ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 11, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

FORMULATION AND OPTIMIZATION OF BIOACTIVE-LOADED POLYMERIC NANOPARTICLE – QUALITY BY DESIGN (QBD) STRATEGY EMPLOYING CENTRAL COMPOSITE DESIGN

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Received: 11 July 2025, Revised and Accepted: 15 September 2025

ABSTRACT

Objective: This study aimed to apply a quality by design (QbD) framework, utilizing central composite design (CCD), to systematically develop and optimize resveratrol-loaded polymeric nanoparticles. The overarching goal was to enhance the solubility, stability, and bioavailability of resveratrol, thereby improving its anti-depressant activity.

Methods: Resveratrol nanoparticles were formulated using the solvent evaporation technique, incorporating poly(lactic-co-glycolic acid) (PLGA) as the biodegradable polymer and Poloxamer 188 as the stabilizing surfactant. High-shear homogenization was employed to ensure uniform particle formation. The nanoparticles were subsequently freeze-dried and characterized for key physicochemical parameters, including particle size, polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency (EE), and *in vitro* drug release profile.

Results: The CCD trial formulations containing PLGA and Poloxamer 188 at 60–80 mg and 2–3% concentration, respectively, exhibited particle sizes ranging from 236 to 461 nm with narrow size distribution (PDI: 0.15–0.22), and ZP values between −25 and −30 mV, indicating stable colloidal systems. EE varied from 60% to 90%, depending on the formulation variables. *In vitro* release studies demonstrated a prolonged drug release pattern, with approximately 97% of resveratrol released over a 48-h period. Based on the outcome of the optimization tool, PLGA and Poloxamer 188 were selected at a concentration of 63.56 mg and 2.20%, respectively, and the average particle size at 295.3±8.96 nm, PDI (0.19±0.01), EE (80.33±1.53), zetapotential (−27.00±1.00), and cumulative drug release was 99.33±1.15% for 48 h.

Conclusion: The QbD-driven development approach enabled the successful formulation of resveratrol-loaded PLGA nanoparticles with enhanced physicochemical and release properties. This nanoformulation offers a promising strategy for improving the bioavailability and therapeutic efficacy of resveratrol, supporting its potential application in clinical settings.

Keywords: Resveratrol, Poly(lactic-co-glycolic acid), Nanoparticles, Quality by design, Central composite design, Drug delivery, Bioavailability.

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INTRODUCTION

Despite of therapeutic potential, bioactive compounds often lack their druggability due to poor aqueous solubility, instability, and limited bioavailability. These pharmaceutical properties challenges pose significant obstacles in translating effective bioactive agents into clinically useful drugs. In this scenario, a game-changer nanoparticle-based delivery system has emerged as a translational approach to overcome these limitations. By improving solubility, enhancing stability, enabling targeted delivery, and allowing controlled release, nanoparticles dramatically enhance the clinical efficacy of various phytochemicals and synthetic bioactives [1-6].

Resveratrol, a naturally occurring polyphenol found in grapes, berries, and peanuts, has garnered considerable attention for its diverse therapeutic properties, including antioxidant, anti-inflammatory, cardioprotective, and anticancer effects. However, its clinical utility remains limited by its poor bioavailability, rapid metabolism, and limited systemic retention. To bridge this translational gap, researchers have turned to innovative chemical and formulation-based strategies designed to improve resveratrol's pharmacological profile. This research attempts the use of current nanoparticle drug delivery advancements aimed at enhancing the bioclinical efficacy of resveratrol, with the goal of harnessing its full therapeutic potential in clinical settings [7-10].

MATERIALS AND METHODS

Materials

Resveratrol, poly(lactic-co-glycolic acid) (PLGA), Poloxamer 188, and dichloromethane (DCM) were procured from Sigma-Aldrich (India). All other chemicals and solvents used in this study were of analytical grade and obtained from certified commercial suppliers.

Methods

Screening of suitable polymer and surfactants for polymeric nanoparticles

To identify the most suitable polymer and surfactant for the development of resveratrol-loaded nanoparticles, a preliminary screening was performed using a two-level mixture design within the framework of design of experiments. The independent variables included (1) type of polymer (PLGA and polycaprolactone [PCL]), (2) type of surfactant (Poloxamer 188 and polyvinyl alcohol), (3) polymer quantity, and (4) surfactant concentration. The key responses evaluated were encapsulation efficiency (EE) (%) and particle size (nm), both of which are critical quality attributes impacting nanoparticle performance and stability. This screening enabled systematic assessment of formulation variables across 15 experimental runs (F1-F15; data not shown). The results show that the nanoparticles size was more with PCL than the PLGA, irrespective of the surfactant used. This phenomenon might be due to the differences in the inherent viscosity of polymers. The inherent viscosity of the PCL (1.0–1.4 dL/g) is more when compared

