RESEARCH PAPER

Formulation, Optimisation, and In Vitro Evaluation of Polymeric Nanoparticles for Enhanced Oral Delivery of Semaglutide

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ABSTRACT
Oral delivery of peptide therapeutics is limited by enzymatic degradation in the gastrointestinal tract and poor intestinal permeability. Semaglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist used for the treatment of type 2 diabetes and obesity, is primarily administered via injection, which may reduce patient compliance. In this study, semaglutide-loaded polymeric nanoparticles were prepared using the solvent evaporation method and optimized using a Box–Behnken design. The effects of polymer concentration, surfactant concentration, and homogenization speed were evaluated on particle size, polydispersity index (PDI), and encapsulation efficiency. The optimized formulation was further characterized for physicochemical properties, drug loading, and in vitro drug release behaviour. The optimized nanoparticles exhibited a particle size of 145 ± 8 nm, PDI of 0.18 ± 0.02, and zeta potential of −22.5 ± 1.6 mV. Encapsulation efficiency and drug loading were found to be 78.4 ± 2.3% and 9.6 ± 0.8%, respectively. In vitro release studies showed an initial release of 28.3% within 6 hours, followed by sustained release reaching 85.6% over 48 hours. Stability studies demonstrated minimal changes in physicochemical properties over 30 days. Overall, the developed polymeric nanoparticle system shows promise as an effective strategy for enhancing the oral delivery of semaglutide. Further in vivo studies are required to confirm its bioavailability and therapeutic potential.

Keywords: Semaglutide; Polymeric nanoparticles; Oral drug delivery; Encapsulation efficiency; Controlled drug release; PLGA.
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INTRODUCTION

Peptide-based therapeutics have gained considerable attention in recent years due to their high specificity, potent pharmacological activity, and relatively favourable safety profiles (1,2). Among these, glucagon-like peptide-1 (GLP-1) receptor agonists have emerged as an important class of drugs for the management of type 2 diabetes mellitus and obesity (3). Semaglutide, a long-acting GLP-1 analogue, has demonstrated significant clinical efficacy in improving glycemic control and promoting weight loss (4). Despite these advantages, its administration is largely limited to subcutaneous injection, which can negatively affect patient compliance, particularly in long-term therapy (5). The oral delivery of peptide drugs such as semaglutide presents multiple physiological and biochemical barriers. In the gastrointestinal tract, peptides are highly susceptible to enzymatic degradation by proteolytic enzymes, including pepsin, trypsin, and chymotrypsin (6). Additionally, the acidic gastric environment contributes to peptide instability and denaturation (7). Even when degradation is partially minimized, the large molecular size and hydrophilic nature of peptides restrict their permeability across the intestinal epithelium, resulting in very low oral bioavailability (8). These challenges necessitate the development of advanced delivery systems that can protect peptide drugs and enhance their absorption.

Several formulation strategies have been investigated to overcome these barriers, including the use of enzyme inhibitors, permeation enhancers, and lipid-based systems (9). However, these approaches often present limitations such as safety concerns, variability in absorption, and insufficient protection of the drug molecule (10). In this context, polymeric nanoparticles have emerged as a promising platform for oral peptide delivery due to their

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ability to encapsulate and protect sensitive biomolecules (11). Polymeric nanoparticles, typically prepared from biodegradable and biocompatible polymers such as poly(lactic-co-glycolic acid) (PLGA), chitosan, and alginate, offer multiple advantages, including enhanced stability, mucoadhesion, and controlled drug release (12,13). These systems can improve drug transport across the intestinal mucosa by increasing residence time and facilitating interaction with epithelial cells (14). Moreover, the physicochemical properties of nanoparticles, such as particle size, surface charge, and hydrophobicity, can be tailored to optimize drug delivery performance (15).

