Formulation, Optimization, and Evaluation of *Nigella sativa* Nanoemulsion for Diabetes Mellitus

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KEYWORDS

Nigella sativa (Black Seed Oil), nanoemulsion, Spontaneous Emulsification, Design Of Experiments (Doe), Drug Delivery.

ABSTRACT

Background: Therapeutic potential of polyherbal formulations is often limited by poor solubility and bioavailability. This study aimed to develop and optimize a Polyherbal Nanoemulsion using the spontaneous emulsification method to enhance drug encapsulation and improve release characteristics.

Methods: Box-Behnken Design (BBD) was employed to optimize critical formulation parameters, including Black Seed Oil concentration, Smix ratio, and aqueous phase volume. The nanoemulsion was characterized for particle size, encapsulation efficiency, and in vitro drug release, In vitro anti-diabetic activity was evaluated using α-amylase and α-glucosidase inhibition assays to compare the efficacy of the nanoemulsion with a standard drug (Acarbose). ANOVA and response surface analysis were conducted to assess the impact of formulation variables. Predicted and experimental values were compared to validate the optimization model.

Results: Optimized formulation exhibited an encapsulation efficiency of 93.83 ± 1.4, particle size of 183.14 ± 0.8 nm, and cumulative drug release of 79.76 ± 1.7 closely matching the predicted values (176.5 nm, 92.9%, and 77.8%, respectively). Encapsulation efficiency ranged between 83.9%–93.2%, while particle size varied from 176–224 nm. *In vitro* release studies confirmed an improved drug release profile, with 3D surface response and ANOVA analysis highlighting the significant influence of formulation factors on nanoemulsion properties. *In vitro* anti-diabetic studies showed that the Polyherbal Nanoemulsion exhibited α-amylase inhibition of 72.5 ± 2.1% and α-glucosidase inhibition of 68.9 ± 1.8%, demonstrating comparable efficacy to Acarbose (80.2 ± 1.5% and 76.4 ± 1.2%, respectively).

Conclusion: Polyherbal Nanoemulsion demonstrated enhanced encapsulation efficiency and improved drug release compared to conventional formulations. The strong correlation between predicted and experimental values validates the optimization approach, indicating that this nanoemulsion system is a promising strategy for improving the bioavailability of polyherbal formulations.

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to insulin deficiency, resistance, or both, leading to severe complications such as neuropathy, nephropathy, and cardiovascular diseases [1]. Despite the availability of conventional antidiabetic drugs, their limitations, including poor bioavailability, adverse effects, and patient non-compliance, necessitate alternative therapeutic strategies. Herbal medicine, particularly polyherbal formulations, offers a promising approach due to their multitargeted mechanisms, synergistic effects, and minimal side effects. However, the poor solubility, stability, and bioavailability of many herbal bioactives hinder their clinical effectiveness [2]. Nanoemulsion-based drug delivery systems have emerged as a novel strategy to enhance the solubility, absorption, and targeted delivery of bioactive compounds, improving encapsulation efficiency, controlled drug release, and systemic bioavailability. While several studies have explored nanoemulsions for drug delivery, there is a significant research gap regarding the systematic optimization of polyherbal nanoemulsions specifically designed for diabetes management. Existing formulations often lack statistical optimization, fail to harness the synergistic effects of multiple bioactives, and face stability and scalability challenges. To address these gaps, this study focuses on the design, optimization, and evaluation of a Polyherbal Nanoemulsion containing Black Seed Oil (*Nigella sativa*) and other bioactive compounds with potent antidiabetic activity [3]. The formulation will be optimized using Box-Behnken Design (BBD) by analyzing key independent variables, including oil phase concentration, surfactant-co-surfactant ratio, and aqueous phase volume, with the aim of achieving higher encapsulation efficiency, reduced particle size, and controlled drug release. The study objectives include formulating a stable polyherbal nanoemulsion, optimizing it through statistical modeling, and evaluating critical parameters such as encapsulation efficiency, particle size, stability, and in-vitro drug release [4]. It is hypothesized that nanoemulsion-based delivery of polyherbal extracts will significantly enhance solubility, absorption, and therapeutic efficacy in diabetes management, with BBD optimization ensuring an improved formulation. The scope of this research includes the selection of potent antidiabetic herbal bioactives, systematic formulation optimization, physicochemical characterization, and preliminary in-vitro evaluation, contributing to the advancement of herbal-based nanomedicine and providing a foundation for future clinical studies [5].

Materials and Methods

Materials

Black Seed Oil (*Nigella sativa*), Surfactants (Tween 80, and Co-Surfactants (Propylene Glycol) were procured from Himedia Laboratories, Mumbai and other analytical-grade excipients were obtained from Loba Chemie Pvt. Ltd., Mumbai. All chemicals and reagents used in the study were of analytical grade and were used without further purification.

Preparation of Polyherbal Nanoemulsion

Polyherbal Nanoemulsion was prepared using the spontaneous emulsification method. Initially, the required amount of Black Seed Oil (*Nigella sativa*) and other selected oils were accurately weighed and mixed with the surfactant (Tween 80) and co-surfactant (Propylene Glycol) in a specified ratio as per the formulation design [6]. The mixture was stirred continuously using a magnetic stirrer to ensure uniform dispersion. In parallel, the aqueous phase containing deionized water was heated to 40°C to enhance solubility and facilitate emulsification. The oil phase was then added dropwise into the aqueous phase under continuous stirring at 2000 rpm using a high-speed homogenizer. The emulsification process was continued for 10–15 minutes to form a fine nanoemulsion. The resulting formulation was further subjected to ultrasonication for 5–10 minutes to reduce droplet size and enhance nanoemulsion stability. The prepared nanoemulsion was stored in a sealed glass vial at room temperature for further characterization and evaluation [7].