Table 1: Factors for optimization design for resveratrol polymeric nanoparticles

Factor	Ingredient	Units	Variable type	Minimum level	Maximum level	Mean	Standard deviation
A	PLGA amount	mg	Numeric	55.86	84.14	70.00	8.16
B	Poloxamer 188 concentration	Percentage	Numeric	1.29	2.71	2.00	0.4082

PLGA: Poly (lactic-co-glycolic acid)

to the PLGA (0.16–0.24 dL/g); due to this lower inherent viscosity, less energy is required during the homogenization process, which could have led to smaller particle size nanoparticles with PLGA. It was also noted that the particle size of PNPs prepared with PVA was larger when compared to PNPs prepared with Poloxamer 188, which can be attributed to the hydrophilic-lipophilic balance (HLB) value of the surfactant. The HLB value of PVA was less compared to Poloxamer 188; as the expected emulsion to be formed during formulation is oil in water, surfactant with higher HLB value (more hydrophilic surfactant like Poloxamer 188) will have more potential to reduce the interfacial tension when compared to the surfactant with lower HLB value (more lipophilic surfactant like PVA). Thus, more particle size reduction might have happened with the same level of energy with high reduction in the interfacial tension in the case of formulation prepared with Poloxamer 188; hence, the particle size was less with Poloxamer 188. This made the selection of PLGA as the polymer and Poloxamer 188 as the surfactant for further optimization studies.

Optimization of formulation using central composite design (CCD)

Statistical design and analysis of the formulation optimization were performed using Stat-Ease Design Expert® software (Version 11.1.2.0, Stat-Ease®, Inc., USA). The optimization employed a CCD with two independent variables: The concentration of polymer (PLGA) and the concentration of surfactant (Poloxamer 188). Each variable was studied at five levels ($-\alpha$, –1, 0, +1, $+\alpha$), where the axial distance α (1.41421) was generated by the software to ensure orthogonality and rotatability of the design and the details were mentioned in Table 1.

A total of 13 experimental runs were conducted as per the CCD matrix (Table 2). The design process comprised four phases: Design generation, statistical analysis, optimization, and post-optimization validation. Analysis of variance (ANOVA) was employed to identify the best-fit model based on F-values and p-values. The relationship between independent variables (X_1 and X_2) and responses (Y) was modeled using a second-order polynomial equation:

$$Y=\beta_0+\beta_1X1+\beta_2X2+\beta_{11}X12+\beta_{22}X22+\beta_{12}X1X2$$

where Y represents the response, β_0 is the intercept, β_1 and β_2 are the linear coefficients, β_{11} and β_{22} are the quadratic coefficients, and β_{12} is the interaction coefficient.

Model fitness was evaluated based on adjusted R^2 and predicted R^2 values (difference <0.2) and adequate precision (>4), ensuring reliable predictability. Response surface methodology (RSM) plots were generated to visualize factor interactions and guide optimization. The optimization criteria incorporated EE, polydispersity index (PDI), particle size, zeta potential (ZP), and percentage drug release at 6, 12, 24, and 48 h. The precision and validity of the optimized formulation were confirmed by conducting triplicate experiments and comparing observed results with model predictions.

Preparation of resveratrol-loaded polymeric nanoparticles

Resveratrol-loaded polymeric nanoparticles were prepared using the solvent evaporation technique. Briefly, 10 mg of resveratrol and the required amount of polymer were dissolved in 10 mL of DCM to form the organic phase. Separately, the designated concentration of surfactant was dissolved in 100 mL of ultrapure water to prepare the aqueous phase. The organic phase was added dropwise into the aqueous phase under high-shear homogenization using a Polytron® PT-6100-D homogenizer (Kinematica® AG, Switzerland) at 25°C and 20,000 rpm for 15 min, resulting in the formation of an oil-in-water (o/w) nanoemulsion. The dispersion was then stirred magnetically at 750 rpm for 60 min to facilitate evaporation of the organic solvent and nanoparticle formation.

Table 2: Central composite design matrix for resveratrol polymeric nanoparticles

Formulation code	Run	Factors			
		Coded values		Actual values	
		A: PLGA amount (mg)	B: Poloxamer 188 Concentration (%)	A: PLGA amount (mg)	B: Poloxamer 188 Concentration (%)
PNP19	12	−1	−1	60	1.5
PNP20	5	+1	−1	80	1.5
PNP21	8	−1	+1	60	2.5
PNP22	2	+1	+1	80	2.5
PNP23	3	$-\alpha$	0	55.86	2
PNP24	1	$+\alpha$	0	84.14	2
PNP25	4	0	$-\alpha$	70	1.29
PNP26	9	0	$+\alpha$	70	2.71
PNP27	11	0	0	70	2
PNP28	7	0	0	70	2
PNP29	10	0	0	70	2
PNP30	13	0	0	70	2
PNP31	6	0	0	70	2

PLGA: Poly (lactic-co-glycolic acid)

The nanoparticle suspension was centrifuged at 9,000 rpm at 25°C for 30 min, whereby the formed nanoparticles are settled down based on their density, size, and shape by the action of centrifugal force. The formed pellet of particles is washed with distilled water to remove unencapsulated drug and residual surfactant and subsequently freeze-dried using an FTS LyoStar™ 3 freeze dryer (SP Scientific) for further analysis.

