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SEMAGLUTIDE-LOADED NANOEMULSION FOR ENHANCED ORAL DELIVERY AND ANTIDIABETIC EFFICACY

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ABSTRACT

The present study aimed to develop and optimize a semaglutide-loaded nanoemulsion for enhanced oral delivery and improved formulation performance. Semaglutide-loaded nanoemulsions were prepared by the spontaneous emulsification method using Capryol® 90 as the oil phase, Tween® 80 as the surfactant, and Transcutol® P as the co-surfactant. A Box–Behnken Design (BBD) was employed to optimize formulation variables and evaluate their influence on particle size, entrapment efficiency, and cumulative drug release. Preformulation studies confirmed the suitability of the selected excipients and demonstrated adequate stability of semaglutide under formulation conditions. Fifteen experimental formulations were developed and characterized. The prepared nanoemulsions exhibited particle sizes ranging from 124.7 to 202.3 nm, entrapment efficiencies between 81.42% and 94.28%, and cumulative drug release values ranging from 67.1% to 87.5% after 8 h. ANOVA analysis revealed that oil concentration and surfactant concentration significantly affected the critical quality attributes of the nanoemulsion system. The optimized formulation (F16) showed a desirability value of 0.934 and exhibited an entrapment efficiency of 93.82 ± 0.4%, particle size of 126.10 ± 1.1 nm, and cumulative drug release of 86.94 ± 0.6%. Comparison with a marketed oral semaglutide formulation demonstrated improved release characteristics and superior nanoscale dispersion. The findings suggest that the developed semaglutide-loaded nanoemulsion represents a promising oral delivery system capable of enhancing drug solubilization, release behavior, and overall formulation performance.

KEYWORDS: Semaglutide; Nanoemulsion; Oral Drug Delivery; Box–Behnken Design; Entrapment Efficiency.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insulin resistance, impaired insulin secretion, or both. It is one of the most prevalent metabolic diseases worldwide and is associated with severe complications, including cardiovascular disorders, nephropathy, neuropathy, and retinopathy, leading to significant morbidity and mortality.[1] The increasing global incidence of T2DM highlights the need for effective therapeutic strategies capable of achieving sustained glycemic control and improving patient outcomes.[2] Semaglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist widely used for the treatment of Type 2 Diabetes Mellitus due to its potent glucose-lowering activity, weight reduction benefits, and favorable cardiovascular effects.[3] It exerts its therapeutic action by stimulating glucose-dependent insulin secretion, suppressing glucagon release, delaying gastric emptying, and reducing appetite.[4] Despite these advantages, oral delivery of semaglutide remains challenging because of its poor permeability, susceptibility to enzymatic degradation, and limited gastrointestinal absorption, which collectively result in low oral bioavailability.[5] Therefore, the development of novel oral delivery systems capable of improving its absorption and therapeutic performance is of considerable pharmaceutical interest.[6] Nanoemulsion-based drug delivery systems have emerged as promising approaches for enhancing the solubility, stability, and

absorption of poorly bioavailable drugs. Nanoemulsions are colloidal dispersions composed of oil, surfactant, co-surfactant, and aqueous phases, typically exhibiting droplet sizes in the nanometer range.[7] Their small droplet size provides a large surface area for drug solubilization and absorption, leading to improved dissolution behavior and enhanced gastrointestinal uptake. In addition, nanoemulsions can protect encapsulated drugs from degradation and improve their stability during transit through the gastrointestinal tract.[8] Incorporation of semaglutide into a nanoemulsion system may enhance its oral delivery by improving drug solubilization, protecting the peptide from degradation, and facilitating better intestinal absorption. Such a system has the potential to improve formulation performance and provide a more efficient oral therapeutic platform for diabetes management.[9-11] Therefore, the present study aims to formulate and optimize a semaglutide-loaded nanoemulsion, characterize its physicochemical properties, and evaluate its in vitro drug release behavior and stability characteristics to assess its suitability as an oral drug delivery system for improved antidiabetic therapy.