Although nanoparticle-based approaches have shown promise in improving the oral delivery of peptides, studies specifically focusing on semaglutide-loaded polymeric nanoparticles remain limited. In particular, there is a lack of systematic optimization studies that assess the influence of formulation variables on critical quality attributes such as particle size, polydispersity index, and encapsulation efficiency. Such optimization is essential to ensure reproducibility and scalability of the formulation. Furthermore, comprehensive in vitro evaluation under simulated gastrointestinal conditions is necessary to understand the release behaviour and stability of the developed system (16). These evaluations provide important insights into the potential in vivo performance of the formulation and its ability to maintain drug integrity during transit through the gastrointestinal tract. Therefore, the present study aims to formulate and optimize semaglutide-loaded polymeric nanoparticles using a Design of Experiments (DoE) approach. The optimized formulation is further characterized for key physicochemical properties, including particle size, surface charge, morphology, and encapsulation efficiency. In addition, in vitro drug release and stability studies are conducted to evaluate the suitability of the system for oral delivery.

2. MATERIALS AND METHODS

2.1 Materials

Semaglutide was procured from a certified pharmaceutical supplier and used as received. Poly(lactic-co-glycolic acid) (PLGA) was selected as the biodegradable polymer. Polyvinyl alcohol (PVA) was used as a stabilizer. Organic solvents such as dichloromethane and acetone were used for nanoparticle preparation. All other reagents were of analytical grade and used without further purification (17,18).

2.2 Preparation of Polymeric Nanoparticles

Semaglutide-loaded polymeric nanoparticles were prepared using the solvent evaporation method. Briefly, PLGA was dissolved in an organic solvent to form the organic phase. Semaglutide was dispersed in an aqueous phase containing PVA. The organic phase was added dropwise into the aqueous phase under high-speed homogenization to form an oil-in-water (O/W) emulsion. The emulsion was stirred continuously to allow solvent evaporation, resulting in nanoparticle formation. The nanoparticles were collected by centrifugation, washed to remove excess stabilizer, and lyophilised for further analysis (19,20).

Table 2.1: Composition of Optimized Nanoparticle Formulation

Component	Quantity
Semaglutide	10 mg
PLGA	100 mg
PVA	1.0 %
Organic solvent	10 mL
Aqueous phase	50 mL

Figure 2.1 illustrates the stepwise process involved in the preparation of polymeric nanoparticles using the solvent evaporation method. Initially, the polymer (PLGA) and drug (semaglutide) are dissolved in an organic solvent to form the organic phase. This phase is then emulsified into an aqueous phase containing a stabiliser (PVA) under high-speed homogenization, resulting in the formation of an oil-in-water (O/W) emulsion. Subsequently, the organic solvent is removed through evaporation under continuous stirring, leading to the solidification of polymer droplets and the formation of nanoparticles. The formed nanoparticles are then collected by centrifugation, followed by washing and drying (typically via lyophilisation) to obtain stable polymeric nanoparticles. The final product consists of uniformly distributed drug-loaded nanoparticles suitable for oral delivery applications.

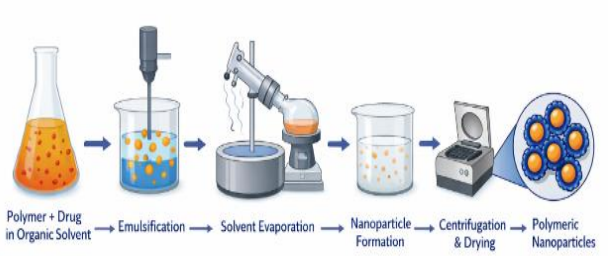

Figure 2.1: Schematic representation of polymeric nanoparticle preparation using solvent evaporation method

2.3 Experimental Design and Optimization

A Box–Behnken design was employed to evaluate the effect of formulation variables on nanoparticle characteristics. Independent variables included polymer concentration (X_1), surfactant concentration (X_2), and homogenization speed (X_3). The responses measured were particle size (Y_1), polydispersity index (Y_2), and encapsulation efficiency (Y_3).

