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FACTORIAL DESIGN-BASED OPTIMIZATION AND CHARACTERIZATION OF COLON-TARGETED NANOFORMULATION OF ELUXADOLINE FOR IRRITABLE BOWEL SYNDROME: IN-VITRO AND IN-VIVO STUDIES

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ABSTRACT
Eluxadoline, a mixed opioid receptor modulator, is used for the treatment of irritable bowel syndrome with diarrhea (IBS-D). However, its systemic metabolism and potential adverse effects necessitate a colon-targeted drug delivery approach to enhance therapeutic efficacy while minimizing off-target effects. This study aims to develop, optimize, and characterize a colon-targeted nanoformulation of eluxadoline using a factorial design approach for improved localized drug delivery, enhanced bioavailability, and therapeutic efficacy in IBS treatment. A factorial design-based optimization strategy was employed to develop eluxadoline-loaded nanoparticles with desirable particle size, entrapment efficiency, and drug release profile. The formulation was characterized using particle size analysis, zeta potential measurement, drug loading efficiency, and surface morphology evaluation.
In-vitro drug release studies were conducted under simulated gastrointestinal conditions to assess the release kinetics and colonic specificity. Ex-vivo permeability studies were performed using intestinal tissue models. In-vivo pharmacokinetic and pharmacodynamic studies in IBS-induced animal models evaluated bioavailability, colonic targeting efficiency, and therapeutic efficacy. Pharmacodynamic evaluations in IBS-induced animal models revealed significant symptom relief, improved motility regulation, and enhanced therapeutic outcomes. The factorial design-based nanoformulation of eluxadoline successfully improved colonic targeting, prolonged drug retention, and enhanced bioavailability, offering a promising strategy for IBS management. Further clinical evaluation is required to validate its therapeutic potential in humans.
Keywords: Eluxadoline, Colon-Targeted Delivery, Nanoparticles, Factorial Design, Irritable Bowel Syndrome, Bioavailability Enhancement, Pharmacokinetics, Pharmacodynamics

1. INTRODUCTION
Irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal disorder characterized by abdominal pain, bloating, and altered bowel habits, significantly impacting patients' quality of life. Among the subtypes, IBS with diarrhea (IBS-D) is particularly challenging to manage due to its unpredictable symptoms and limited treatment options. Eluxadoline, a mixed opioid receptor modulator, has emerged as an effective therapeutic agent for IBS-D by reducing bowel motility and alleviating pain. However, its conventional oral administration is associated with systemic metabolism, reduced bioavailability, and potential adverse effects, necessitating a more targeted and efficient delivery system.

Need for Colon-Targeted Drug Delivery
Colon-targeted drug delivery offers several advantages in IBS therapy by ensuring localized drug action, minimizing systemic side effects, and enhancing therapeutic efficacy. Nanotechnology-based drug delivery systems, particularly nanoparticles, have gained attention due to their ability to improve drug solubility, protect the drug from premature degradation, and facilitate controlled release at the target site. Designing an optimized nanoparticle formulation for colonic delivery of eluxadoline could enhance its therapeutic potential by ensuring sustained release and improved bioavailability at the intended site of action.

Role of Factorial Design in Optimization

The development of an efficient nanoformulation requires systematic optimization of multiple formulation and process variables. Factorial design, a statistical approach in experimental design, allows for the identification of critical factors influencing nanoparticle characteristics such as particle size, entrapment efficiency, and drug release profile. By employing a factorial design-based approach, an optimized formulation can be developed with minimal experimental runs, ensuring a cost-effective and efficient formulation strategy.

The present study aims to develop, optimize, and characterize a colon-targeted nanoformulation of eluxadoline using factorial design to enhance its therapeutic efficacy for IBS-D. The formulated nanoparticles will be evaluated for their physicochemical properties, in-vitro drug release behavior, and in-vivo pharmacokinetic and pharmacodynamic performance in IBS-induced animal models.

This research is expected to contribute to the advancement of targeted drug delivery for IBS management by providing a novel nanotechnology-based approach for eluxadoline administration. A successfully developed nanoformulation with enhanced colon-specific release and bioavailability could potentially improve treatment outcomes and patient compliance.