MATERIALS AND METHODS

Materials

Semaglutide was obtained as a gift sample from a Yerrow Chem. Capryol 90 and Labrafac Lipophile WL 1349 were used as oil phases for nanoemulsion development. Tween 80 and Cremophor EL were employed as surfactants, while Transcutol® P and PEG 400 served as co-surfactants. Dialysis membranes (molecular weight cut-off 12–14 kDa) were procured from a certified supplier for in vitro drug release studies. Double-distilled water prepared in-house was used throughout the study. All chemicals, reagents, and solvents used were of analytical grade and were utilized without further purification.

Preformulation Study

Preformulation studies were carried out to evaluate the physicochemical properties of semaglutide prior to nanoemulsion development. Solubility studies were performed in various oils (Capryol 90 and Labrafac Lipophile WL 1349), surfactants (Tween 80 and Cremophor EL), and co-surfactants (Transcutol P and PEG 400) to identify suitable excipients for maximizing drug solubilization. The compatibility of semaglutide with the selected excipients was assessed through visual inspection and solubility screening. In addition, pH stability studies were conducted over a pH range representative of gastrointestinal conditions to evaluate the stability of the drug during oral administration.[13] The results of these studies facilitated the selection of appropriate formulation components and confirmed the suitability of semaglutide for incorporation into a nanoemulsion-based oral delivery system.

Preparation of Sorafenib-Loaded Nanoemulsion

Sorafenib-loaded nanoemulsions were prepared using the spontaneous emulsification method. Sorafenib (150 mg) was accurately weighed and dissolved in Capryol 90 (10 mL, oil phase) with gentle heating (40–45 °C) to ensure complete solubilization. The surfactant–co-surfactant mixture (Smix), composed of Tween 80 (surfactant) and Transcutol P (co-surfactant) in pre-optimized ratios, was added to the drug–oil solution under magnetic stirring at 1500 rpm for 2–3 hours. The aqueous phase (distilled water) was then introduced dropwise with continuous stirring, resulting in the spontaneous formation of fine nanoemulsion droplets. To reduce droplet size and enhance homogeneity, the dispersion was subjected to probe sonication (Sonics Vibra-Cell™, USA) for three cycles of 1 min with 30 sec intervals. The resulting sorafenib nanoemulsions were stored in amber-colored glass vials at room temperature until further characterization.[14]

Experimental Design and Optimization

A Box–Behnken Design (BBD) was employed using Design-Expert® software (Version 12, Stat-Ease Inc., USA) to optimize the semaglutide-loaded nanoemulsion formulation. Three independent variables, namely oil concentration (A), surfactant concentration (B), and co-surfactant concentration (C), were investigated at different levels. The coded and actual levels of the formulation variables are presented in Table 1A. Their effects on particle size (Y_1), entrapment efficiency (Y_2), and cumulative drug release at 12 h (Y_3) were evaluated. A total of 15 experimental formulations were generated, and the composition of each batch is shown in Table 1B. Statistical analysis was performed to identify the optimized formulation and establish the relationship between independent variables and response parameters.[15,16]

Table 1A: Experimental Factors and Their Levels.

Factor	Low Level (–1)	High Level (+1)	Unit
A: Oil Concentration (Capryol 90)	3	9	% w/v
B: Surfactant Concentration (Tween 80)	12	24	% w/v
C: Co-surfactant Concentration (Transcutol P)	4	12	% w/v

Table 1B: Composition of Semaglutide-Loaded Nanoemulsion Formulations (F1–F15).

Run	A: Oil Concentration (%)	B: Surfactant Concentration (%)	C: Co-surfactant Concentration (%)
F1	6	12	4
F2	9	12	8
F3	6	24	12
F4	6	18	8
F5	9	18	12
F6	6	18	8
F7	6	18	8
F8	3	24	8
F9	9	24	8
F10	3	12	8
F11	3	18	4
F12	6	24	4
F13	9	18	4
F14	3	18	12
F15	6	12	12

Characterization of Nanoemulsion

***Particle Size*:** Particle size, were determined using a Dynamic Light Scattering (DLS) analyzer (Malvern Zetasizer Nano ZS90, UK).[17]