Table 2.2: Experimental Variables and Responses

Factor	Variable	Symbol	Levels (Low–Medium–High)
Independent	Polymer concentration	X_1	50–100–150 mg

Independent	Surfactant concentration	X_2	0.5–1.0–1.5 %
Independent	Homogenization speed	X_3	8000–12000–16000 rpm
Dependent	Particle size	Y_1	Measured
Dependent	PDI	Y_2	Measured
Dependent	Encapsulation efficiency	Y_3	Measured

Statistical analysis and optimization were performed using response surface methodology to obtain an optimized formulation with minimum particle size and maximum encapsulation efficiency (21,22).

2.4 Characterization of Nanoparticles

The prepared nanoparticles were characterized for physicochemical properties using standard analytical techniques.

Table 2.3: Characterization Techniques and Instruments

Parameter	Technique	Instrument
Particle size, PDI	Dynamic Light Scattering (DLS)	Malvern Zetasizer
Zeta potential	Electrophoretic mobility	Zetasizer
Morphology	Scanning Electron Microscopy (SEM)	SEM Instrument
Drug content	High-Performance Liquid Chromatography (HPLC)	HPLC System

Particle size and PDI were measured using dynamic light scattering after appropriate dilution (23). Zeta potential was determined to assess surface charge and stability (24). Morphology was examined using SEM (25). Drug content and encapsulation efficiency were quantified using HPLC analysis (26).

2.5 In Vitro Drug Release Study

The in vitro release of semaglutide from nanoparticles was evaluated using a dialysis bag method. The study was conducted in simulated gastric fluid (pH 1.2) followed by simulated intestinal fluid (pH 6.8). Samples were withdrawn at predetermined intervals and analyzed using HPLC. Sink conditions were maintained throughout the study (27,28).

2.6 Stability Studies

Stability studies were carried out under refrigerated (4°C) and accelerated (25°C ± 2°C/60% RH) conditions. Samples were analyzed periodically for particle size, PDI, and drug content to assess formulation stability (29).

2.7 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical significance was evaluated using ANOVA, with $p < 0.05$ considered significant (30).

3. RESULTS

3.1 Optimization of Formulation Variables

The formulation of semaglutide-loaded polymeric nanoparticles was optimized using a Box–Behnken design. The experimental design matrix, along with observed responses, is presented in Table 1. The results demonstrate that formulation variables significantly influenced particle size, polydispersity index (PDI), and encapsulation efficiency. An increase in polymer concentration resulted in larger particle sizes, which may be attributed to increased viscosity of the organic phase, limiting efficient droplet breakup during emulsification (31). In contrast, higher surfactant concentration reduced particle size and PDI due to improved stabilization of the emulsion system (32). Increased homogenization speed further contributed to reduced particle size, likely due to enhanced shear forces.

Table 1: Experimental Design (DoE Matrix)

Run	Polymer Conc. (X_1)	Surfactant Conc. (X_2)	Homogenization Speed (X_3)	Particle Size (nm)	PDI	Encapsulation Efficiency (%)
1	Low	Medium	High	165	0.22	72.5
2	High	Low	Medium	210	0.30	81.2

3	Medium	High	Low	140	0.18	75.6
4	Medium	Medium	Medium	145	0.19	78.4
5	High	High	High	175	0.24	79.8

The statistical validity of the optimization model was confirmed using ANOVA, as summarized in Table 2. All responses showed high regression coefficients ($R^2 > 0.90$) and statistically significant p-values ($p < 0.05$), indicating good model fit and predictive capability (31,32).

Table 2: Optimization Model Summary (ANOVA Results)

Response	R^2	Adjusted R^2	p-value	Model Significance
Particle Size	0.96	0.94	<0.001	Significant
PDI	0.93	0.90	<0.01	Significant
Encapsulation Efficiency	0.95	0.92	<0.001	Significant

3.2 Characterisation of Optimized Nanoparticles

The optimized nanoparticle formulation exhibited a mean particle size of 145 ± 8 nm with a PDI of 0.18 ± 0.02, indicating a narrow size distribution (Table 3). Such nanoscale dimensions are considered favourable for oral drug delivery systems (33). Zeta potential was found to be −22.5 ± 1.6 mV, suggesting moderate colloidal stability due to electrostatic repulsion between particles (34). Morphological analysis (SEM) revealed spherical nanoparticles with smooth surfaces, indicating uniform formation and absence of aggregation (35).