2. LITERATURE REVIEW

1. Irritable Bowel Syndrome (IBS) and its Management

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder that affects a significant proportion of the global population, with IBS-D (diarrhea-predominant) being one of the most common subtypes. The pathophysiology of IBS-D is multifactorial, involving dysregulation of gut motility, visceral hypersensitivity, and changes in the gut microbiota. The condition is associated with symptoms like abdominal pain, bloating, and altered bowel movements, significantly impacting patients' quality of life (Camilleri et al., 2017).

Treatment of IBS-D often focuses on alleviating symptoms, as the underlying causes are complex and not fully understood. Pharmacological approaches include antidiarrheals, antispasmodics, and mixed opioid receptor modulators such as eluxadoline. Eluxadoline, a μ-opioid receptor agonist and δ-opioid receptor antagonist, works by regulating bowel motility and alleviating abdominal discomfort (Lacy et al., 2021).

2. Colon-Targeted Drug Delivery

Colon-specific drug delivery is of particular importance in treating diseases that affect the colon, such as IBS, inflammatory bowel disease (IBD), and colorectal cancer. Conventional oral formulations are rapidly absorbed in the upper gastrointestinal tract, resulting in poor colonic bioavailability. Therefore, the development of drug delivery systems that can target the colon, ensuring localized release of the drug, is essential for improving therapeutic outcomes while reducing systemic side effects (Patel et al., 2020).

Various approaches have been employed to achieve colon-targeted delivery, including pH-sensitive, time-dependent, and microbiome-based systems. Among these, nanotechnology-based drug delivery systems offer several advantages, such as increased solubility, protection from enzymatic degradation, and the ability to control release profiles (Bhattarai & Kim, 2019).

3. Nanoparticle-Based Drug Delivery Systems

Nanoparticles, due to their small size and large surface area, offer a promising strategy for drug delivery. Nanoparticle systems, including liposomes, solid lipid nanoparticles (SLNs), and polymeric nanoparticles, have been explored for the delivery of both hydrophobic and hydrophilic drugs. These systems are capable of enhancing bioavailability, controlling drug release, and improving patient compliance by reducing the frequency of dosing (Singh & Lillard, 2018).

In the context of IBS-D treatment, nanoparticles can be engineered to target the colon by incorporating pH-sensitive polymers, such as Eudragit, which dissolve under the alkaline pH conditions of the colon, ensuring that the drug is released specifically at the site of action (Saharan & Kukkar, 2020).

4. Role of Factorial Design in Formulation Optimization

The optimization of nanoparticle formulations involves a careful selection of key variables, including drug-to-polymer ratio, surfactant concentration, and the type of polymer used. Traditional optimization methods often require numerous trial-and-error experiments, making the process time-

consuming and resource-intensive. Factorial design, a statistical experimental design technique, has been widely adopted in pharmaceutical research to optimize formulations efficiently.

Factorial design allows for the simultaneous evaluation of multiple factors and their interactions, helping to identify the most significant variables that affect the formulation's properties. By using this approach, researchers can achieve an optimal balance between particle size, drug release profile, and other critical characteristics with fewer experimental runs (Box & Wilson, 1951). This strategy has been successfully applied in the development of various drug delivery systems, including those targeting the colon (Bansal & Bansal, 2021).

The development of colon-targeted nanoformulations for the delivery of eluxadoline offers significant potential for improving the management of IBS-D. By utilizing factorial design for formulation optimization, it is possible to create a formulation that not only enhances the bioavailability of eluxadoline but also ensures localized drug release, reducing the risk of systemic side effects. Further preclinical and clinical studies will be necessary to validate these formulations and fully establish their clinical benefits.

3. METHODOLOGY

1. UV spectrophotometric analysis: Eluxadoline working standard was received as gift sample from Zydus cadila healthcare Ltd, Thane, India. Methanol AR grade, Hydrochloric acid (HCl) was purchased from LobaChemiePvt. Ltd. (India). Elico SL-159 UV-visible spectrophotometer equipped with a matched quartz cells ultrasonic bath was used to carry out the assay. The solvent used for the assay was spectroscopic-grade methanol. About 10 µg/mL of Eluxadoline drug substance was accurately prepared in spectroscopic-grade methanol solvent. This preparation was then scanned in the 200-350 nm UV region. The wavelength maxima (λ_{max}) was observed at 245 nm and this wavelength was adopted for absorbance measurement. UV analysis was used to determine the concentration of EXD at various dilutions by plotting a calibration curve of concentration versus absorbance.