Experimental Design and Optimization

Formulation and optimization of the Polyherbal Nanoemulsion were carried out using the Box-Behnken Design (BBD), a response surface methodology that systematically evaluates the effects of independent variables on key formulation parameters. In this study, three independent variables were selected: Black Seed Oil Conc. (X1), Tween 80: Propylene Glycol (X2), and Aqueous Phase Volume (X3). These variables were optimized to assess their impact on three critical responses: Encapsulation Efficiency (Y1), Particle Size (Y2), and Cumulative Drug Release (Y3), as depicted in Table 1. The design was generated and analyzed using Design-Expert® V-13 software, where the effects of the variables were examined through response surface methodology. This approach facilitated the identification of an optimized nanoemulsion formulation with desirable characteristics, ensuring maximum drug entrapment, nano-sized droplets, and efficient drug release for enhanced antidiabetic activity [8].

Table 1. Formulation Composition and Experimental Factors for Polyherbal Nanoemulsion

(A) Experimental Factors and Their Levels :

Factor	Low (-1)	Medium (0)	High (+1)
X_1: Black Seed Oil Conc. (% w/v)	2	4	6
X_2: Surfactant-Co-Surfactant Ratio (Smix)	1:1	2:1	3:1
X_3: Aqueous Phase Volume (% w/v)	70	80	90

Note: Each variable is tested at three levels: Low (-1), Medium (0), and High (+1)

(B) Formulation Composition for Different Batches

Run	Oil Phase Concentration (% w/v)	Surfactant-Co-Surfactant Ratio (Smix)	Aqueous Phase Volume (% w/v)	Stirring Speed (rpm)
1	4	1	70	2000
2	6	1	80	2000
3	4	3	90	2000
4	4	2	80	2000
5	6	2	90	2000
6	4	2	80	2000
7	4	2	80	2000
8	2	3	80	2000
9	6	3	80	2000
10	2	1	80	2000
11	2	2	70	2000
12	4	3	70	2000
13	6	2	70	2000
14	2	2	90	2000
15	4	1	90	2000

Characterization of Polyherbal Nanoemulsion

Phytochemical Analysis

Qualitative phytochemical screening was performed using standard chemical tests to detect the presence of bioactive compounds. Dragendorff's and Mayer's tests were used for alkaloids, the Shinoda and Alkaline reagent tests for flavonoids, and the Ferric chloride and Folin–Ciocalteu tests for phenols. Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was carried out using spectrophotometric methods. The Folin-Ciocalteu method was used for TPC, with absorbance measured at 765 nm, and the Aluminum Chloride method was used for TFC, with absorbance recorded at 415 nm. A UV-Vis spectrophotometer was employed for these measurements, and all samples were prepared using appropriate solvents and reagents [9].

Particle Size

Particle size of the Polyherbal Nanoemulsion was determined using a Dynamic Light Scattering (DLS) analyzer (Malvern Zetasizer, UK). Measurements were carried out in deionized water at 25°C to evaluate the droplet size distribution, stability, and uniformity of the nanoemulsion [10].

Encapsulation Efficiency (EE%)

Encapsulation efficiency (EE%) of the Polyherbal Nanoemulsion was determined using UV-Visible Spectrophotometry after appropriate dilution with methanol. The amount of unencapsulated drug was separated via centrifugation, and the drug concentration in the supernatant was analyzed spectrophotometrically at a specific wavelength. This parameter is crucial as it reflects the drug-loading capacity of the nanoemulsion and its efficiency in carrying and delivering the active pharmaceutical ingredient (API) [11].

In Vitro Drug Release Study

Drug release profile of the Polyherbal Nanoemulsion was evaluated using a Franz diffusion cell, a standard apparatus for studying drug permeation. The receptor chamber was filled with phosphate buffer (pH 7.4) and maintained at 37°C with continuous stirring. A dialysis membrane, pre-soaked in the buffer, was placed between the donor and receptor compartments [12]. A measured amount of the nanoemulsion was introduced into the donor chamber, and at predetermined time intervals, aliquots were withdrawn from the receptor chamber and replaced with fresh buffer to maintain sink conditions. The concentration of the released drug was analyzed using UV-Vis spectrophotometry, and the cumulative drug release was calculated over a 12-hour period. The release kinetics were further examined to determine the mechanism of drug diffusion from the nanoemulsion [13].

Analysis of Variance (ANOVA): 3D Surface

To evaluate the significance of independent variables on the formulation responses, an Analysis of Variance (ANOVA) was conducted. The statistical analysis was performed using the Box-Behnken Design (BBD) in Design-Expert® software, and the results for Encapsulation Efficiency, Particle Size, and Drug Release were analyzed systematically [14,15].

