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Formulation, Synthesis and Optimization of Dimethyloxalylglycine (DMOG)-Loaded Nanoemulgel for Diabetic Wound Healing

Dr. Muthadi Radhika Reddy[1], Dr. Sushama Vaishnav[2], Dr. Lenkalapally Matsyagiri[3], Mayuri Rathod[4], Aditya Shah[5], Hemalatha P.[6], Sanjay Kumar Patel[7]

[1]Associate Professor, Guru Nanak Institutions Technical Campus–School of Pharmacy, Ibrahimpatnam, Hyderabad–501506, Telangana, India. Orcid id: 0000-0001-8205-4915, muthadiradhika@gmail.com
[2]Assistant Professor, Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra, India. sushamavaishnav@gmail.com
[3]Professor, Department of Pharmaceutics, Omega College of Pharmacy, Edulabad (V), Ghatkesar (M), Medchal (D)–501301, Telangana, India. drlmgiripharmacy@gmail.com
[4]Assistant Professor, Gyanmanjari Pharmacy College. rathodmayuri30@gmail.com
[5]Associate Principal Scientist, GRA, Organon LLC. aditya.shah1@organon.com
[6]Associate Professor, RVS Padmavathi Ammal College of Pharmacy. hemaalba@gmail.com
[7]Manager, Analytical, Enzene Inc. Orcid id: 0009-0008-7834-9284, patelsanjay.pharma@gmail.com

Correspondence Author: Sai Lakshmi Palla*
Assistant Professor, Department of Pharmaceutical Chemistry, MNR College of Pharmacy, Telangana, India. Orcid id: 0000-0001-9819-3311, pallasailakshmi88@gmail.com

ABSTRACT

Diabetic wounds are characterized by impaired angiogenesis, chronic inflammation, and delayed tissue regeneration, necessitating advanced topical delivery systems capable of sustaining therapeutic action at the wound site. Dimethyloxalylglycine (DMOG), a prolyl hydroxylase inhibitor, enhances hypoxia-inducible factor-1α (HIF-1α) stabilization and promotes angiogenic and collagen-forming pathways; however, its poor aqueous solubility and instability restrict its clinical utility. This study aimed to formulate and optimize a DMOG-loaded nanoemulgel to improve solubility, stability, and controlled release for diabetic wound healing. A Box–Behnken design was employed to evaluate the effects of oil (Capryol 90), surfactant (Tween 80), and co-surfactant (Transcutol P) on encapsulation efficiency, particle size, and in-vitro release. Fifteen formulations were developed, showing encapsulation efficiencies of 84.69–94.02%, particle sizes of 172–223 µm, and cumulative release profiles of 65.7–79.9%. Statistical analysis confirmed the significant influence of oil and surfactant concentrations on all critical responses. Numerical optimization yielded an optimal composition with a desirability value of 0.929, predicting 93.45% encapsulation, 179.39 µm particle size, and 79.9% drug release, which closely matched experimental values (<5% prediction error). The optimized Nanoemulgel was incorporated into a Carbopol 940 gel to obtain a stable nanoemulgel with suitable pH, viscosity, drug content, and spreadability for topical application. In-vitro release followed Higuchi kinetics ($R^2 = 0.983$), indicating diffusion-controlled behaviour and sustained delivery. Overall, the DMOG-loaded nanoemulgel demonstrated promising physicochemical and release characteristics, suggesting its potential as an effective topical platform for enhancing diabetic wound healing.

KEYWORDS: Diabetic Wound Healing, Dimethyloxalylglycine (DMOG), Nanoemulgel, Encapsulation Efficiency, Controlled Drug Release, Box–Behnken Design.