2. HPLC Quantification: The HPLC method for analyzing Eluxadoline (EXD) was developed using an Agilent 1100 series instrument equipped with a Quaternary G1311A pump, a COLCOM G1316A thermostat column temperature control, a G1329A thermostatic auto sampler, and a G1314A variable programmable UV detector. The system was operated using Agilent ChemStation software. For sample preparation, tablets of EXDL (100 mg each) were powdered, with 10 mg dissolved in a diluent to create a 1.0 mg/mL solution, which was further diluted to 200 µg/mL using methanol for analysis. The method employed a C18 column after testing various column types, utilizing a mobile phase of methanol, water, and acetonitrile in optimized ratios of 55:30:15. The pH and concentration of a 0.1 M Sodium acetate buffer were fine-tuned to achieve the best peak shape and plate count, achieving a resolution greater than 6.0 between the drug and its impurities. A solution containing 100 µg/mL of EXD along with lower concentrations of specific impurities was also prepared to ensure adequate separation and analysis.

Eluxadoline-Loaded PLGA Nanoparticles for IBS-D Treatment: Factorial Design, In Vitro Characterization, and Colon-Targeting Potential

A1. Preparation of Eluxadoline-Loaded PLGA Nanoparticles (EXD-PLGA NPs): Eluxadoline-loaded PLGA nanoparticles (EXD-PLGA NPs) were prepared using the single emulsion-solvent evaporation technique, as described by Khalil et al., with slight modifications. Briefly, 100-200 mg of PLGA polymer was dissolved in 10 mL of dichloromethane (DCM) in a glass tube. The desired amount of Eluxadoline (10 mg) was then added to the polymer/solvent mixture and allowed to dissolve for 30 minutes with intermittent vortexing to ensure complete dissolution. The organic phase containing PLGA and Eluxadoline was rapidly added dropwise into a glass tube containing 10 mL of PVA (1.5% or 3% w/v) (polyvinyl alcohol) in an aqueous solution while continuously vortexing. After the entire drug/polymer mixture was added to the PVA solution, the contents were vortexed for an additional 10 seconds at a high setting to ensure proper mixing. The tube contents were then emulsified using specific sonication time (5 and 7 minutes) at 40% amplitude in an ice-water bath, utilizing a probe sonicator (Vibra-Cell VCX 750 sonicator, Sonics & Materials Inc., Newtown, CT, USA). The resulting fine oil-in-water (O/W) emulsion was immediately poured into 20 mL of an aqueous PVA solution (1.5% or 3% w/v) under rapid stirring using a magnetic stirrer. Dichloromethane was then evaporated from the emulsion droplets under continuous magnetic stirring

at 800 rpm for 3 hours, allowing for the formation of solid nanoparticles. The nanoparticles were collected by centrifugation at 20,000 rpm for 15 minutes and washed three times with Milli-Q water to remove any residual PVA and unencapsulated drug. The supernatants were collected to evaluate the encapsulation efficiency of Eluxadoline. Finally, the pellet of the nanoparticles was resuspended in 5 mL of Milli-Q water, providing a suspension of Eluxadoline-loaded PLGA nanoparticles suitable for further characterization and use in colon-targeted drug delivery studies.

A2.Experimental Design for EXD-PLGA NPs: Design-Expert® software was used to implement the 2^3 factorial design approach to optimize the Eluxadoline-loaded PLGA nanoparticles (EXD-PLGA NPs). The independent variables selected for optimization were: 1) the amount of PLGA in the organic phase (X1 = Qty of PLGA); 2) Conc. of PVA in the aqueous phase (X2 = % of PVA), and 3) the amount of Eluxadoline in the organic phase (X3 = c ELX). The chosen responses for the optimization process were: 1) the mean particle size of the E-PLGA NPs (Y1), 2) the zeta potential of the E-PLGA NPs (Y2), 3) the encapsulation efficiency (Y3), and 4) the percentage (%) of the cumulative drug released after 24 hours (Y4). Each independent variable was assigned a high and low level value to explore their effects on the formulation's performance, as detailed in Table 1. This factorial design approach allows for the systematic evaluation of the effects of these independent variables on the desired nanoparticle characteristics, facilitating the identification of optimal conditions for preparing Eluxadoline-loaded nanoparticles for targeted drug delivery.

