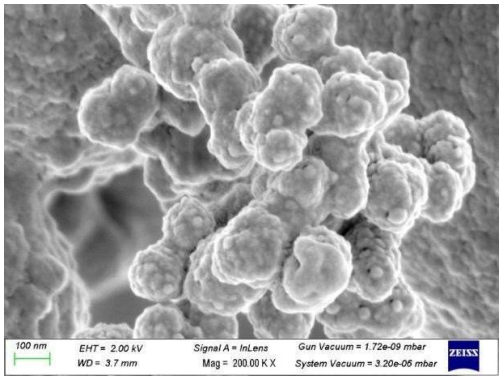


Figure 19: SEM of In situ gel formulation

3.8.5 Fourier Transforms Infrared Spectroscopy (FTIR of formulation)

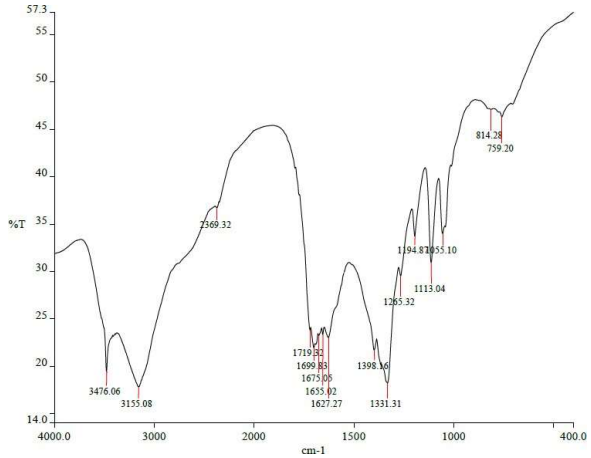


Figure 20: FTIR of In-situ gel formulation

3.8.6 *In Vitro* drug release study Table 21:

***In-vitro* drug release studies**

S. No	Time (min.)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
1.	0	0	100	0.000	2.000	0.000	0.000
2.	30	23.11	76.89	5.477	1.886	1.477	1.364
3.	60	36.1	63.9	7.746	1.806	1.778	1.558
4.	90	43.13	56.87	9.487	1.755	1.954	1.635
5.	120	57.59	42.41	10.954	1.627	2.079	1.760
6.	180	66.83	33.17	13.416	1.521	2.255	1.825
7.	240	78.17	21.83	15.492	1.339	2.380	1.893
8.	300	82.41	17.59	17.321	1.245	2.477	1.916
9.	360	97.73	2.27	18.974	0.356	2.556	1.990

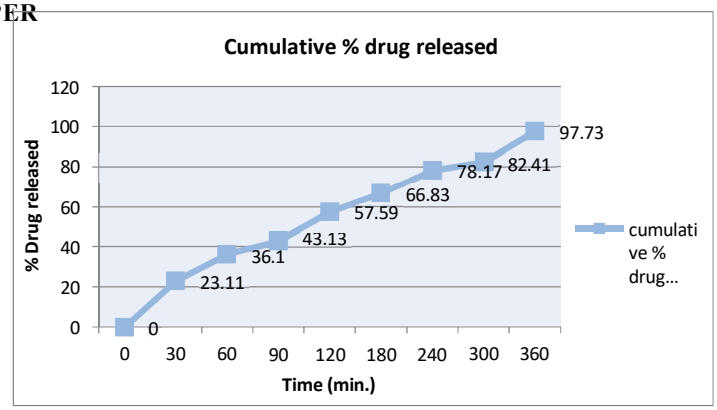


Figure 21: Drug release study Table 6.1: Correlation value (R^2 value)

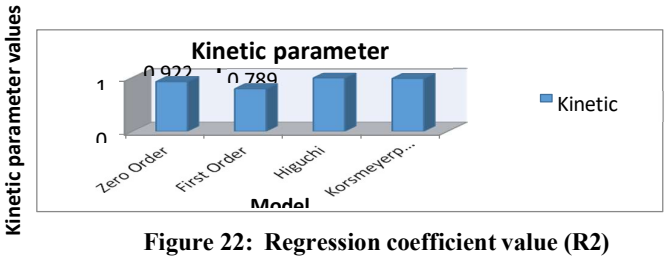


Figure 22: Regression coefficient value (R2)