Evaluation of nanoparticles

Formulations were prepared according to the experimental runs defined in the design matrix. The prepared polymeric nanoparticles were evaluated for EE, particle size distribution (PSD), ZP, PDI, and *in vitro* drug release. All measurements were performed in triplicate, (n=3).

Following the selection of suitable constraints (Table 3), optimization tools generated contour and overlay plots, identifying optimal polymer and surfactant concentrations along with predicted response values. The model was validated by preparing formulations using the optimized variables and comparing the experimentally observed results with predicted values to confirm the accuracy of the design.

Kinetic studies of drug release

To determine the drug release mechanism from nanoparticles, a study was carried out by dialysis bag method using a diffusion membrane [11] in pH6.8 phosphate buffer for 48 h. The release data were fitted into various models such as zero order, first order, Higuchi, and Korsmeyer-Peppas models. The criteria were selected based on the factor goodness-of-fit test. For determining mechanism of release, the drug release was plotted as per the Korsmeyer equation to determine

Table 3: Constraints used for the optimization of polymer amount and surfactant concentration for resveratrol PNPs

Ingredient	Target	Lower level	Upper level
A: PLGA quantity (mg)	Is in range	60 mg	80 mg
B: Poloxamer concentration (%)	Is in range	1.5	2.5
Particle size (nm)	minimize	100 nm	300 nm
PDI (polydispersity index)	Minimize	0	0.2
Encapsulation efficiency (%)	Maximize	75	100
Zeta potential (mV)	Is in range	−30 mV	30 mV
Dissolution at 6 h (%)	Maximize	50	60
Dissolution at 12 h (%)	Maximize	60	70
Dissolution at 24 h (%)	Maximize	70	85
Dissolution at 48 h (%)	Maximize	85	100

Table 4: Model terms: Design evaluation matrix for central composite design

Model terms	Standard error	Variance inflation factor (VIF)	(Ri^2)	Power (%)
A	0.35360	1.0	0.0	98.7
B	0.35360	1.0	0.0	98.7
AB	0.5	1.0	0.0	85.0
A^2	0.3791	1.01731	0.0170	99.9
B^2	0.3791	1.01731	0.0170	99.9

Table 5: Design evaluation of central composite design: Degrees of freedom

Parameter	Value
Model	5
Residuals (RSLs)	7
Lack of fit (LOF)	3
Pure error (PE)	4
Corrected total sum of squares (SOS)	12

the diffusion exponent (n).

$$Mt/M = K_t^n$$

Particle size and ZP determination

PSD, ZP, and PDI are estimated by DLS method using Malvern Zeta sizer (Model: Nano ZS; Malvern Instruments, UK) at 25°C temperature. The samples were diluted appropriately in water before carrying out the analysis, and the measurement was carried out at 173° angle in triplicate.

Scanning electron microscopy (SEM) studies for polymeric nanoparticles

The surface morphology (roundness and formation of aggregates) of the optimized nanoparticle formulation was measured according to the reported procedure using SEM (Make: JEOL, Model: JCM-6000PLUS Benchtop SEM) equipped with 15 kV. Polymeric nanoparticles were deposited onto sample stubs and coated with gold and the samples were processed with SEM and images were captured at various magnification levels.

RESULTS AND DISCUSSION

Optimization of resveratrol polymeric nanoparticles

Optimization of the composition was carried out by implementing CCD considering the concentration of lipid and surfactants as factors.

CCD: Design phase

Model evaluation

Results indicated that the SE is similar to each other, stating that the model is a balanced design. VIF near 1 indicates that the model is good to evaluate the relationship between the factors. The results showed that R_i^2 in near to zero showed as a good fit model. Power >80% shows that the sample size is enough to predict the interactions between the factors.

LOF of 3 and PE 4 indicated the model selected gives a good design (respective data is mentioned in table 4 and 5).

Table 6: %encapsulation efficiency (EE), particle size (PS), zeta potential (ZP), polydispersity index (PDI), and drug release of the resveratrol PNP optimization design matrix