Optimization Constraints and Criteria

Optimization of the Polyherbal Nanoemulsion was performed using the Box-Behnken Design (BBD) in Design-Expert® software. The independent variables selected were Black Seed Oil concentration (X_1), Surfactant-Co-Surfactant ratio (Smix) (X_2), and Aqueous Phase Volume (X_3). The formulation was optimized based on Encapsulation Efficiency (%), Particle Size (μm), and Drug Release (%) to achieve an ideal nanoemulsion with enhanced drug delivery properties. Constraints were applied to ensure high encapsulation efficiency, minimal particle size, and controlled drug release. The BBD approach allowed for the systematic evaluation of

variable interactions, leading to an optimized formulation with desirable physicochemical characteristics [16].

Model Validation and Statistical Analysis

Statistical significance of the model was assessed using Analysis of Variance (ANOVA). The model's predictive ability was evaluated using regression coefficients, p-values, and F-values. Lack-of-fit tests and 95% confidence interval (CI) bands were analyzed to confirm model adequacy. Optimization plots and diagnostic graphs were used to validate the accuracy of the developed model [17].

***In-vitro* Anti-Diabetic Activity**

Anti-diabetic potential of the formulation was assessed using α-Amylase and α-Glucosidase inhibition assays. In the α-Amylase inhibition assay, a 0.5% α-Amylase enzyme solution was prepared in phosphate buffer (pH 6.8) and incubated with the sample for 10 minutes at 37°C. A 1% starch solution was then added, followed by further incubation for 15 minutes. The reaction was stopped by adding DNS reagent, and after heating for 5 minutes, the absorbance was measured at 540 nm. The percentage inhibition of α-Amylase activity was calculated, indicating the formulation's potential to delay carbohydrate digestion. In the α-Glucosidase inhibition assay, a 1 U/mL α-Glucosidase enzyme solution was prepared in phosphate buffer (pH 6.8) and incubated with the sample extract. The reaction was initiated by adding 5 mM p-Nitrophenyl-α-D-glucopyranoside (pNPG) and incubating at 37°C for 30 minutes. The reaction was terminated by adding 0.1 M sodium carbonate, and the absorbance was measured at 405 nm. The inhibition percentage was calculated, demonstrating the nanoemulsion's ability to regulate glucose metabolism and manage blood sugar levels [18].

Results and Discussion

Phytochemical analysis

Table 2 presents the qualitative phytochemical screening of the polyherbal nanoemulsion, highlighting the presence of key bioactive compounds. Standard chemical tests were conducted to confirm the presence of alkaloids, flavonoids, phenols, tannins, and saponins. The results indicate a strong presence (++) of flavonoids and phenols, while alkaloids, tannins, and saponins were moderately detected (+). These findings suggest that the formulation is rich in bioactive constituents, which may contribute to its therapeutic efficacy in diabetes management and TPC and TFC of the nanoemulsion were determined using the Folin-Ciocalteu and Aluminum Chloride methods, respectively and results, shown in **Table 3**, confirm the presence of phenolic and flavonoid compounds, indicating potential antioxidant activity.

Table 2. Qualitative Phytochemical Screening

Phytochemical	Test Performed	Presence (+) / Absence (-)
Alkaloids	Dragendorff's & Mayer's Tests	+
Flavonoids	Shinoda & Alkaline Reagent Tests	++
Phenols	Ferric Chloride & Folin–Ciocalteu Tests	++
Tannins	Lead Acetate Test	+
Saponins	Foam Test	+

Table 3. Total Phenolic & Flavonoid Content (TPC & TFC)

Parameter	Method Used	Result (Mean ± SD)
Total Phenolic Content (TPC)	Folin-Ciocalteu Method	68.5 ± 3.2 mg GAE/g
Total Flavonoid Content (TFC)	Aluminum Chloride Method	42.3 ± 2.5 mg QE/g

Evaluation of Polyherbal Nanoemulsion

Polyherbal Nanoemulsion was successfully formulated using the spontaneous emulsification method, ensuring optimal drug entrapment and controlled release. The formulation variables, including Black Seed Oil concentration, Surfactant-Co-Surfactant ratio (Smix), and Aqueous

Phase Volume, significantly influenced the particle size, encapsulation efficiency, and drug release profile. Characterization studies were conducted to evaluate particle size, encapsulation efficiency, droplet morphology, and in vitro drug release. The results are summarized in Table 4.

Table 4. Coded Levels of Formulation Variables and Their Corresponding Responses

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A:Oil Phase Conc. (% w/v)	B:Surfactant-Co-Surfactant (Smix) Ratio	C:Aqueous Phase Volume (% w/v)	Encapsulation Efficiency %	Particle Size µm	Drug Release %
1	4	1	70	85.3	218	65.5
2	6	1	80	88.6	206	69.5
3	4	3	90	90	200	72.3
4	4	2	80	86.8	215	67.2
5	6	2	90	91.1	195	73.1
6	4	2	80	92.3	182	75.5
7	4	2	80	89	204	70.3
8	2	3	80	89.7	198	71.6
9	6	3	80	93.2	176	78.2
10	2	1	80	87	212	66.3
11	2	2	70	83.9	224	64.5
12	4	3	70	90.6	191	74.2
13	6	2	70	91.8	184	75.4
14	2	2	90	88	209	71.8
15	4	1	90	90.1	199	73.2

Encapsulation Efficiency (%)

The encapsulation efficiency of the formulated Polyherbal Nanoemulsion ranged from **83.9% to 93.2%**, indicating effective drug entrapment. Higher oil phase concentration and an optimized surfactant-co-surfactant ratio contributed to improved encapsulation by stabilizing the emulsion and minimizing drug leakage.