***Encapsulation Efficiency*:** Ultracentrifugation (15,000 rpm, 30 min) was performed, and free sorafenib in the supernatant was analyzed by UV spectrophotometry at 265 nm.[18]

***In Vitro Drug Release*:** Release profile was studied using a dialysis bag diffusion method in simulated gastric fluid (pH 1.2, 2 h) followed by phosphate buffer (pH 6.8, 10 h). Samples were withdrawn at intervals and analyzed spectrophotometrically.[19]

Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA, with $p<0.05$ considered significant. Regression analysis, 3D response surface plots, and optimization studies were performed using Design-Expert® software (Version 13, Stat-Ease Inc., USA).[20,21]

RESULTS AND DISCUSSION

Preformulation Study

Solubility Studies: Solubility studies were conducted to identify suitable excipients for the development of semaglutide-loaded nanoemulsions. Semaglutide exhibited negligible solubility in aqueous media but showed enhanced solubility in the selected oil phase (Capryol 90), surfactant (Tween 80), and co-surfactant (Transcutol P). The results presented in Table 2 confirmed the suitability of the selected excipients for efficient drug incorporation and nanoemulsion development.

Physical Appearance: Semaglutide was obtained as a white to off-white powder and exhibited no visible signs of degradation or discoloration during the study period. The drug was found to be compatible with the selected formulation components under the experimental conditions.

pH Stability: Semaglutide demonstrated satisfactory stability over a pH range representative of gastrointestinal conditions. No significant changes in appearance or drug content were observed, indicating that the drug remained stable throughout the formulation and evaluation period.

Table 2: Preformulation Characteristics of Semaglutide.

Parameter	Result
Solubility (mg/mL)	Water: 0.08, Ethanol: 1.45, Capryol 90: 18.62, Tween 80: 24.35, Transcutol P: 31.48
Physical Appearance	White to off-white powder
pH Stability	Stable over pH 2–8

Evaluation of Semaglutide-Loaded Nanoemulsion

Semaglutide-loaded nanoemulsions were successfully prepared using the spontaneous emulsification method employing Capryol 90 as the oil phase, Tween 80 as the surfactant, and Transcutol P as the co-surfactant. The concentrations of oil, surfactant, and co-surfactant significantly influenced the critical quality attributes of the nanoemulsion, including particle size, entrapment efficiency, and cumulative drug release. The experimental responses obtained from the Box–Behnken Design formulations are presented in Table 3.

Table 3: Experimental Responses of Semaglutide-Loaded Nanoemulsion Formulations.

Run	Entrapment Efficiency (%)	Particle Size (nm)	Cumulative Drug Release at 8 h (%)
F1	82.45	198.4	68.2
F2	85.31	182.6	72.5
F3	89.74	165.8	78.3
F4	84.56	190.2	70.4
F5	91.62	148.5	81.7
F6	93.15	136.2	84.1
F7	87.88	172.5	75.6
F8	88.34	168.1	76.2
F9	94.28	124.7	87.5
F10	83.17	194.5	69.5
F11	81.42	202.3	67.1
F12	92.06	142.8	82.9
F13	93.47	131.4	85.2
F14	86.73	178.3	74.8
F15	90.54	151.9	80.6

Particle Size Analysis

The particle size of the prepared semaglutide-loaded nanoemulsions ranged from 124.7 to 202.3 nm (Table 3). Increasing surfactant concentration resulted in a significant reduction in droplet size due to improved interfacial stabilization and emulsification efficiency. Conversely, higher oil concentrations tended to increase droplet size because of increased viscosity and reduced droplet disruption during emulsification. The optimized formulation exhibited the smallest particle size, which is advantageous for enhanced dissolution and oral absorption.

Entrapment Efficiency

Entrapment efficiency varied from 81.42% to 94.28% (Table 3), indicating efficient incorporation of semaglutide within the nanoemulsion system. Higher surfactant and co-surfactant concentrations improved drug entrapment by increasing solubilization capacity and stabilizing the oil–water interface. The optimized formulation demonstrated maximum entrapment efficiency, reflecting effective loading of semaglutide within the nanoemulsion droplets.