Formulation code	Responses							
	Particle size (nm)	Polydispersity index	Encapsulation efficiency (%)	Zeta potential (mV)	Percentage CDR at 6 h	Percentage CDR at 12 h	Percentage CDR at 24 h	Percentage CDR at 48 h
PF19	386±2.5	0.200±0.005	70±1.5	−25.5±0.02	56±1.5	60±2.0	72±1.2	88±0.9
PF20	431±3.7	0.198±0.010	90±0.9	−28.6±0.04	35±2.5	58±3.5	68±2.1	84±1.1
PF21	236±2.1	0.154±0.008	74±0.8	−25.7±0.08	68±3.5	77±5.2	90±1.2	103±0.9
PF22	316±4.5	0.201±0.011	93±1.1	−30.5±0.06	61±2.5	71±3.5	82±3.4	104±0.8
PF23	290±6.1	0.187±0.004	60±1.0	−23.8±0.07	49±3.5	60±2.1	69±2.1	90±1.1
PF24	441±2.7	0.221±0.008	91±0.7	−30.5±0.08	48±3.4	65±4.1	68±1.9	86±1.7
PF25	461±3.8	0.200±0.004	86±1.2	−27.5±0.12	36±6.8	48±6.4	64±2.1	81±1.1
PF26	266±4.6	0.185±0.008	90±1.1	−28.0±0.02	66±3.3	73±2.1	84±1.4	102±0.8
PF27	351±5.4	0.194±0.009	84±0.9	−28.5±0.05	55±1.1	65±2.0	78±1.9	95±1.1
PF28	340±2.5	0.192±0.014	89±1.2	−28.9±0.08	52±2.1	61±1.4	72±2.0	95±1.0
PF29	349±3.4	0.195±0.011	85±1.1	−26.7±0.07	58±4.2	69±2.5	76±1.6	97±0.7
PF30	336±4.1	0.199±0.016	83±1.0	−27.1±0.06	53±3.3	66±3.4	76±2.4	100±0.5
PF31	356±3.4	0.189±0.011	88±0.8	−27.5±0.04	61±2.0	69±3.3	80±1.5	95±1.6

Information given as mean±Standard deviation; n=3 for PSD: Particle size distribution, PDI, EE, Zeta potential, and n=6 for % CDR

Table 7: Analysis of variance analysis of particle size: Linear model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	50880.94	2	25440.47	93.74	<0.0001	Significant
A-PLGA amount (mg)	14326.70	1	14326.70	52.79	<0.0001	
B-Poloxamer 188 concentration (%)	36554.25	1	36554.25	134.70	<0.0001	
Residuals	2713.83	10	271.38			
Lack of fit	2444.63	6	407.44	6.05	0.0515	Insignificant
Pure error	269.20	4	67.30			
Corrected sum of squares	53594.77	12				

Table 8: Analysis of variance analysis of polydispersity index: 2FI model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	0.0022	3	0.0007	14.71	0.0008	Significant
A-PLGA amount (mg)	0.0011	1	0.0011	21.73	0.0012	
B-Poloxamer 188 concentration (%)	0.0005	1	0.0005	10.34	0.0106	
AB	0.0006	1	0.0006	12.05	0.0070	
Residuals	0.0004	9	0.0000			
Lack of fit	0.0004	5	0.0001	5.75	0.0575	Insignificant
Pure error	0.0001	4	0.0000			
Corrected sum of squares	0.0026	12				

Table 9: Analysis of variance analysis of encapsulation efficiency: Quadratic model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	1084.81	5	216.96	50.70	<0.0001	Significant
A-PLGA amount (mg)	857.82	1	857.82	200.46	<0.0001	
B-Poloxamer 188 concentration (%)	20.02	1	20.02	4.68	0.0673	
AB	0.2500	1	0.2500	0.0584	0.0815	
A^2	184.50	1	184.50	43.12	0.0003	
B^2	8.42	1	8.42	1.97	0.2035	
Residuals	29.95	7	4.28			
Lack of fit	3.15	3	1.05	0.1569	0.9200	Insignificant
Pure error	26.80	4	6.70			
Corrected sum of squares	1114.77	12				

Table 10: Analysis of variance analysis of zeta potential: Linear model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	44.77	2	22.39	53.95	<0.0001	Significant
A-PLGA amount (mg)	44.65	1	44.65	107.61	<0.0001	
B-Poloxamer 188 concentration (%)	0.1250	1	0.1250	0.3013	0.5951	
Residuals	4.15	10	0.4149			
Lack of fit	1.35	6	0.2249	0.3212	0.8960	Insignificant
Pure error	2.80	4	0.7000			
Corrected sum of squares	48.92	12				

Table 11: Analysis of variance analysis of % CDR at 6 h: Linear model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	864.19	2	432.10	14.33	0.0012	Significant
A-PLGA amount (mg)	87.21	1	87.21	2.89	0.1198	
B-Poloxamer 188 concentration (%)	776.98	1	776.98	25.77	0.0005	
Residuals	301.50	10	30.15			
Lack of fit	240.30	6	40.05	2.62	0.1854	Insignificant
Pure error	61.20	4	15.30			
Corrected sum of squares	1165.69	12				

Table 12: Analysis of variance analysis of % CDR at 12 h: Linear model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	620.31	2	310.16	15.25	0.0009	Significant
A-PLGA amount (mg)	19.04	1	19.04	0.9364	0.3560	
B-Poloxamer 188 concentration (%)	601.27	1	601.27	29.56	0.0003	
Residuals	203.38	10	20.34			
Lack of fit	154.18	6	25.70	2.09	0.2482	Insignificant
Pure error	49.20	4	12.30			
Corrected sum of squares	823.69	12				