Table 3: Characterization of Optimized Nanoparticles

Parameter	Value (Mean ± SD)
Particle Size (nm)	145 ± 8
PDI	0.18 ± 0.02
Zeta Potential (mV)	−22.5 ± 1.6
Encapsulation Efficiency (%)	78.4 ± 2.3
Drug Loading (%)	9.6 ± 0.8

3.3 Encapsulation Efficiency and Drug Loading

The encapsulation efficiency and drug loading of the optimized formulation were found to be 78.4 ± 2.3% and 9.6 ± 0.8%, respectively (Table 3). High encapsulation efficiency suggests effective incorporation of semaglutide within the polymeric matrix, which may be attributed to rapid polymer solidification and reduced drug diffusion into the external phase during formulation (36).

3.4 In Vitro Drug Release Profile

The in vitro release profile of semaglutide from polymeric nanoparticles is presented in Table 4. The release pattern showed an initial burst release followed by sustained drug release over 48 hours. Approximately 28.3% of the drug was released within the first 6 hours, followed by a gradual release reaching 85.6% at 48 hours. The initial burst release may be associated with drug molecules adsorbed on the nanoparticle surface, while the sustained phase is governed by diffusion through the polymer matrix (37,38).

Table 4: In Vitro Drug Release Data

Time (hrs)	% Drug Released (Mean ± SD)
0	0
2	12.5 ± 1.2
6	28.3 ± 2.1
12	45.7 ± 2.8
24	62.4 ± 3.0
48	85.6 ± 3.5

3.5 Stability Studies

The stability study results are summarized in Table 5. The optimized formulation remained stable under both refrigerated and room temperature conditions over 30 days. No significant variation was observed in particle size, PDI, or drug content, indicating good physicochemical stability. This stability may be attributed to the inherent properties of PLGA and optimized surfactant concentration, which help maintain nanoparticle integrity (39,40).

Table 5: Stability Study Results

Storage Condition	Time	Particle Size (nm)	PDI	Drug Content (%)
4°C	0	145	0.18	100
	30 d	148	0.19	98.7
25°C	0	145	0.18	100
	30 d	152	0.21	97.9

4. DISCUSSION

The present study demonstrated the successful formulation and optimization of semaglutide-loaded polymeric nanoparticles using a systematic Design of Experiments approach. The observed relationships between formulation variables and response parameters (Table 1 and Table 2) confirm that polymer concentration, surfactant level, and

homogenization speed play a critical role in determining nanoparticle characteristics. The increase in particle size with higher polymer concentration (up to ~210 nm in Table 1) can be attributed to increased viscosity of the organic phase, which limits efficient droplet breakdown during emulsification (41). Conversely, formulations containing higher surfactant concentrations showed reduced particle size (~140–165 nm), indicating improved stabilization of the emulsion droplets and prevention of coalescence (42). Similarly, increased homogenization speed contributed to a smaller particle size due to enhanced shear forces, which promote finer dispersion. These findings are consistent with the statistically significant models observed in Table 2 ($R^2 > 0.90$, $p < 0.05$), confirming the reliability of the optimization approach (41,42).