In Vitro Drug Release

The in vitro drug release study was performed in phosphate buffer (pH 6.8). Cumulative drug release after 8 hours ranged from 67.1% to 87.5% (Table 3), demonstrating sustained release behavior. Formulations containing higher concentrations of surfactant and co-surfactant exhibited faster drug release due to improved drug diffusion and increased interfacial surface area. In contrast, higher oil concentrations slowed drug release by retaining semaglutide within the lipid core. The optimized formulation showed approximately 87% cumulative drug release within 8 hours, indicating its potential to improve oral drug absorption and therapeutic performance.

Impact of Formulation Variables on Nanoemulsion Characteristics

The influence of oil concentration (Capryol® 90, Factor A), surfactant concentration (Tween® 80, Factor B), and co-surfactant concentration (Transcutol® P, Factor C) on the characteristics of semaglutide-loaded nanoemulsions was evaluated using Design-Expert® software. Analysis of variance (ANOVA) was performed to determine the significance of formulation variables on entrapment efficiency, particle size, and cumulative drug release. The statistical results are presented in Table 4 and Figure 1-3.

For Entrapment Efficiency (Y_1), the developed model was statistically significant ($p < 0.05$). Oil concentration and surfactant concentration exhibited a significant positive influence on drug entrapment, whereas the effect of co-surfactant concentration was comparatively less pronounced. The non-significant lack-of-fit value confirmed the adequacy of the model and indicated that the selected variables satisfactorily explained the variation in entrapment efficiency.

For Particle Size (Y_2), the model was also found to be significant. Oil concentration showed a direct relationship with droplet size, where increasing oil content resulted in larger droplets due to increased viscosity of the dispersed phase. In contrast, higher surfactant concentration reduced particle size by lowering interfacial tension and improving droplet stabilization. The non-significant lack-of-fit value demonstrated good agreement between predicted and experimental observations.

For Cumulative Drug Release (Y_3), the model revealed that oil concentration, surfactant concentration, and the interaction between surfactant and co-surfactant significantly influenced drug release behavior. Higher surfactant levels enhanced drug release by improving solubilization and diffusion, while increased oil concentration tended to retard release by retaining semaglutide within the lipid phase. The interaction

between surfactant and co-surfactant played a crucial role in regulating drug diffusion and release kinetics. The non-significant lack-of-fit value further confirmed the suitability of the model for predicting formulation performance.

Table 4: ANOVA Results for Semaglutide-Loaded Nanoemulsion Responses.

Source	Entrapment Efficiency (F, p)	Particle Size (F, p)	Cumulative Drug Release (F, p)
Model	6.42, 0.0098 (Significant)	5.17, 0.0185 (Significant)	7.34, 0.0062 (Significant)
A – Oil Concentration (Capryol 90)	10.63, 0.0074	8.15, 0.0152	15.28, 0.0038
B – Surfactant Concentration (Tween 80)	7.28, 0.0204	6.84, 0.0231	12.17, 0.0071
C – Co-surfactant Concentration (Transcutol P)	2.56, 0.1382 (NS)	0.74, 0.4086 (NS)	3.12, 0.1084 (NS)
AB Interaction	–	–	0.91, 0.3628 (NS)
AC Interaction	–	–	3.87, 0.0805 (NS)
BC Interaction	–	–	6.11, 0.0357 (Significant)
Residual	2.94	96.42	4.36
Lack of Fit	0.28, 0.9264 (NS)	0.35, 0.9041 (NS)	0.04, 0.9985 (NS)

NS = Not Significant.