Table 13: Analysis of variance analysis of % CDR at 24 h-linear model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	596.31	2	298.16	15.17	0.0009	Significant
A-PLGA amount (mg)	47.11	1	47.11	2.40	0.1527	
B-Poloxamer 188 concentration (%)	549.20	1	549.20	27.93	0.0004	
Residuals	196.61	10	19.66			
Lack of fit	155.41	6	25.90	2.51	0.1956	Insignificant
Pure error	41.20	4	10.30			
Corrected sum of squares	792.92	12				

Table 14: Analysis of variance analysis of % CDR at 48 h: Linear model

Model source	Sum of squares (SOS)	Degree of freedom (DOF)	Mean squares	F-value	p-value	Impact
Design model	1002.56	2	501.28	26.99	<0.0001	Significant
A-PLGA amount (mg)	8.35	1	8.35	0.4494	0.5178	
B-Poloxamer 188 concentration (%)	994.22	1	994.22	53.53	<0.0001	
Residuals	185.74	10	18.57			
Lack of fit	159.74	6	26.62	4.10	0.0968	Insignificant
Pure error	26.00	4	6.50			
Corrected sum of squares	1188.31	12				

All the above terms indicated that the selected model is statistically significant with $p<0.05$

Table 15: Central composite design: Fit statistics of responses

Response	Particle size (nm)	Polydispersity index	Encapsulation efficiency (%)	Zeta potential (mV)	Percentage CDR at 6 h	Percentage CDR at 12 h	Percentage CDR at 24 h	Percentage CDR at 48 h
Standard deviation	13.97	0.0057	2.55	0.6441	5.49	4.51	4.43	4.31
Average	399.69	0.21	79.15	-25	51.08	61.46	72.38	83.68
% covariance	3.51	2.82	3.24	2.48	10.73	7.41	6.16	5.21
R^2	0.9618	0.9689	0.9631	0.9152	0.7414	0.7531	0.7520	0.8437
Adjusted R^2	0.9542	0.9468	0.9368	0.8982	0.6896	0.7037	0.7025	0.8124
Predicted R^2	0.9221	0.8208	0.8581	0.8736	0.5099	0.5319	0.5247	0.6842
Adequate precision	31.9678	23.1527	20.0885	21.5940	10.5675	11.3186	11.0021	15.2299

Table 16: Constraints used for the optimization using the central composite design

Factor	Target	Lower level	Upper level	Significance
A. PLGA amount (mg)	To be in range	50	60	3
B. Concentration of Poloxamer 188 (%)	To be in range	1.5	2.5	3
Particle size distribution (nm)	To minimize	100	300	3
Poly dispersity index	To be in range	0	0.2	3
Encapsulation efficiency (%)	To maximize	75	100	3
Zeta potential (mV)	To be in range	−30	30	3
Percentage CDR at 6 h	To be in range	50	60	3
Percentage CDR at 12 h	To be in range	60	70	3
Percentage CDR at 24 h	To be in range	70	85	3
Percentage CDR at 48 h	To be in range	85	100	3

Table 17: Solutions obtained from the optimization tool - central composite design

Number	PLGA amount (mg)	Poloxamer 188 concentration (%)	Particle Size (nm)	PDI	EE (%)	Zeta potential (mV)	Percentage CDR at 6 h	Percentage CDR at 12 h	Percentage CDR at 24 h	Percentage CDR at 48 h	Desirability	
1	63.557	2.196	296.943	0.180	77.842	−26.338	60.000	68.045	79.340	97.712	0.56	Selected
2	63.485	2.195	296.817	0.180	77.714	−26.322	60.000	68.024	79.333	97.698	0.56	
3	63.635	2.197	297.080	0.180	77.981	−26.356	60.000	68.067	79.349	97.726	0.56	
4	63.185	2.189	296.289	0.179	77.172	−26.253	60.000	67.938	79.300	97.642	0.55	
5	64.022	2.204	297.761	0.180	78.660	−26.445	60.000	68.178	79.390	97.799	0.55	

Table 18: Analytical results of optimized formulation

Formulation code	Particle size (nm)	Polydispersity index	Encapsulation efficiency (%)	Zeta potential (mV)	Percentage CDR			
					At 6 h	At 12 h	At 24 h	At 48 h
PF32	300	0.18	80.00	−26.00	63.00	70.00	85.00	100.00
PF33	301	0.19	82.00	−27.00	66.00	75.00	81.00	98.00
PF34	285	0.20	79.00	−28.00	61.00	72.00	86.00	100.00
Average±SD	295.33±8.96	0.19±0.01	80.33±1.53	−27.00±1.00	63.33±2.52	72.33±4.04	84.00±2.65	99.33±1.15