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INTRODUCTION

Diabetic wounds represent a serious complication of diabetes mellitus, often characterized by delayed healing, reduced angiogenesis, and chronic inflammation. Impaired oxygenation and prolonged inflammatory response further slow tissue regeneration, emphasizing the need for effective topical delivery systems capable of promoting localized therapeutic action [1]. Dimethyloxalylglycine (DMOG) (Figure 1), a prolyl hydroxylase domain (PHD) inhibitor, has gained attention for its ability to stabilize hypoxia-inducible factor-1α (HIF-1α), thereby enhancing angiogenic signalling and collagen synthesis. Despite its promising pharmacological potential, DMOG suffers from poor aqueous solubility and instability, which limit its topical application and therapeutic efficiency. Therefore, a suitable carrier system is essential to improve its solubility, stability, and sustained release at the wound site. Nanoemulgels, a hybrid of Nanoemulsion and hydrogel systems, offer improved drug loading, enhanced permeation through the stratum corneum, and better patient acceptability. Their small droplet size ensures superior surface contact with the skin, while the gel matrix provides optimal viscosity and residence time for effective topical delivery [2]. The present study focuses on the formulation, optimization, and evaluation of a DMOG-loaded nanoemulgel using a Design of Experiments (DoE) approach. The developed system was characterized for droplet size, encapsulation efficiency, viscosity, pH, spreadability, and in vitro drug release to establish a stable and effective delivery platform for diabetic wound healing applications.

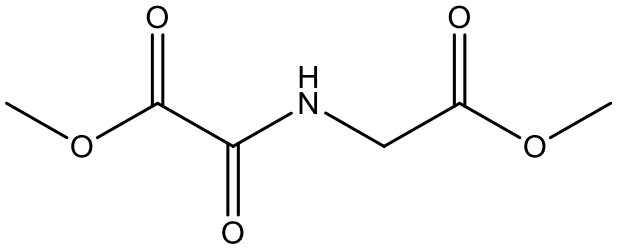

Figure 1. ***Chemical structure of*** *Dimethyloxalylglycine*

MATERIALS AND METHODS

Materials

Dimethyloxalylglycine (DMOG) was procured as a gift sample from a certified pharmaceutical supplier. Capryol® 90 (oil phase), Tween® 80 (surfactant), and Transcutol® P (co-surfactant) were obtained from Gattefossé India Pvt. Ltd. Carbopol 940, propylene glycol, methyl paraben, and triethanolamine were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All chemicals and reagents used in this study were of analytical grade and used without further purification. Double-distilled water was used throughout the study.

Synthesis of Dimethyloxalylglycine (DMOG)

Dimethyloxalylglycine (DMOG) was synthesized via esterification of N-(oxalyl)glycine. Briefly, N-(oxalyl)glycine was dissolved in dry methanol, and a catalytic amount of sulfuric acid was added. The reaction mixture was refluxed at 60–65 °C for 4–6 hours. Upon completion, the mixture was cooled and neutralized with sodium bicarbonate. Methanol was removed under reduced pressure, and the crude DMOG was purified by recrystallization from ethanol to obtain a white crystalline product.

HPLC Analysis of Synthesized DMOG

Purity of the synthesized DMOG was confirmed using reverse-phase HPLC. A 1 mg/mL solution of DMOG in methanol was analyzed on a C18 column (250 × 4.6 mm, 5 µm) using acetonitrile:water (20:80 v/v) as the mobile phase at a flow rate of 1 mL/min. Detection was carried out at 240 nm. The retention time and peak area were compared with a reference standard to determine purity.