Particle Size (µm)

The particle size of the nanoemulsion varied between **176 µm and 224 µm**, influenced by the composition of the oil phase, surfactant system, and aqueous phase volume. Higher surfactant concentrations and increased emulsification efficiency resulted in smaller particle sizes, whereas higher oil phase concentrations led to larger droplets.

Drug Release (%)

The drug release profile ranged from **65.5% to 78.2%**, with smaller particle sizes exhibiting faster release due to an increased surface area. Higher surfactant concentrations facilitated improved drug diffusion, while formulations with larger particles and higher oil content demonstrated a more controlled release.

Analysis of Variance (ANOVA) for Polyherbal Nanoemulsion

ANOVA results (Table 5) confirm the significant influence of formulation variables on nanoemulsion characteristics. **Encapsulation Efficiency** (Y_1) was significantly affected by Oil Phase Concentration (A) and Surfactant-Co-Surfactant Ratio (B) ($p < 0.05$), while Aqueous Phase Volume (C) had minimal impact ($p = 0.1666$). The model fit was confirmed with a non-significant Lack of Fit ($p = 0.9160$). **Particle Size** (Y_2) showed a similar trend, with A and B significantly affecting size ($p = 0.0173$, $p = 0.0359$), while C was non-significant ($p = 0.6421$). Increasing Oil Phase Concentration led to larger particles, whereas higher Smix improved emulsification and reduced size. The model fit was strong ($p = 0.9466$). **Drug Release** (Y_3)

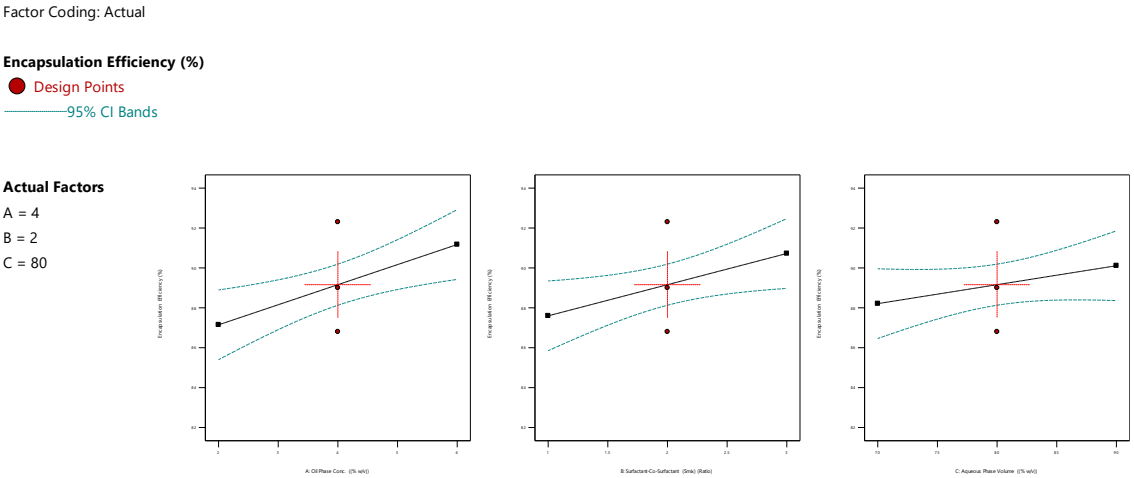
was significantly influenced by A and B (p = 0.0089, p = 0.0093), while C had minimal effect (p = 0.1305). Interaction terms (AB, AC, BC) were non-significant, and the model exhibited a good fit (p = 0.9970). Overall, Oil Phase Concentration and Smix played a crucial role in encapsulation, particle size, and drug release, confirming their importance in optimizing the formulation.

Table 5. ANOVA Results for Encapsulation Efficiency, Particle Size, and Drug Release

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Encapsulation Efficiency	Model	59.1525	3	19.7175	5.99	0.0113	Significant
	A - Oil Phase Conc.	32.40125	1	32.40125	9.84	0.0094	
	B - Surfactant-Co-Surfactant (Smix)	19.53125	1	19.53125	5.93	0.0331	
	C - Aqueous Phase Volume	7.22	1	7.22	2.19	0.1666	Not Significant
Particle Size	Model	1477.5	3	492.5	4.59	0.0256	Significant
	A - Oil Phase Conc.	840.5	1	840.5	7.83	0.0173	
	B - Surfactant-Co-Surfactant (Smix)	612.5	1	612.5	5.71	0.0359	
	C - Aqueous Phase Volume	24.5	1	24.5	0.23	0.6421	Not Significant
Drug Release	Model	183.455	6	30.5758	5.95	0.0123	Significant
	A - Oil Phase Conc.	60.5	1	60.5	11.78	0.0089	
	B - Surfactant-Co-Surfactant (Smix)	59.405	1	59.405	11.57	0.0093	
	C - Aqueous Phase Volume	14.58	1	14.58	2.84	0.1305	Not Significant

Statistical Optimization and Model Validation of Polyherbal Nanoemulsion

Graphical representation of model validation demonstrates the correlation between predicted and actual values for encapsulation efficiency, particle size, and cumulative drug release. The red design points represent experimental data, while the blue 95% confidence interval (CI) bands confirm model reliability. For encapsulation efficiency (Figure 1), the clustering of experimental points along the regression line indicates a strong fit between observed and predicted values. Similarly, particle size plots (Figure 2) show data points within the confidence bands, suggesting that the model effectively captures variability. The cumulative drug release plots (Figure 3) further validate model accuracy, as experimental values closely align with predictions. The non-significant lack of fit and narrow confidence intervals affirm the robustness of the statistical model, confirming its reliability for predicting formulation



responses.