SD: Standard deviation

Table 19: Comparison of predicted values versus results - central composite design

Response	Mean-predicted	Standard deviation	Number repetitions	Predicted standard error	PI<95%	Mean data	PI>95%
Particle size (nm)	296.401	16.4737	3	11.4382	270.915	295.333	321.887
Polydispersity index	0.179602	0.00705929	3	0.00498512	0.168325	0.19	0.190879
Encapsulation efficiency (%)	77.8687	2.06862	3	1.5169	74.2818	80.3333	81.4556
Zeta potential (mV)	−26.3416	0.727852	3	0.505372	−27.4677	−27	−25.2156
Percentage CDR 6 h	60.0815	5.40434	3	3.75241	51.7206	63.3333	68.4424
Percentage CDR 12 h	68.1118	3.82472	3	2.65563	62.1947	72.3333	74.0289
Percentage CDR 24 h	79.4018	4.3128	3	2.99452	72.7295	84	86.074
Percentage CDR 48 h	97.778	3.80904	3	2.64474	91.8851	99.3333	103.671

CCD: Analysis phase

Trials were executed as per the design matrix and the samples (in triplicate) were analyzed for PS (Particle Size) and EE (%). The obtained results were tabulated and analysis phase was carried out.

ANOVA analysis is carried out for each response and the statistical details along with the statistics are presented in the table 6-14.

All responses Fit statistics are presented in table 15: Regression predicted is in line with Regression adjusted with a difference of <0.2. Desired signal: noise ratio measured by adequate precision is 4 where the values above 4 indicate signal adequacy. Hence, the model could be used for design space navigation.

CCD: Optimization phase

The optimization phase was carried out by selecting the appropriate constraints and also based on the analytical results to get the desired responses, i.e., EE, PSD, ZP, PDI, and *in vitro* release at various time intervals. Constraints and solutions obtained were tabulated in table 16 and 17 respectively and the obtained contour plots are given in figure 1.

Based on the outcome of the optimization tool, PLGA and Poloxamer 188 were selected at concentrations of 63.56 mg and 2.20%, respectively.

CCD: Post analysis phase

Resveratrol nanoparticles in triplicate were manufactured with a composition obtained from the optimization phase to evaluate the model's validity (results are mentioned in table 18 and 19).

From the results, it is noticed that the obtained values were found to be comparable with predicted ones with individual values within ±95% prediction limits.

where G is the PLGA amount and H is Poloxamer 188 concentration.

The interactions between the factors and responses are depicted by the polynomial equations in Table 20. Equations with negative coefficients show that the independent variable has a negative impact on the response, while equations with positive coefficients show that the independent variable has a positive impact on the response. The viscosity of the formulation increases as the amount of PLGA increases, requiring more energy and stirring time to achieve the desired particle size and PDI. Larger particle sizes and PDI were consequently noted. Higher PLGA concentration ensures that the lipid needed to entrap the drug is available; as a result, higher EE was seen.

Table 20: Mathematical modelling: Relationship between the variables and responses - resveratrol polymeric nanoparticles

Response	Mathematical equation
Particle size (nm)	350.69+42.32*G−67.60*H
Polydispersity index	0.1935+0.0116*G−0.0080*H+0.0123*GH
Encapsulation efficiency (%)	85.80+10.36*G+1.58*H−0.25*GH−5.15*G2+1.10*H2
Zeta potential (mV)	−27.60−2.17*G-0.3509*H
Percentage CDR 6 h	53.69−3.68*G+10.05*H
Percentage CDR 12 h	64.77−0.1161*G+8.17*H
Percentage CDR 24 h	75.31−1.68*G+7.54*H
Percentage CDR 48 h	93.85−1.08*G+8.09*H

Table 21: Drug release kinetic data of resveratrol nanoparticles

Model parameters	Resveratrol PNPs
Zero-order model's r^2	0.8487
First-order model's r^2	0.9810
Higuchi model's r^2	0.9424
Korsmeyer-Peppas model's r^2	0.9748
Korsmeyer-Peppas model's Release exponent (n)	0.288

According to the experimental findings and formulation analysis, Poloxamer 188 demonstrated superior surfactant efficiency due to its higher HLB value and greater hydrophilicity, which facilitate a more effective reduction of interfacial tension with less energy input. This characteristic leads to the formation of smaller and more uniform nanoparticles, particularly at higher concentrations of Poloxamer 188. A clear inverse relationship was observed between surfactant concentration and particle size, aligning with the established principle that nanoparticle dissolution is inversely proportional to particle size. Consequently, increased concentrations of Poloxamer 188 in the formulation resulted in reduced particle size and PDI, along with improved EE.