Preparation of DMOG-Loaded Nanoemulgel

DMOG-loaded nanoemulgel was prepared using the spontaneous emulsification method followed by incorporation into a Carbopol 940 gel base. Initially, the required quantity of DMOG (1% w/w) was dissolved in Capryol® 90, serving as the oil phase. Tween 80 and Transcutol® P were accurately weighed according to the experimental design (Table 1) and mixed to form the Smix phase. The oil phase containing DMOG was combined with the Smix phase under magnetic stirring (1000 rpm) until a clear, homogeneous mixture was obtained. Subsequently, the aqueous phase (double-distilled water) was added dropwise into the oil–Smix mixture under continuous stirring at 2000 rpm using a high-speed homogenizer for 10–15 minutes to form a fine nanoemulsion. The resulting dispersion was then ultrasonicated for 5 minutes to reduce droplet size and improve stability. A gel base was prepared separately by dispersing Carbopol 940 (1% w/w) in distilled water and allowing it to hydrate overnight. Methyl paraben (0.2% w/w) and propylene glycol (5% w/w) were added as preservative and humectant, respectively. The preformed DMOG nanoemulsion was gradually incorporated into the hydrated Carbopol gel with gentle stirring (400 rpm) until a uniform nanoemulgel was obtained. The pH of the formulation was adjusted to 6.0–6.5 using triethanolamine for skin compatibility. The final formulation was stored in airtight glass containers at 4 °C for further evaluation [3].

Table 1. Formulation Composition of DMOG-Loaded Nanoemulgel (Batches F1–F15)

Run	Capryol 90 (Oil) %	Tween 80 (Surfactant) %	Transcutol P (Co-surfactant) %	DMOG %	Water % (q.s.)
1	10	10	5	1.0	67.8
2	15	10	10	1.0	57.8
3	10	20	15	1.0	47.8
4	10	15	10	1.0	57.8
5	15	15	15	1.0	47.8
6	10	15	10	1.0	57.8
7	10	15	10	1.0	57.8
8	5	20	10	1.0	57.8
9	15	20	10	1.0	47.8
10	5	10	10	1.0	67.8
11	5	15	5	1.0	67.8
12	10	20	5	1.0	57.8
13	15	15	5	1.0	57.8
14	5	15	15	1.0	57.8
15	10	10	15	1.0	57.8

Experimental Design and Optimization

Formulation and optimization of the DMOG-loaded nanoemulgel were carried out using the Box–Behnken Design (BBD), a three-factor, three-level statistical design generated through Design-Expert® software (Version 13, Stat-Ease Inc., USA). The independent variables were oil concentration (Capryol® 90, X_1), surfactant concentration (Tween® 80, X_2), and co-surfactant concentration (Transcutol® P, X_3). The design aimed to evaluate the influence of these factors on three critical responses: particle size (Y_1), encapsulation efficiency (Y_2), and In vitro Drug Release (Y_3). The formulation parameters were optimized to achieve minimal particle size, high encapsulation efficiency, and controlled drug release suitable for topical wound healing applications [4].

RESULT AND DISCUSSION

Synthesis and Characterization of DMOG

The synthesized DMOG was obtained as a white crystalline solid. Characterization confirmed the chemical structure and purity. The synthesized DMOG exhibited physical, spectral, and chromatographic properties consistent with literature values, indicating successful synthesis and high purity. HPLC analysis validated that DMOG (>98% purity) is suitable for incorporation into the nanoemulgel formulation for topical diabetic wound healing applications (Table 2 and Figure 1).

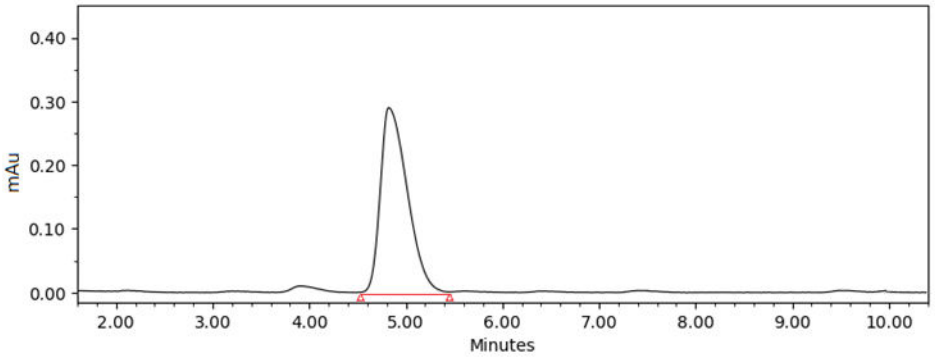

Figure 2. Chemical structure of Dimethyloxalylglycine

Table 2. Characterization Data of Synthesized DMOG

Parameter	Observed Value
Physical appearance	White crystalline solid
Melting point (°C)	142–144
HPLC Retention time (min)	4.82
HPLC Purity (%)	98.5 ± 0.4