A3. Characterization of PLGA encapsulated EXD Nps:

A 3.1 % Yield, Particle Size, PDI, and Zeta Potential of Optimized NPs: The percent yield of EXD-PLGA-Nps-3 and EXD-PLGA-Nps-7 was determined using a traditional gravimetric method. The average particle size, polydispersity index (PDI), and zeta potential of these optimized formulations were measured using a Zetasizer Nano ZS (Malvern Instruments, Southborough, UK). The nanoparticles were placed in sample cells designed specifically for particle size and zeta potential measurements and introduced into the Malvern device. The parameters were measured at 25 °C using dynamic light scattering (DLS) after achieving temperature equilibrium. Each experiment was conducted in triplicate, and the results are expressed as mean ± standard deviation (SD).

RESULTS

Uv absorbance maxima of EXD

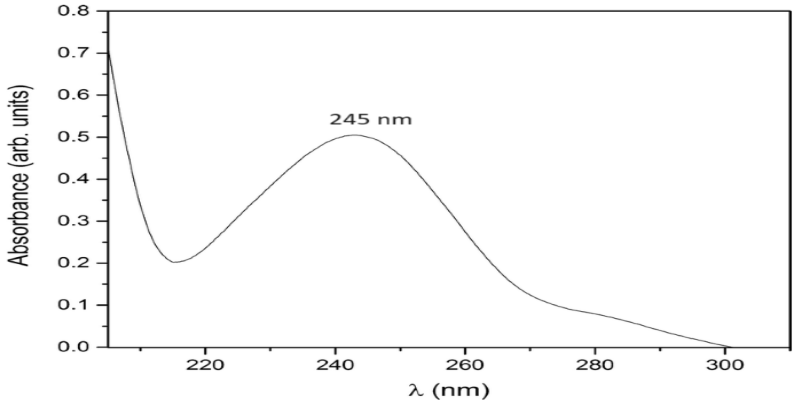


Figure 1. UV absorbance spectra of EXD

2. Calibration of EXD by UV spectra: The calibration curve of EXD was prepared in methanol and detected at λ_{max} at 245 nm. Slope and regression value (r^2) was found to be 0.215 and 0.997 respectively (Table 1 & Figure 2).

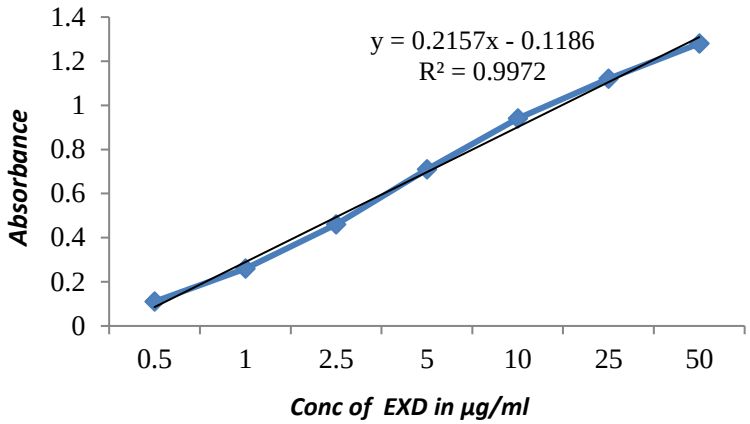


Figure 2. UV-Calibration curve of EXD

Table 1. The absorbance of EXD at different concentrations determined by UV

Conc. of EXD in µg/ml	Absorbance @ 245 nm
0.5	0.11
1	0.26
2.5	0.46
5	0.71
10	0.94
25	1.12
50	1.28

3. HPLC method quantification: The HPLC method for analyzing Eluxadoline (EXD) was developed using an Agilent 1100 series instrument equipped with a Quaternary G1311A pump, a COLCOM G1316A thermostat column temperature control, a G1329A thermostatic auto sampler, and a G1314A variable programmable UV detector. The system was operated using Agilent ChemStation software. For sample preparation, tablets of EXDL (100 mg each) were powdered, with 10 mg dissolved in a diluent to create a 1.0 mg/mL solution, which was further diluted to 200 µg/mL using methanol for analysis. The method employed a C18 column after testing various column types, utilizing a mobile phase of methanol, water, and acetonitrile in optimized ratios of 55:30:15. The pH and concentration of a 0.1 M Sodium acetate buffer were fine-tuned to achieve the best peak shape and plate count, achieving a resolution greater than 6.0 between the drug and its impurities. A solution containing 100 µg/mL of EXD along with lower concentrations of specific impurities was also prepared to ensure adequate separation and analysis.