Furthermore, formulations containing higher amounts of Poloxamer 188 enhanced the solubility of resveratrol in the dissolution medium, likely due to improved surface wettability and stabilization of the nanoparticles. In contrast, increasing the concentration of PLGA led to a slower drug release profile. This can be attributed to the extended degradation time of the polymer matrix, which forms a denser and

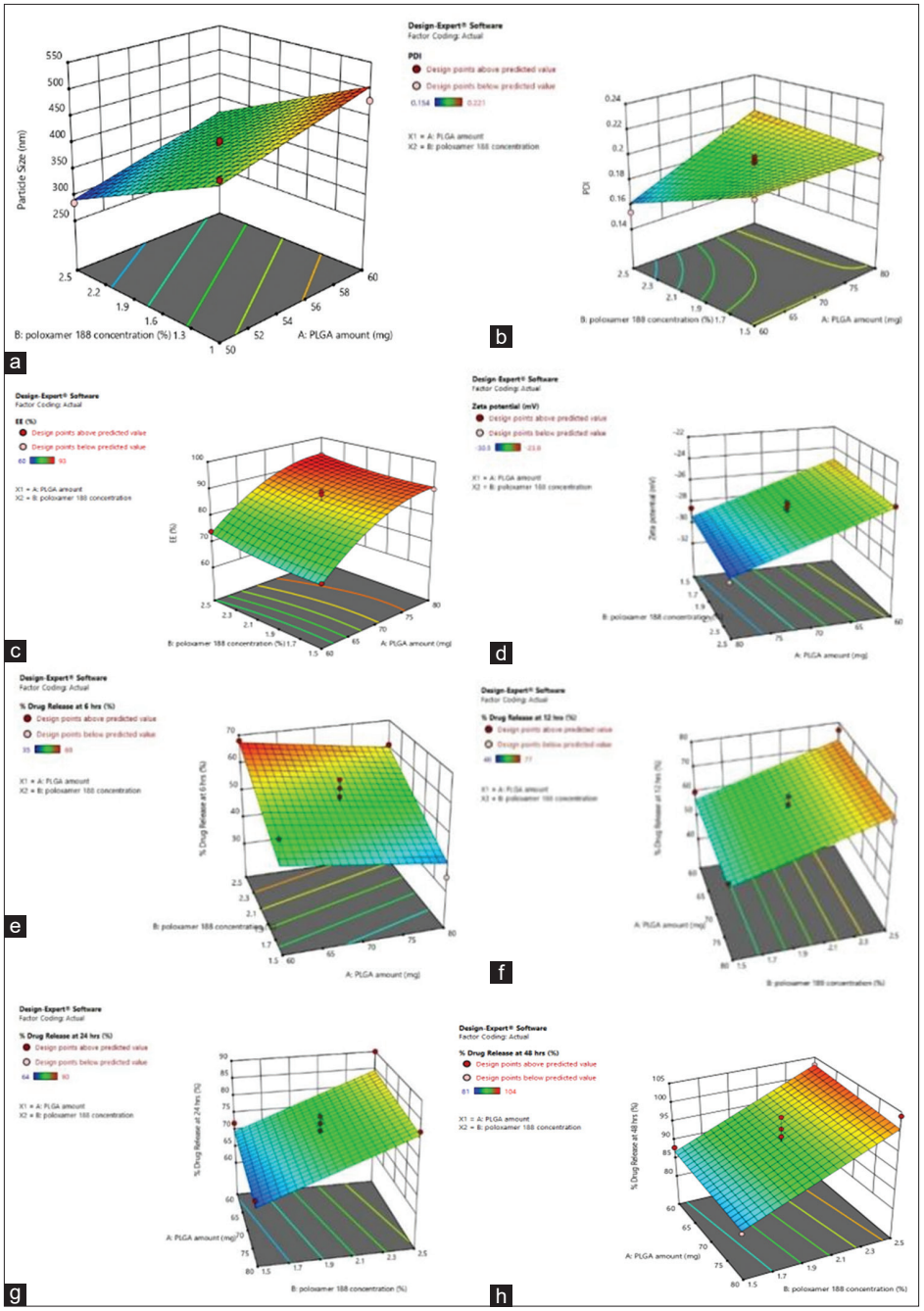

Fig. 1: Contour plots of all responses for resveratrol PNPs (a) Particle size (b) Poly dispersity index (c) Encapsulation efficiency (d) Zeta potential (e) % CDR at 6 h, (f) % CDR at 12h, (g) % CDR at 24 h, (h) % CDR at 48 h

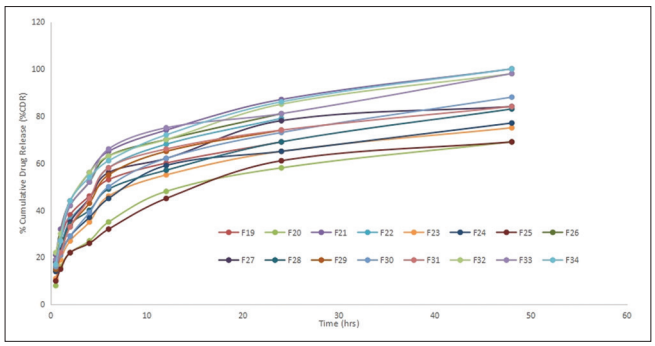

Fig. 2: *In vitro* drug release profiles of resveratrol polymeric nanoparticles

more sustained-release system at higher polymer loadings. These findings underscore the critical influence of both surfactant and polymer concentrations on the physicochemical properties and release behavior of resveratrol-loaded nanoparticles.