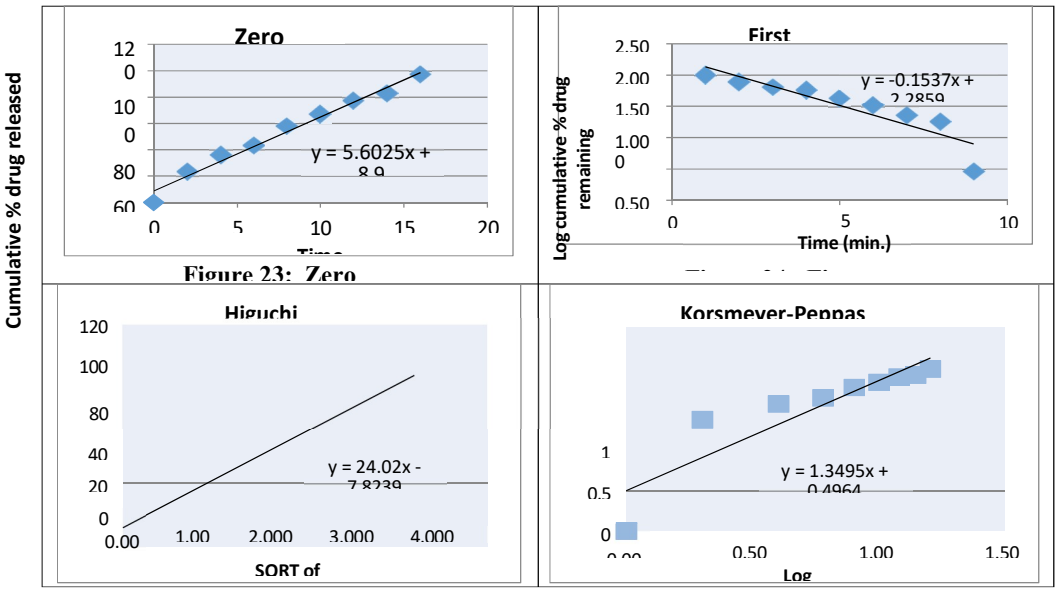


Figure 23: Zero

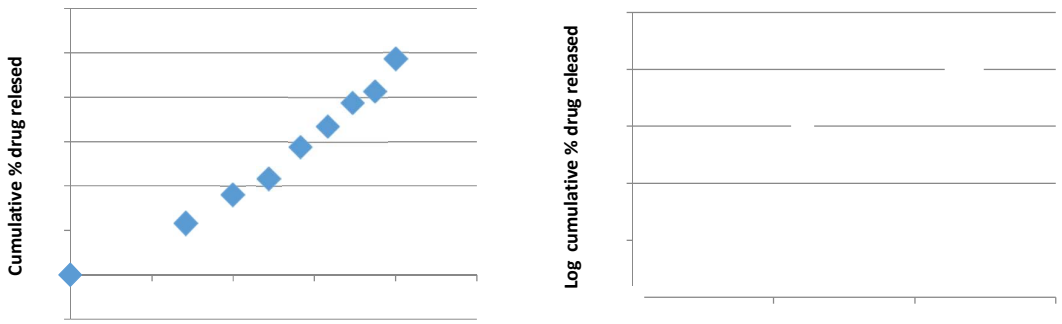


Figure 25: Higuchi	Figure 26: Korsmeyerpeppas

3.8.7 Stability study of optimized In situ gel formulation Table 22:

Result of Stability study

S. No	Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH			40^0C±2 ^{0}C and 70 ±5% RH		
		Viscosity (cps)	pH	*In-vitro* release studies (%)	Viscosity (cps)	pH	*In-vitro* release studies (%)
1.	0	2952.28	6.7	97.73	2952.28	6.7	97.73
2.	30	2948.56	6.7	97.71	2958.63	6.5	97.70
3.	45	2945.78	6.6	97.69	2977.35	6.7	91.70
3.	60	2928.08	6.8	97.70	2985.28	6.8	97.65
4.	90	2866.17	6.6	97.70	2995.35	6.9	97.50

***3.9 In-Vitro* Study antimicrobial activity**

3.9.1 Well diffusion assay

Table 23: Antimicrobial activity of Ellagic acid gel formulation against gram positive bacteria (*Bacillus subtilis)*

Sample	Different concentrations			
	25 µg/ml	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**
1% Ellagic acid gel	0 mm	0 mm	0 mm	11.2 mm

Table 24: Antimicrobial activity of Ellagic acid gel formulation against gram negative bacteria (*P. aureoginosa)*

Sample	Different concentrations			
	25 µg/ml	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**
1% Ellagic acid gel	0 mm	0 mm	6.5 mm	12.4 mm

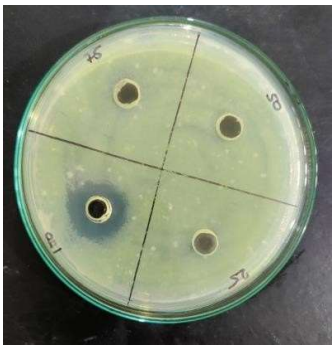


Figure 27: Antimicrobial activity of Ellagic acid gel formulation against gram positive bacteria (Bacillus Subtilis)