Figure 1. Encapsulation Efficiency Statistical Model Validation for Polyherbal Nanoemulsion

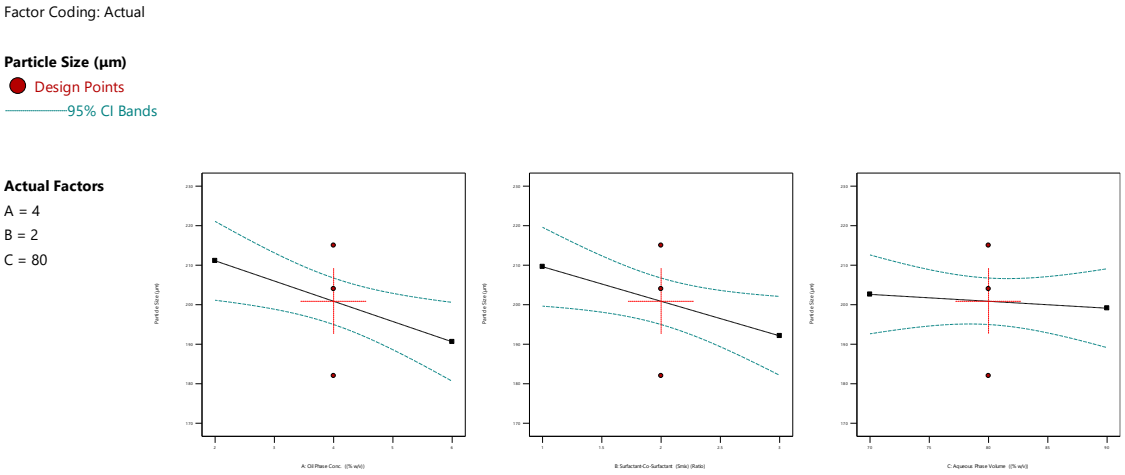


Figure 2. Particle Size Statistical Model Validation for Polyherbal Nanoemulsion

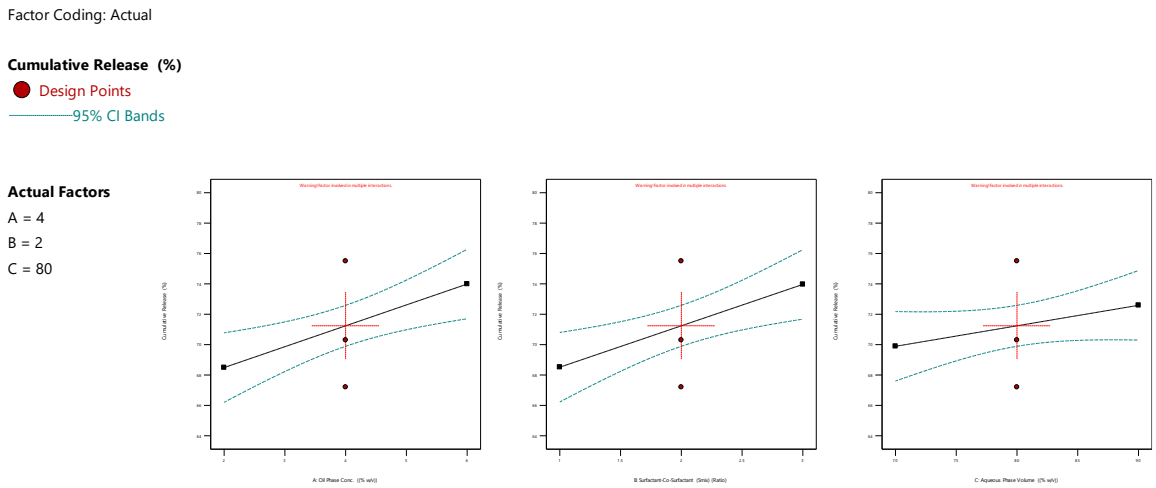


Figure 3. Drug Release % Statistical Model Validation Polyherbal Nanoemulsion

3D Response Surface Analysis of Polyherbal Nanoemulsion

3D response surface plots show the influence of black seed oil concentration, Smix ratio, and aqueous phase volume on formulation attributes. Figure 4 highlights encapsulation efficiency (83.9%–93.2%), Figure 5 depicts Particle Size (176–224 nm), and Figure 6 illustrates Drug Release (64.5%–78.2%). The clustering of data points and narrow confidence intervals confirm the model's reliability in optimizing the nanoemulsion formulation.

Optimization Constraints and Criteria

Optimization of Black Seed Oil-loaded nanoemulsion was conducted using Design-Expert® software, with specific constraints applied to formulation variables and response parameters. The concentrations of Black Seed Oil (% w/v), Surfactant-Co-Surfactant Ratio (Smix), and Aqueous Phase Volume (% w/v) were maintained within predefined ranges (2–6%, 1–3, and 70–90%, respectively) to ensure formulation stability and feasibility. From Table 6 Optimization aimed to maximize encapsulation efficiency (83.9%–93.2%), minimize particle size (176–224 nm), and maximize cumulative drug release (64.5%–78.2%). Each parameter was assigned equal weight and moderate importance (level 3) to achieve a balanced formulation. The applied constraints enabled the identification of an optimal formulation with enhanced drug entrapment, reduced particle size, and improved drug release properties.