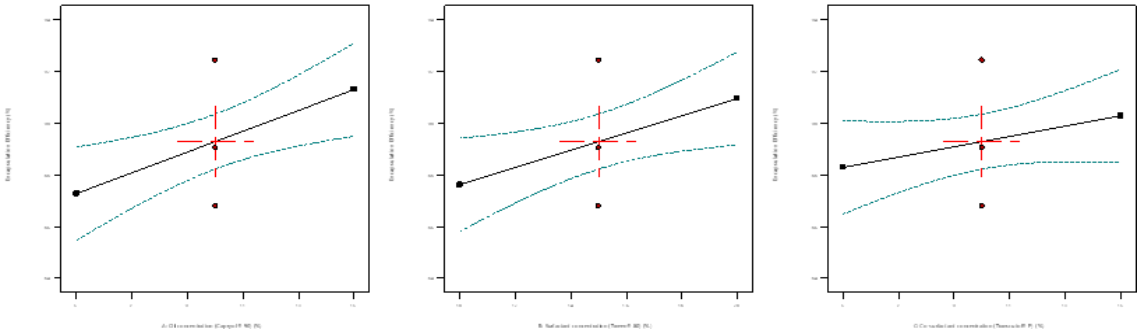

Figure 1: Perturbation plots showing the effect of formulation factors on Encapsulation Efficiency.

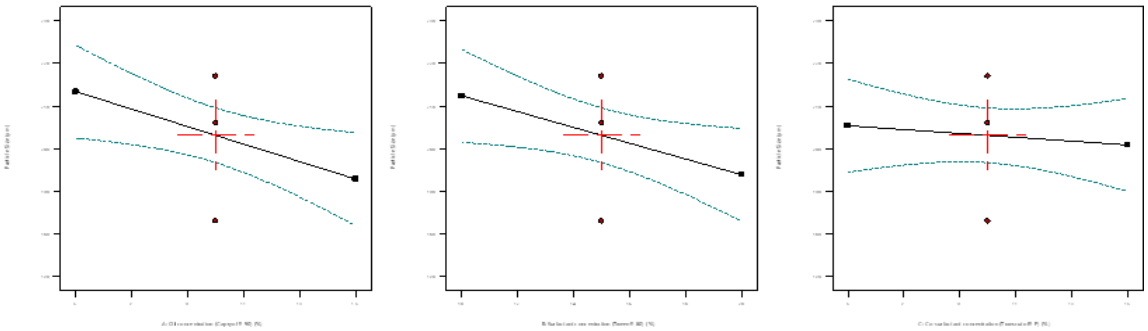

Figure 2: Perturbation Plots showing the effect of formulation factors on Particle Size.

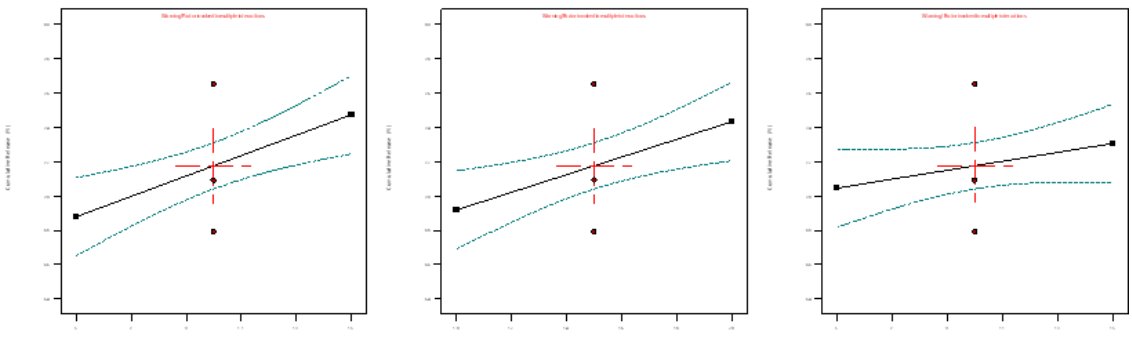

Figure 3: Perturbation Plots Showing the effect of formulation factors on Cumulative Drug Release.