The optimized formulation exhibited a particle size of 145 ± 8 nm with a PDI of 0.18 ± 0.02 (Table 3), indicating a uniform nanoparticle population. Nanoparticles within this size range are reported to enhance interaction with the intestinal epithelium and facilitate uptake via transcellular or endocytic pathways, which is beneficial for oral drug delivery (43). The zeta potential value of −22.5 ± 1.6 mV suggests moderate colloidal stability, which helps prevent aggregation without compromising biocompatibility (44). The spherical morphology observed in SEM analysis further confirms the uniformity and structural integrity of the nanoparticles, which is essential for predictable drug release behaviour (45). The encapsulation efficiency of 78.4 ± 2.3% and drug loading of 9.6 ± 0.8% (Table 3) indicate effective incorporation of semaglutide within the polymer matrix. The increase in encapsulation efficiency with polymer concentration observed in Table 1 supports the hypothesis that a larger polymer matrix facilitates greater drug entrapment. This behaviour is commonly attributed to rapid polymer solidification during solvent evaporation, which minimises drug diffusion into the external aqueous phase (46).

The in vitro drug release profile (Table 4) exhibited a biphasic pattern, with an initial release of approximately 28.3% within the first 6 hours, followed by sustained release reaching 85.6% at 48 hours. The initial burst release can be associated with a surface-bound drug, while the sustained phase is governed by diffusion of the drug through the polymer matrix and gradual polymer degradation (47,48). Such a release profile is advantageous for oral peptide delivery, as it may provide an initial therapeutic effect followed by prolonged drug availability. The release kinetics observed in this study are consistent with diffusion-controlled mechanisms typically described in matrix-based systems. The polymeric network acts as a barrier that regulates drug diffusion, thereby protecting semaglutide from rapid enzymatic degradation in the gastrointestinal environment (47). This controlled release behavior is particularly important for improving the stability and bioavailability of peptide drugs. Stability studies (Table 5) demonstrated minimal changes in particle size (145 to 152 nm), PDI (0.18 to 0.21), and drug content (~98% retention) over 30 days under both refrigerated and room temperature conditions. These results indicate that the optimized formulation possesses good physicochemical stability. The stability can be attributed to the inherent properties of PLGA and the optimized surfactant concentration, which together help maintain nanoparticle integrity and prevent aggregation (49,50). Overall, the results demonstrate that the developed polymeric nanoparticle system effectively addresses key formulation challenges associated with oral delivery of semaglutide. The optimized formulation exhibits suitable particle size, high encapsulation efficiency, controlled drug release, and good stability, all of which are critical parameters for successful oral delivery systems. However, it is important to note that in vitro findings may not fully translate to in vivo performance. Factors such as gastrointestinal motility, enzymatic activity, and absorption barriers may influence drug bioavailability. Therefore, further in vivo studies are required to confirm the pharmacokinetic and therapeutic potential of the developed formulation.

5. CONCLUSION

This study successfully developed and optimized semaglutide-loaded polymeric nanoparticles using a systematic experimental approach. The optimized formulation exhibited desirable physicochemical characteristics, including nanoscale particle size, uniform size distribution, and high encapsulation efficiency, indicating effective drug incorporation. The in vitro release profile demonstrated a controlled biphasic pattern with sustained drug release over 48 hours, which is advantageous for maintaining therapeutic levels and reducing dosing frequency. The stability of the formulation under different storage conditions further confirms its robustness and suitability for pharmaceutical applications. Overall, the findings suggest that polymeric nanoparticles provide an effective platform for improving the oral delivery of semaglutide by enhancing drug protection and modulating release behavior. This approach holds potential as an alternative to conventional injectable therapy.

6. FUTURE PERSPECTIVES

Although the developed nanoparticle system demonstrated promising in vitro performance, further studies are required to establish its clinical relevance. In vivo investigations should be conducted to evaluate pharmacokinetic parameters, bioavailability, and therapeutic efficacy. Additionally, scale-up of the formulation process needs to be explored to ensure reproducibility and industrial feasibility. Future research may also focus on advanced strategies such as surface functionalization, mucoadhesive coatings, or targeting ligands to further enhance intestinal absorption and bioavailability. From a regulatory standpoint, comprehensive safety and toxicity assessments will be essential for clinical translation. The developed system can also be extended to other peptide-based therapeutics, offering a versatile platform for oral drug delivery.

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