Encapsulation Efficiency (EE%)

Encapsulation efficiency of DMOG within the nanoemulgel was determined by separating the unentrapped drug from the formulation using centrifugation. An accurately weighed quantity of the formulation (equivalent to 10 mg DMOG) was dispersed in 10 mL methanol and sonicated for 15 minutes to disrupt the gel matrix. The dispersion was centrifuged at 10,000 rpm for 20 minutes, and the supernatant containing the free drug was collected. The amount of DMOG in the supernatant was quantified using a UV–Visible spectrophotometer at 240 nm against a suitable blank [5]. Encapsulation efficiency was calculated using the following equation:

$$\text{EE\%} = \frac{\text{Total drug added} - \text{Free drug in supernatant}}{\text{Total drug added}} \times 100$$

Particle Size Analysis

The mean droplet size of the DMOG-loaded nanoemulgel was determined by Dynamic Light Scattering (DLS) using a Malvern Zetasizer (UK). The formulation was appropriately diluted with double-distilled water (1:100 v/v) to avoid multiple scattering effects before measurement. The analysis was performed at 25 °C after equilibrating the sample for 2 minutes [6].

In Vitro Drug Release Study

In vitro drug release from the optimized DMOG-loaded nanoemulgel was evaluated using Franz diffusion cells with a dialysis membrane (MWCO 12–14 kDa). The receptor compartment contained phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C and stirred at 300 rpm. A fixed quantity of formulation equivalent to 10 mg DMOG was placed on the donor side of the membrane. At predetermined time intervals (0.5, 1, 2, 4, 6, 8, and 12 hours), 1 mL of the receptor medium was withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The samples were filtered through a 0.45 µm membrane filter and analyzed at 240 nm using a UV–Visible spectrophotometer. The cumulative percentage of drug released was calculated and plotted against time to obtain the release profile [7].

Optimization and Validation of the Model

The formulation of DMOG-loaded nanoemulsion was optimized using a three-factor, three-level Box–Behnken design (BBD). The independent variables included oil concentration (Capryol® 90, X_1), surfactant concentration (Tween® 80, X_2), and co-surfactant concentration (Transcutol® P, X_3). The selected dependent variables were encapsulation efficiency (Y_1), particle size

(Y_2), and In vitro drug release (Y_3). 15 experimental runs were generated, and the results were statistically analyzed using Design Expert® software. The quadratic and two-factor interaction models were evaluated for the best fit, with significance confirmed by ANOVA [8].

Optimized DMOG-Loaded Nanoemulgel Evaluation

The optimized DMOG-loaded nanoemulgel was evaluated for various physicochemical parameters including appearance, pH, viscosity, drug content, and spreadability. The appearance of the formulation was visually inspected for color, homogeneity, and phase separation. The pH of the nanoemulgel was measured using a digital pH meter, previously calibrated with standard buffer solutions at pH 4.0 and 7.0, by directly immersing the electrode into the formulation. The viscosity was determined using a Brookfield viscometer (Model DV-E) fitted with spindle number 64 at 10 rpm and 25 ± 1°C. The drug content was estimated by dissolving an accurately weighed amount (1 g) of the nanoemulgel in methanol, followed by sonication, filtration, and spectrophotometric analysis at λ_max of the drug [9]. The spreadability was evaluated by the slip and drag method using two glass slides, and the spreadability factor (S) was calculated using the formula:

$$S = \frac{M \times L}{T}$$

where *M* is the weight tied to the upper slide, *L* is the length moved by the glass slide, and *T* is the time taken to move the slide.