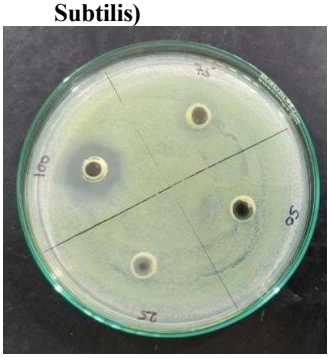
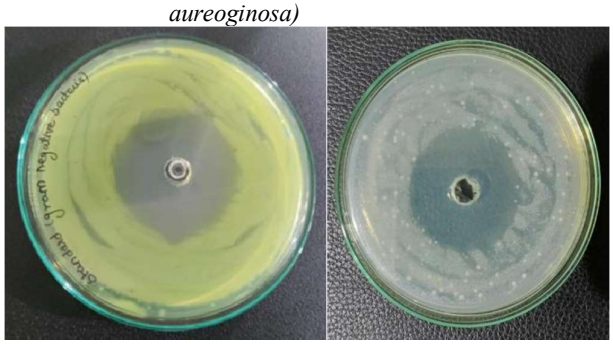


Figure 28: Antimicrobial activity of Ellagic acid gel formulation against gram negative bacteria *(P. aureoginosa)*



(a) **(b)**

Figure 29: (a) Antimicrobial activity of standard (Ofloxacin) against Gram negative (b) Antimicrobial activity of standard (Amoxycillin) against Gram positive

3.9.2 MIC of *Ellagic acid Gel*

Table 25: MIC against *Bacillus subtilis and P. aeruginosa*

S.NO	Concentration (µg/ml)	Absorbance (640 nm) MIC against *Bacillus subtilis*	Absorbance (640 nm) MIC against *P. aeruginosa*
1.	100	0.123	0.127
2.	50	0.154	0.171
3.	12.5	0.184	0.154
4.	6.25	0.238	0.194
5.	3.12	0.267	0.223
6.	1.56	0.291	0.257
7.	0.78	0.332	0.286
8.	0.39	0.376	0.326
9.	0.19	0.403	0.351

3.9.3 MIC of standard (Amoxycillin) against gram positive bacteria

Table 26: Results of MIC of standard (Amoxycillin) against gram positive bacteria

S.NO	Concentration (µg/ml)	Absorbance (640 nm)
1.	25	0.036
2.	12.5	0.062
3.	6.25	0.091
4.	3.12	0.114
5.	1.56	0.127
6.	0.78	0.139
7.	0.39	0.191
8.	0.19	0.150
9.	0.09	0.163
10	0.004	0.188

3.9.4 MIC of standard (Ofloxacin) against gram negative bacteria

Table 27: Results of MIC of standard (Ofloxacin) against gram negative bacteria

S.NO	Concentration (µg/ml)	Absorbance (640 nm)
1.	25	0.029
2.	12.5	0.053
3.	6.25	0.086
4.	3.12	0.102
5.	1.56	0.133
6.	0.78	0.159
7.	0.39	0.176
8.	0.19	0.161
9.	0.09	0.170
10	0.004	0.181

3.10 *In-Vivo* Anti Ulcer Activity Table 28:

Effect on weight of animals

Group	Weight of animals (mean ± S.D.) On Day				
	0	3	6	9	12
A (Control)	198±0.47	184±0.94	177±0.67	180±1.31	216.51±2.93
B (Standard)	238±0.67	227±0.73	233±1.03	240±1.31	240.34±1.78
C (gel 1%)	236±075	222±0.60	232±0.76	238±0.76	238.75±2.95
D (Gel 0.5%)	198±0.47	184±0.94	177±0.67	180±1.31	224.1±1.49

Table 29: Effect on Ulcer Diameter

Group	Ulcer diameter (mean ±S.D.) On Day				
	0	3	6	9	12
A (Control)	4.09±0.3	5.81±0.26	6.31±0.55	4.09±0.3	3.14±0.26
B (Standard)	4.31±0.26	2.0±0.3	1.14±0.26	0	0
C (gel 10%)	4.3±0.76	3.0±0.86	2.0±0.3	4.09±0.3	0
D (Gel 5%)	4.09±0.3	4.08±0.26	4.31±0.55	3.3±0.3	2.30±0.26

Table 30: Effect on thickness of epithelium

Day	Group	N	Thickness of epithelium (mm)			Mean	S,D
3	A (Control)	3	22.15	18.99	12.66	17.933	4.832
6	B (Standard)	3	189.9	227.88	221.15	212.976	20.266
9	C (gel 10%)	3	164.58	132.93	145.59	147.7	15.930
12	D (Gel 5%)	3	151.92	145.59	132.93	143.48	9.669