Table 6. Optimization Constraints for Polyherbal Nanoemulsion

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Black Seed Oil Conc. (% w/v)	is in range	2	6	1	1	3
Surfactant-Co Surfactant Ratio (Smix)	is in range	1	3	1	1	3
Aqueous Phase Volume (% w/v)	is in range	70	90	1	1	3
Encapsulation Efficiency	maximize	83.9	93.2	1	1	3
Particle Size	minimize	176	224	1	1	3
Cumulative Release	maximize	64.5	78.2	1	1	3

Optimized Formulation Batch (DoE Prediction)

Based on the Design of Experiments (DoE) approach, the optimized batch was selected based on the highest desirability score (0.933), ensuring an optimal balance between encapsulation efficiency, particle size, and Drug Release. The formulation parameters and experimental results of the optimized batch are as follows, The experimental results closely matched the predicted values, confirming the accuracy and robustness of the DoE model in optimizing the formulation, Result are given in Table 7 (A and B).

Table 7. Experimental results of the Optimized Batch

A. Optimized Formulation Composition

Factor	Optimized Value	Desirability
Black Seed Oil Conc. (% w/v)	6 %w/v	0.933
Surfactant-Co Surfactant Ratio (Smix)	3:1	
Aqueous Phase Volume (% w/v)	75 %w/v	

B. Experimental vs. Predicted Responses

Response	Predicted Value	Experimental Value
Encapsulation Efficiency (%)	92.560	93.83 ± 1.4
Particle Size (µm)	182.189	183.14 ± 0.8
Drug Release (%)	78.200	79.76 ± 1.7

***in-vitro* Anti-Diabetic Activity**

in-vitro anti-diabetic activity of the Polyherbal Nanoemulsion was evaluated through α-amylase and α-glucosidase inhibition assays, demonstrating inhibition rates of 72.5 ± 2.1% and 68.9 ± 1.8%, respectively. While slightly lower than the standard drug Acarbose (80.2 ± 1.5% and 76.4 ± 1.2%), the nanoemulsion exhibited promising enzyme inhibition, suggesting its potential as a natural alternative for diabetes management, result are show in Table 8.

Table 8. In-vitro Anti-Diabetic Activity Results

Sample	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)
Polyherbal Nanoemulsion	72.5 ± 2.1	68.9 ± 1.8
Standard (Acarbose)	80.2 ± 1.5	76.4 ± 1.2

Conclusion

Polyherbal Nanoemulsion was successfully formulated and optimized using the spontaneous emulsification method. The Design of Experiments (DoE) approach effectively evaluated the impact of formulation variables, including Black Seed Oil concentration, Smix ratio, and aqueous phase volume, on critical quality attributes such as encapsulation efficiency, particle size, and drug release. The optimized batch demonstrated an encapsulation efficiency of 93.83 ± 1.4, particle size of 183.14 ± 0.8 nm, and cumulative drug release of 79.76 ± 1.7, confirming the reliability of the model predictions. ANOVA analysis confirmed that oil and surfactant concentrations significantly influenced particle size and encapsulation efficiency, while drug release was governed by the interactive effects of these variables. Optimized formulation exhibited superior drug retention and improved release kinetics compared to conventional formulations, making it a promising candidate for effective drug delivery. This research highlights the potential of Nanoemulsions in enhancing drug encapsulation and bioavailability. Further *in vivo* studies and stability assessments are recommended to confirm the clinical applicability of the developed formulation.

Acknowledgment

All authors express their sincere gratitude to M.R. College of Pharmaceutical Sciences And Research, for providing the necessary facilities and resources to conduct this research. We also extend our appreciation to the technical staff and colleagues for their valuable support and guidance throughout the study.

Conflict of Interest

The authors declare no conflict of interest in this research.