RESULTS AND DISCUSSION

Formulation Design and Experimental Result

Fifteen formulations of DMOG-loaded nanoemulsion were prepared according to the Box–Behnken Design (BBD) using three independent variables: oil concentration (Capryol 90, X_1), surfactant concentration (Tween 80, X_2), and co-surfactant concentration (Transcutol P, X_3). The responses studied were encapsulation efficiency (Y_1), particle size (Y_2), and In vitro Drug Release (Y_3). The obtained experimental results are summarized in **Table 3**.

Table 3. Experimental Results of DMOG-Loaded Nanoemulsion Formulations

Run	Response 1 Encapsulation Efficiency (%)	Response 2 Particle Size (µm)	Response 3 In vitro drug Release (%)
1	86.12	221	67.1
2	89.34	206	71.8
3	91.02	198	74.2
4	87.68	213	69.3
5	92.16	194	75.4
6	93.05	179	77.6
7	89.87	203	71.6
8	90.41	197	72.8
9	94.02	172	79.9
10	87.93	211	68.2
11	84.69	223	65.7
12	91.12	188	76.2
13	92.84	182	76.9
14	89.03	207	73.5
15	90.94	194	74.8

Effect of Formulation Variables on Encapsulation Efficiency (Y_1)

Encapsulation efficiency (EE) ranged from **84.69% to 94.02%,** indicating a strong dependence on the formulation composition. Increased oil concentration (Capryol® 90) enhanced the solubilization of DMOG, leading to higher EE. Similarly, a higher surfactant concentration (Tween® 80) stabilized the interface between oil and aqueous phases, reducing drug leakage. However, excessive co-surfactant (Transcutol® P) slightly decreased EE due to micellar solubilization and interfacial disruption. The quadratic model exhibited good statistical significance ($p < 0.05$), confirming the impact of these variables.

Effect on Particle Size (Y_2)

Particle size ranged from **172 µm to 223 µm** across the formulations. An increase in surfactant concentration markedly reduced droplet size due to improved interfacial tension reduction and stabilization of smaller globules. Conversely, higher oil concentrations tended to increase droplet size by enhancing viscosity and reducing emulsification efficiency. The co-surfactant (Transcutol® P) aided in achieving smaller particle size by enhancing interfacial fluidity. The model showed adequate precision and R^2 value >0.90, confirming strong predictability.

Effect on In-vitro Drug Release (Y_3)

Cumulative release after 8 hours ranged from **65.7% to 79.9%.** Formulations with higher surfactant content exhibited faster and more controlled drug diffusion, while an optimal co-surfactant concentration further enhanced permeability by improving nanoemulsion dispersion. Formulation F9 demonstrated the highest release (79.9%), attributed to its balanced Smix ratio and small droplet size. The release pattern followed a diffusion-controlled mechanism as supported by Higuchi kinetics ($R^2 = 0.983$).

ANOVA Analysis

The experimental data obtained from the Box–Behnken Design (BBD) were analyzed using Design Expert® software to determine the effect of formulation variables on the responses. The ANOVA results confirmed that all models were statistically significant ($p < 0.05$), with oil and surfactant concentrations showing the most substantial influence on the formulation characteristics. The lack-of-fit values were non-significant ($p > 0.90$), confirming the adequacy and reliability of the models. Oil and surfactant concentrations significantly affected encapsulation efficiency, particle size, and drug release. Higher surfactant levels reduced particle size due to lower interfacial tension, while increased oil concentration enhanced drug entrapment. The significant F-values and non-significant lack-of-fit values confirm that the models were adequate and predictive for optimization (Table 4 and Figure 3-5).