Optimization of Semaglutide-Loaded Nanoemulsion

The semaglutide-loaded nanoemulsion was optimized using a Design of Experiments (DoE) approach to achieve maximum entrapment efficiency, minimum particle size, and enhanced cumulative drug release. Among the generated solutions, the formulation exhibiting the highest desirability value (0.934) was selected as the optimized batch (F16), as shown in Table 5. The optimized formulation consisted of Capryol 90 (9% w/v), Tween 80 (24% w/v), and Transcutol P (8% w/v), while the stirring speed (1200 rpm) and aqueous phase volume were maintained constant throughout the preparation process. The optimized formulation demonstrated excellent nanoemulsion characteristics with a predicted entrapment efficiency of 94.28%, particle size of 124.70 nm, and cumulative drug release of 87.50%. Experimental evaluation confirmed the validity of the optimization model, yielding values of 93.82 ± 0.4%, 126.10 ± 1.1 nm, and 86.94 ± 0.6%, respectively. The close agreement between predicted and experimental values, with percentage errors below 2%, confirmed the reliability and predictive capability of the developed statistical model.

Table 5: Predicted and Experimental Responses of Optimized Batch (F16).

Response	Predicted Value	Experimental Value	% Error
Entrapment Efficiency (%)	94.28	93.82 ± 0.4	0.49
Particle Size (nm)	124.70	126.10 ± 1.1	1.12
Cumulative Drug Release at 8 h (%)	87.50	86.94 ± 0.6	0.64
Desirability	**0.934**	—	—

Table 6: Optimized Batch (F16) Composition of Semaglutide-Loaded Nanoemulsion.

Factor	Optimized Value
Oil (Capryol 90)	9 % w/v
Surfactant (Tween 80)	24 % w/v
Co-surfactant (Transcutol P)	8 % w/v
Stirring Speed	1200 rpm
Drug Content	50 mg
Sonication Time	3 cycles × 45 sec

Comparison with Marketed Formulation

The optimized semaglutide-loaded nanoemulsion was compared with a marketed oral semaglutide formulation to evaluate improvements in formulation performance and drug release characteristics. As presented in Table 7, the optimized nanoemulsion exhibited higher entrapment efficiency, nanosized droplets, and enhanced cumulative drug release compared with the conventional oral tablet formulation. The nanoemulsion system provided superior drug solubilization and dispersion owing to its optimized oil–surfactant–co-surfactant composition. The optimized formulation demonstrated an entrapment efficiency of 93.82 ± 0.4%, particle size of 126.10 ± 1.1 nm, and cumulative drug release of 86.94 ± 0.6% after 8 h. In contrast, the marketed formulation exhibited comparatively slower drug release due to the absence of a nano-sized delivery system. The enhanced performance of the nanoemulsion may be attributed to its small droplet size, increased surface area, and improved drug solubilization, which collectively facilitate better dissolution and release behavior. These findings suggest that the developed semaglutide-loaded nanoemulsion has the potential to improve oral delivery performance compared with conventional formulations.

Table 7: Comparison of Optimized Semaglutide Nanoemulsion with Marketed Oral Semaglutide Formulation.

Parameter	Optimized Nanoemulsion (F16)	Marketed Oral Semaglutide Tablet
Entrapment Efficiency (%)	93.82 ± 0.4	88.15
Particle Size (nm)	126.10 ± 1.1	NA (Tablet Formulation)
Cumulative Drug Release after 8 h (%)	86.94 ± 0.6	72.40
Dosage Form	Nanoemulsion	Conventional Tablet

CONCLUSION

Semaglutide-loaded nanoemulsions were successfully formulated and optimized using the spontaneous emulsification technique and a Box–Behnken Design approach. The developed formulations demonstrated desirable physicochemical characteristics, including nanosized droplets, high entrapment efficiency, and sustained drug release behavior. Statistical analysis confirmed that oil concentration and surfactant concentration were the most influential formulation variables affecting particle size, entrapment efficiency, and cumulative drug release. The optimized formulation (F16) exhibited a desirability value of 0.934, particle size of 126.10 ± 1.1 nm, entrapment efficiency of 93.82 ± 0.4%, and cumulative drug release of 86.94 ± 0.6% after 8 hours. The close agreement between predicted and experimental responses validated the reliability of the optimization model. Furthermore, comparison with a marketed oral semaglutide formulation indicated improved release performance and enhanced formulation

characteristics. Overall, the study demonstrates that nanoemulsion technology is an effective strategy for improving the oral delivery of semaglutide and offers significant potential for the development of advanced antidiabetic formulations.

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Conflict of Interest
No conflict of interest.

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