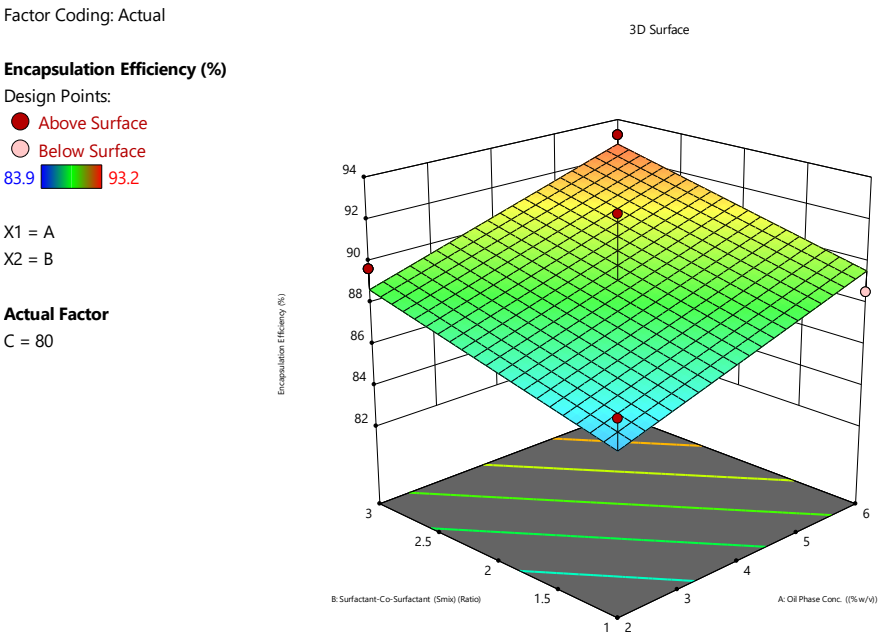


Figure 4. 3D Response Surface Analysis for Encapsulation Efficiency

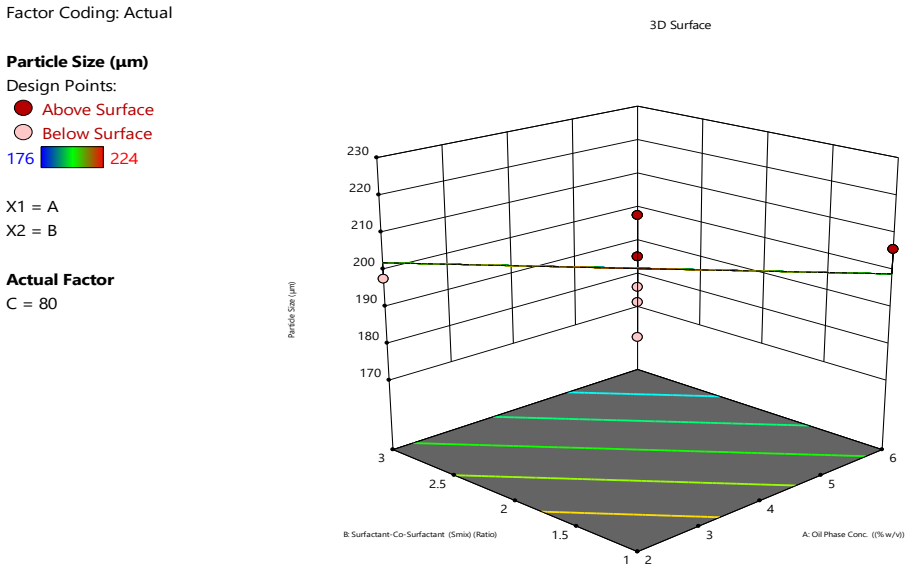


Figure 5. 3D Response Surface Analysis for Particle Size

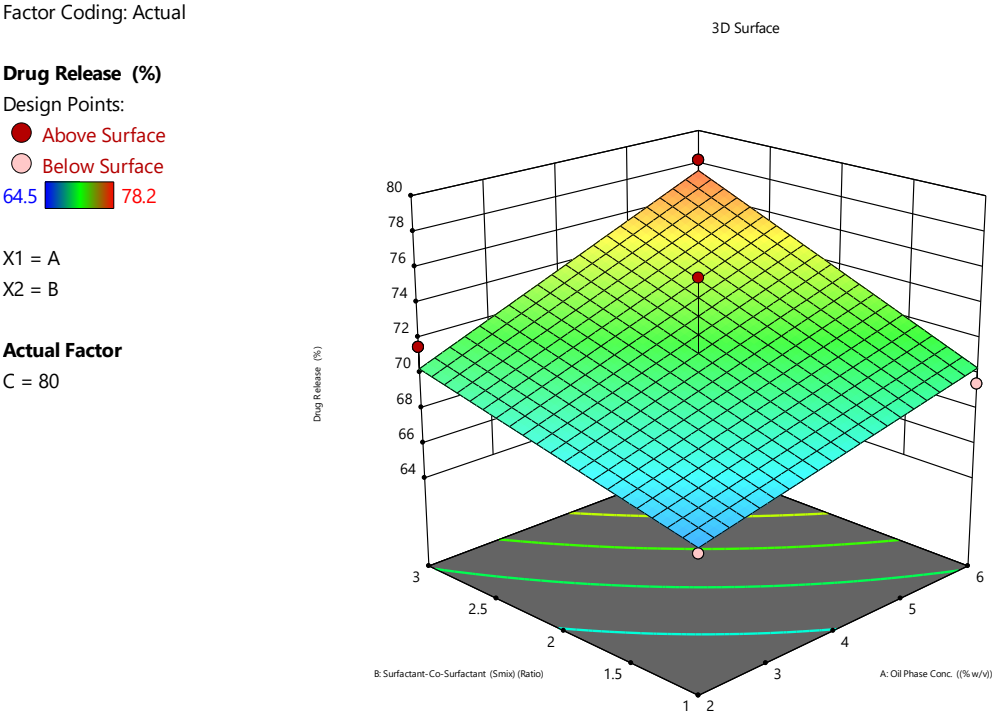


Figure 6. 3D Response Surface Analysis for Drug Release

References

1. Kumar, S., Saha, S., Singh, K., Singh, T., Mishra, A. K., Dubey, B. N., & Singh, S. (2024). Beneficial effects of spirulina on brain health: A systematic review. *Current Functional Foods*, *3*(1), Article e120124225622. https://doi.org/10.2174/0126668629269256231222092721
2. RaviKKumar VR, Rathi S, Singh S, Patel B, Singh S, Chaturvedi K, Sharma B. A Comprehensive Review on Ulcer and Their Treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2023 Dec 21;39:e20230006. doi: 10.62958/j.cjap.2023.006. PMID: 38755116.
3. Singh, V., Arora, S., Akram, W., Alam, S., Kumari, L., Kumar, N., Kumar, B., Kumar, S., Agrawal, M., Singhal, M., Kumar, S., Singh, S., Singh, K., Saha, S., & Dwivedi, V. (2024). Involvement of molecular mechanism and biological activities of Pemirolast: A therapeutic review. *New Emirates Medical Journal*, *5*, Article e02506882308410.
4. Rajput DS, Gupta N, Singh S, Sharma B. A Comprehensive Review: Personalized Medicine for Rare Disease Cancer Treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2023 Dec 23;39:e20230008. doi: 10.62958/j.cjap.2023.008. PMID: 38830754.
5. Singh S, Chaurasia A, Rajput DS, Gupta N. Mucoadhesive Drug Delivery System and There Future Prospective: Are a Promising Approach for Effective Treatment? Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2023 Dec 20;39:e20230005. doi: 10.62958/j.cjap.2023.005. PMID: 38751344.
6. Kumar, S., Saha, S., Sharma, B., Singh, S., Shukla, P., Mukherjee, S., Agrawal, M., Singh, K., & Singh, T. (2023). The role of resveratrol in Alzheimer's disease: A comprehensive review of current research. *Current Functional Foods*, *2*(2), Article e121223224364, 13 pages.
7. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, Bumrela S, Jain PC, Goswami P, Singh S. Recent Innovations and Future Perspectives in Transferosomes for Transdermal Drug Delivery in Therapeutic and Pharmacological Applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Oct 24;40:e20240031. doi: 10.62958/j.cjap.2024.031. PMID: 39442957.

8. Vaghela MC, Rathi S, Shirole RL, Verma J, Shaheen, Panigrahi S, Singh S. Leveraging AI and Machine Learning in Six-Sigma Documentation for Pharmaceutical Quality Assurance. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Jul 18;40:e20240005. doi: 10.62958/j.cjap.2024.005. PMID: 39019923.