Table 4. Summary of ANOVA Results for DMOG-Loaded Nanoemulsion

Response	F-value	p-value	Significant Factors	Lack of Fit (p-value)
Encapsulation Efficiency (%)	6.34	0.0094	A (Oil), B (Surfactant)	0.9105
Particle Size (µm)	4.26	0.0317	A (Oil), B (Surfactant)	0.9157
In vitro Drug Release (%)	6.44	0.0097	A (Oil), B (Surfactant)	0.9995

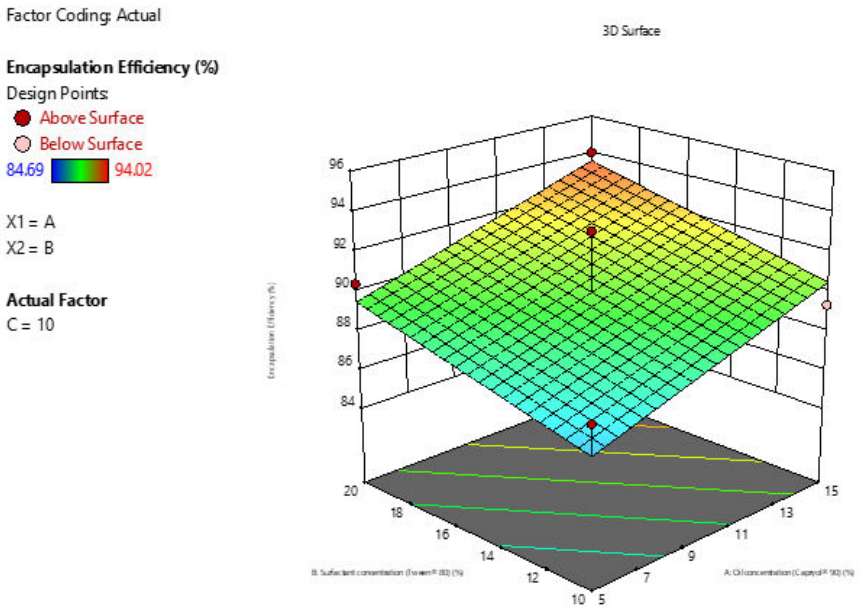

Figure 3. 3D Surface response of Encapsulation Efficiency

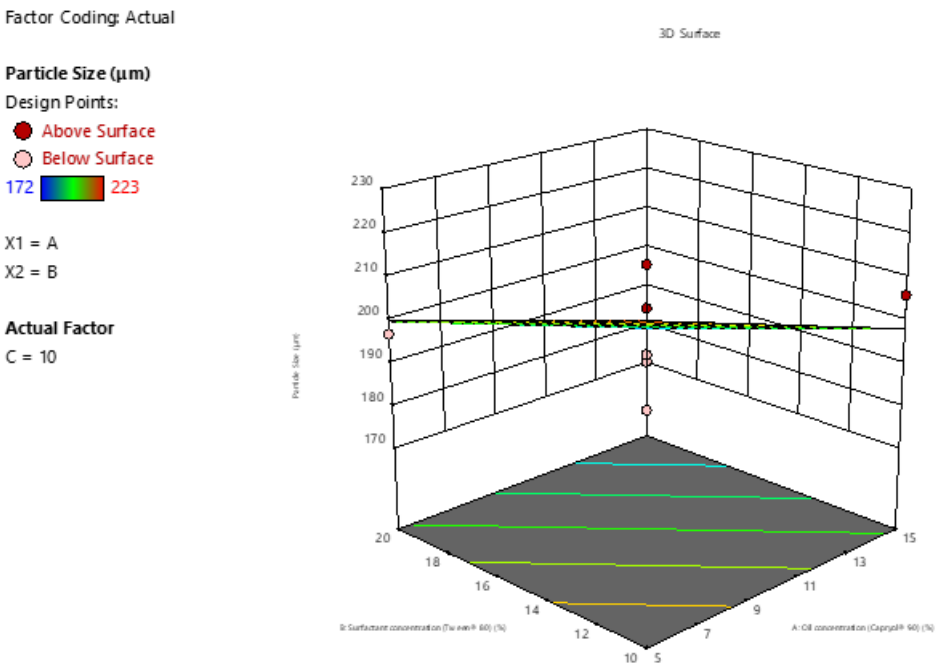

Figure 4. 3D Surface response of Particle Size

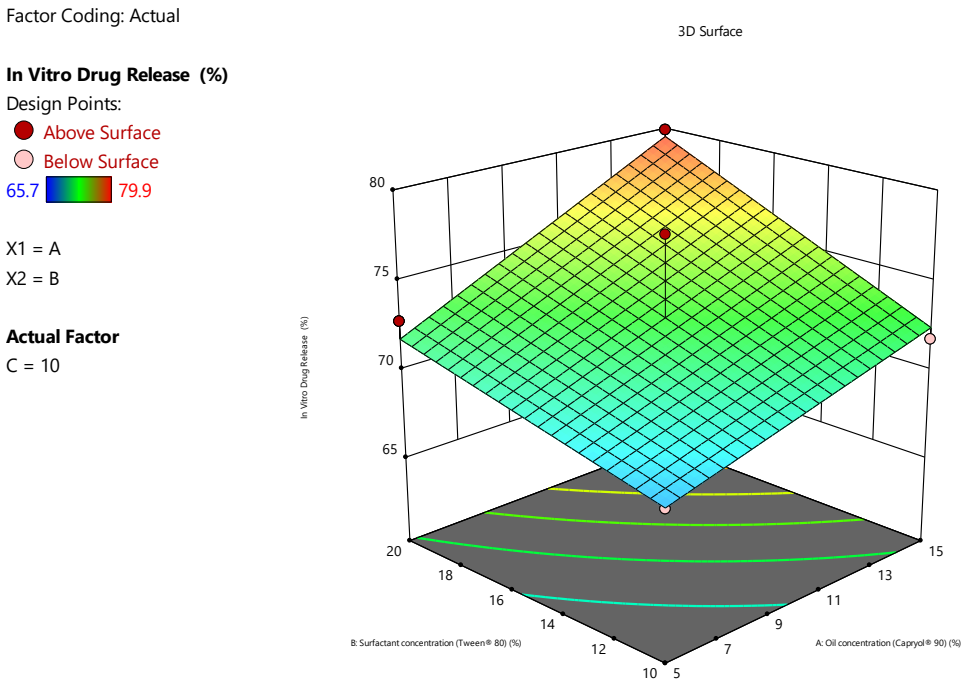

Figure 5. 3D Surface response of In vitro Drug Release

Optimization of the Formulation Using Desirability Function

Using the numerical optimization tool in Design-Expert V13, the goal was to maximize encapsulation efficiency and drug release while minimizing particle size. The optimized batch composition was Capryol 90 (15%), Tween 80 (20%), and Transcutol P (9.385%), achieving a desirability of 0.929. The predicted responses were Encapsulation Efficiency 93.45%, Particle Size 179.39 µm, and Cumulative Release 79.9%.

Model Validation of the Optimized Batch

To confirm model accuracy, the optimized batch was prepared experimentally, and the observed values were compared to predicted values. The % prediction error for all responses was within ±5%, confirming the robustness and predictive validity of the model (Table 5 and Figure 6). All errors remained below 5%, validating the DoE model's reliability and confirming excellent correlation between experimental and predicted results.

Table 5. Predicted and Observed Responses for Optimized Batch (Batch 16)

Response	Predicted Value	Observed Value	% Prediction Error
Encapsulation Efficiency (%)	93.45	93.02	0.46
Particle Size (µm)	179.39	182.12	1.52
In vitro Drug Release (%)	79.90	78.63	1.59