Resveratrol PNPs *in vitro* drug release study

In vitro release studies were carried out as per the suitable method and the obtained results are represented in figure 2.

From the results, it found that maximum drug release was released after 48 h due to the slow release of the drug from matrix.

Drug release kinetic studies – resveratrol nanoparticulate formulations

Drug release data of the optimized formulations were evaluated for release and release mechanism by fitting into various models.

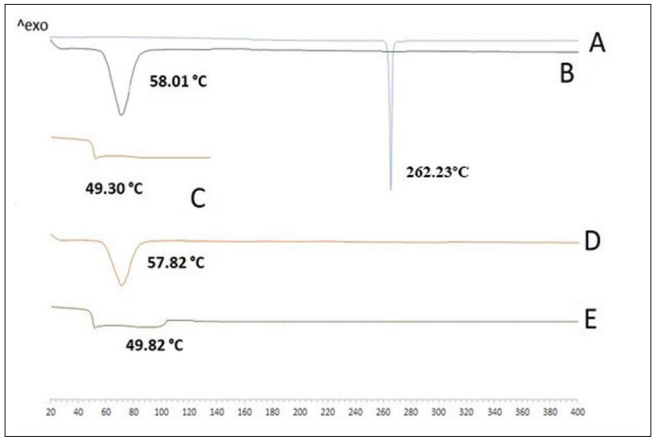

Fig. 3: DSC overlay of resveratrol; (A) Resveratrol; (B) Polycaprolactone (PCL); (C) Poly(lactic-co-glycolic acid) (PLGA); (D) Resveratrol-PCL solid lipid nanoparticles; and (E) Resveratrol-PLGA PNPs

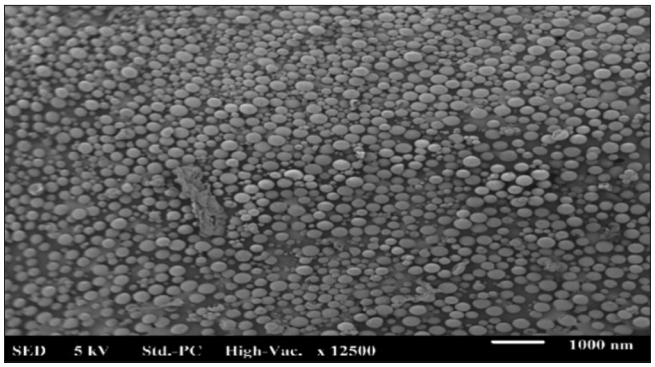

Fig. 4: Scanning electron microscopic image of resveratrol polymeric nanoparticles (formulation: PF33)

The above data found that the first-order model's regression coefficient is higher than the zero-order and Higuchi models. r^2 values of zero order, first order, and Higuchi model's are 0.8487, 0.9810, and 0.9424 for resveratrol PNP formulation, respectively. The release exponent values of resveratrol PNPs are 0.288. From the r^2 values, it was understood that the formulation followed first-order release kinetics, and the release was mechanism was found to be diffusion mediated.

Differential scanning calorimetry

DSC analysis was performed for the pure drug, lipids, polymers, and nanoparticle formulations. The comparisons of DSC thermograms are shown in Fig. 3.

Microscopic characterization of resveratrol nanoparticulate formulations

Optimized polymeric nanoparticles of resveratrol were evaluated for surface morphology using SEM for polymeric nanoparticles (Figure 4). From the evaluation, it was found that the PNPs were spherical with uniform size distribution at the range of 295.33±8.96.

CONCLUSION

This study successfully demonstrated the application of a quality by design approach using CCD for the systematic development and optimization of resveratrol-loaded polymeric nanoparticles. The optimized formulation, developed using PLGA as the polymer and Poloxamer 188 as the surfactant, exhibited favorable physicochemical characteristics, including reduced particle size, narrow PDI, high EE, and sustained drug release.

The results clearly indicated that increasing the concentration of Poloxamer 188 significantly improved nanoparticle dispersion and EE by reducing interfacial tension and particle size. Similarly, higher PLGA content contributed to prolonged drug release due to its slower degradation rate. These findings highlight the critical role of formulation variables in modulating nanoparticle performance and drug release kinetics.

Overall, the developed nanoparticulate system offers a promising strategy for enhancing the solubility, stability, and bioavailability of resveratrol, thereby improving its therapeutic potential in clinical applications.

AUTHORS CONTRIBUTION

All authors have contributed equally.

CONFLICTS OF INTEREST

Declared none.

FUNDING

Nil.

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