9. Singh S, Chaurasia A, Gupta N, Rajput DS. Effect of Formulation Parameters on Enalapril Maleate Mucoadhesive Buccal Tablet Using Quality by Design (QbD) Approach. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Jun 27;40:e20240003. doi: 10.62958/j.cjap.2024.003. PMID: 38925868.
10. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, Bumrela S, Jain PC, Goswami P, Singh S. Recent Innovations and Future Perspectives in Transferosomes for Transdermal Drug Delivery in Therapeutic and Pharmacological Applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Oct 24;40:e20240031. doi: 10.62958/j.cjap.2024.031. PMID: 39442957.
11. Kumar, S., Saha, S., Pathak, D., Singh, T., Kumar, A., Singh, K., Mishra, A. K., Singh, S., & Singh, S. (2024). Cholesterol absorption inhibition by some nutraceuticals. *Recent Advances in Food, Nutrition & Agriculture*, *16*(1), 2–11. https://doi.org/10.2174/012772574X285280240220065812
12. Singh, S., Chaurasia, A., Rajput, D. S., & Gupta, N. (2024). An overview on mucoadhesive buccal drug delivery systems & approaches: A comprehensive review. *African Journal of Biological Sciences (South Africa)*, *6*(5), 522–541, DOI: 10.33472/AFJBS.6.5.2024.522-541
13. Kumar, S., Singh, S., Rajput, D., Sharma, B., Chaturvedi, K., Singh, N., Saha, S., Singh, K., & Mukherjee, S. (2024). Pharmacological approaches and herbal interventions for Alzheimer's disease. *The Natural Products Journal*, *14*(8), Article e220124225945. https://doi.org/10.2174/0122103155275266231123090138
14. Ravikkumar VR, Patel BD, Rathi S, Parthiban S, Upadhye MC, Shah AM, Rehan SSA, Samanta S, Singh S. Formulation and Evaluation of Drumstick Leaves Tablet as An Immunomodulator. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Jun 21;40:e20240004. doi: 10.62958/j.cjap.2024.004. PMID: 38902996.
15. Sharma, A., Bara, G., Keshamma, E., Sharma, B., Singh, S., Singh, S. P., Parashar, T., Rathore, H. S., Sarma, S. K., & Rawat, S. (2023). Cancer biology and therapeutics: A contemporary review. *Journal of Cardiovascular Disease Research*, *14*(10), 1229-1247.
16. Dewangan, H. K., Singh, S., Mishra, R., & Dubey, R. K. (2020). A review on application of nanoadjuvant as delivery system. *International Journal of Applied Pharmaceutics*, *12*(4), 24–33. https://doi.org/10.22159/ijap.2020v12i4.36856
17. Patel SK, Prathyusha S, Kasturi M, Godse KC, Singh R, Rathi S, Bumrela S, Singh S, Goswami P. Optimizing Irbesartan Fast Dissolving Tablets Using Natural Polysaccharides for Enhanced Drug Delivery and Patient Compliance. *Int Res J Multidiscip Scope (IRJMS).* 2025;6(1):1181-1190. https://doi.org/10.47857/irjms.2025.v06i01.02542`
18. Patel S, Alam MI, Shirole RL, Kulkarni PA, Nath J, Prasad M, Singh S, Rathi S. Formulation and Optimization of Piroxicam-Loaded Nanoparticles for Topical Application Using Design of Experiments (DoE). *Cuestiones de Fisioterapia.* 2025;54(4):109-119.