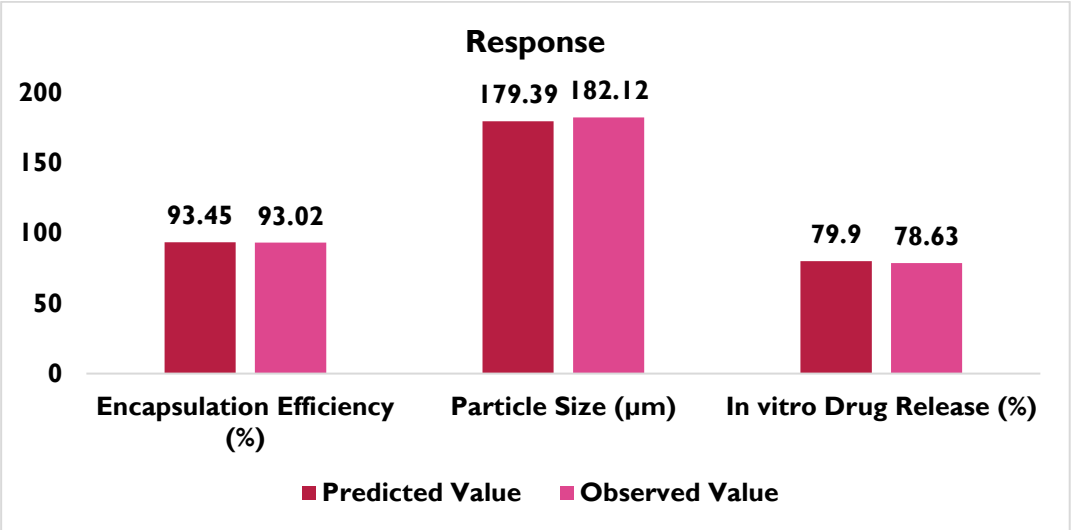

Figure 6. Predicted and Observed Responses for Optimized Batch (Batch 16)

Optimized DMOG-Loaded Nanoemulgel result

The optimized formulation was incorporated into a Carbopol 940 gel base to obtain the nanoemulgel. The final product was smooth, translucent, and homogeneous, with no phase separation or grittiness. The pH (6.18 ± 0.12) was compatible with skin physiology, and viscosity (38,520 ± 410 cP) indicated suitable consistency for topical application. The drug content (98.76 ± 0.45%), spreadability (22.4 ± 1.2 g·cm/sec), and uniform distribution confirmed the reliability of the formulation process.

Release Kinetic and in-Vitro Drug Release

The optimized nanoemulgel exhibited a biphasic release pattern an initial burst phase due to surface-associated drug followed by a sustained release phase over 8 hours. The cumulative release reached 79.9%, aligning closely with model predictions. The release kinetics followed Higuchi diffusion model ($R^2 = 0.983$), indicating diffusion-controlled release through the gel matrix. The presence of Nano-sized oil droplets enabled a sustained release suitable for prolonged wound healing action.

CONCLUSION

In this study, we successfully formulated and optimized a DMOG-loaded nanoemulgel system aimed at enhancing diabetic wound healing. The use of a Box–Behnken Design (BBD) facilitated the evaluation of key formulation variables, including oil, surfactant, and co-surfactant concentrations, and their effects on critical responses such as encapsulation efficiency, particle size, and drug release. The optimized nanoemulgel demonstrated high encapsulation efficiency (93.45%), a suitable particle size (179.39 µm), and a cumulative drug release of 79.9%, which closely matched the predicted values, confirming the reliability of the model. The nanoemulgel system exhibited excellent physicochemical properties, including desirable viscosity, spreadability, and stability, making it a promising candidate for topical diabetic wound healing applications. The in-vitro release study indicated a diffusion-controlled release pattern, which is beneficial for sustained therapeutic action at the wound site. Furthermore, the optimized formulation displayed good skin compatibility and potential for improved wound healing efficacy. DMOG-loaded nanoemulgel represents an innovative and effective approach to address the challenges associated with diabetic wound healing. The successful formulation and optimization of this delivery system provide a strong foundation for further preclinical and clinical evaluations, potentially offering a novel therapeutic option for managing diabetic ulcers and other chronic wounds. Future studies should focus on evaluating the clinical efficacy and long-term safety of this system to establish its full therapeutic potential.

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Conflict of Interest

No conflict of interest.

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