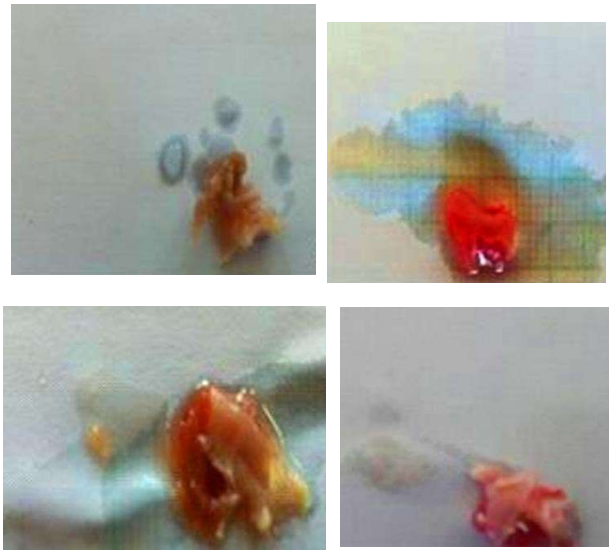


Figure 30: Anti Ulcer Activity- A. Isolation of buccal mucosa from animal of control group (Group A); B. Isolation of buccal mucosa from animal of standard group (Group B); C. Isolation of buccal mucosa from animal of Ellagic Acid Gel (1%) (Group C); D. Isolation of buccal mucosa from animal of Ellagic Acid Gel (0.5%) (Group D)

DISCUSSION

The present study successfully developed and evaluated an ellagic acid-loaded nano ethosomal in-situ gel system for enhanced topical drug delivery and anti-ulcer activity. Pre-formulation studies confirmed the suitability of ellagic acid for pharmaceutical formulation development.

The drug exhibited characteristic organoleptic properties, acceptable pH, good thermal stability, and compatibility with selected excipients. FTIR analysis confirmed the absence of chemical interaction between ellagic acid and formulation polymers, indicating formulation stability and compatibility.

The nano ethosomal formulation demonstrated desirable physicochemical characteristics with suitable particle size, entrapment efficiency, and stable zeta potential, indicating efficient drug incorporation and colloidal stability. SEM analysis revealed a porous and interconnected polymeric network structure, which supports sustained drug release behavior.

Optimization using Box–Behnken design effectively established the influence of formulation variables on viscosity and drug release. The optimized formulation containing 20% Poloxamer 407, 1% HPMC, and 0.35% Carbopol 934P exhibited excellent desirability with optimum viscosity and maximum drug release. Statistical analysis confirmed the reliability and predictive capability of the optimization model.

The developed in-situ gel showed rapid sol-to-gel transition, appropriate gel strength, and excellent physical stability. The optimized formulation demonstrated high drug content uniformity and sustained drug release over 360 minutes. Drug release kinetics predominantly followed the Higuchi diffusion model, suggesting diffusion-controlled release through the polymeric matrix.

The stability studies performed under ICH conditions confirmed that the optimized formulation remained physically and chemically stable throughout the study period. No significant changes were observed in pH, viscosity, appearance, or drug release profile, indicating good formulation stability and potential shelf life.

The antimicrobial study demonstrated concentration-dependent antibacterial activity against both Bacillus subtilis and Pseudomonas aeruginosa. Although the activity was lower than standard antibiotics, the formulation showed appreciable antimicrobial potential, which may contribute to improved ulcer healing.

The in-vivo anti-ulcer study further confirmed the therapeutic efficacy of the developed formulation in streptozotocin-induced diabetic rats. The treated groups exhibited faster ulcer healing, improved epithelial regeneration, and better recovery in body weight compared to the untreated control group. The 1% ellagic acid gel demonstrated superior healing efficacy compared to the 0.5% formulation, indicating dose-dependent therapeutic activity. The observed wound healing effect may be attributed to the antioxidant, anti-inflammatory, and antimicrobial properties of ellagic acid.

4. CONCLUSION

The present study successfully developed and optimized an ellagic acid-loaded nano ethosomal in-situ gel formulation for topical anti-ulcer therapy. The formulation exhibited satisfactory physicochemical properties, rapid gelation, sustained drug release, excellent stability, and good compatibility between the drug and excipients. Optimization using Box–Behnken design produced a formulation with desirable viscosity and maximum drug release characteristics.

The developed formulation demonstrated significant antimicrobial and anti-ulcer activity in both in-vitro and in-vivo studies. Histopathological evaluation confirmed enhanced re- epithelialization and accelerated ulcer healing in treated animals. Overall, the results suggest that the ellagic acid-loaded nano ethosomal in-situ gel system is a promising and effective approach for oral ulcer in diabetic animal management and sustained drug delivery. Further clinical investigations may be carried out to establish its therapeutic potential in human applications.

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