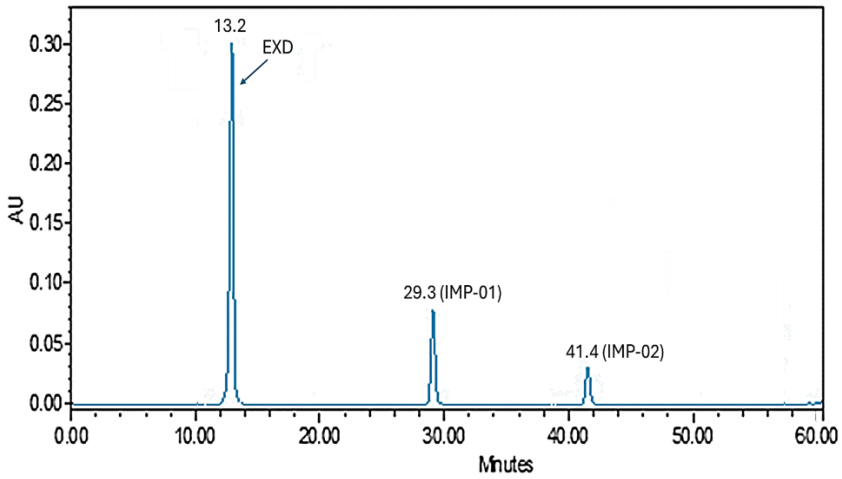


Figure 3. HPLC chromatogram of EXD at a flow rate of 1mL/min

Linearity of EXD Using HPLC

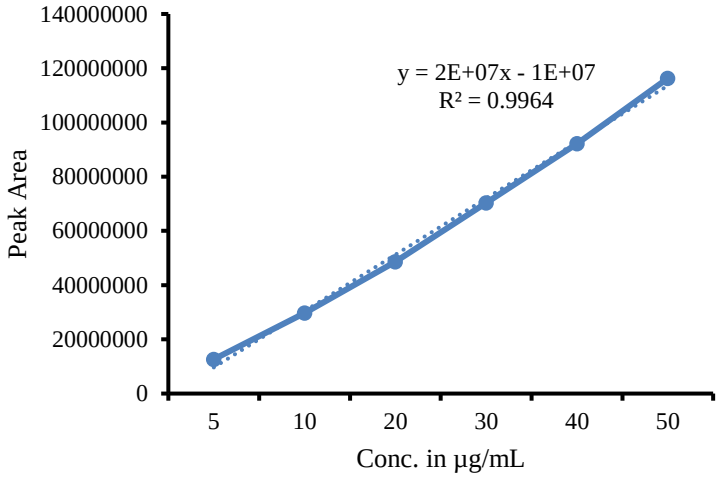


Table 2. Linearity of EXD using HPLC analysis

Concentration (µg/mL)	Peak Area (arbitrary units)
5	12633587 ± 2215
10	29658954 ± 3647
20	48552375 ± 7412
30	70332659 ± 9236
40	92144587 ± 10558
50	116255473 ± 12332

Section-A

Eluxadoline-Loaded PLGA Nanoparticles for IBS-D Treatment: Factorial Design, In Vitro Characterization, and Colon-Targeting Potential

A1. Design of Experiments using 2^3 factorial design

Independent and dependent variables were used for the 2^3-factorial design approach.

Independent variables	Level		Dependent variables
	Low	High	
Qty of PLGA (X_1)	100 mg	200 mg	Particle size in nm (Y1) Zetapotential (Y2) Encapsulation efficiency (%) (Y3) In vitro release of EXD (%) (Y4)
% of PVA (X2)	1.5 %	3 %	
Qty of EXD (X3)	10	20	

A2. 2^3 factorial design formulation protocol

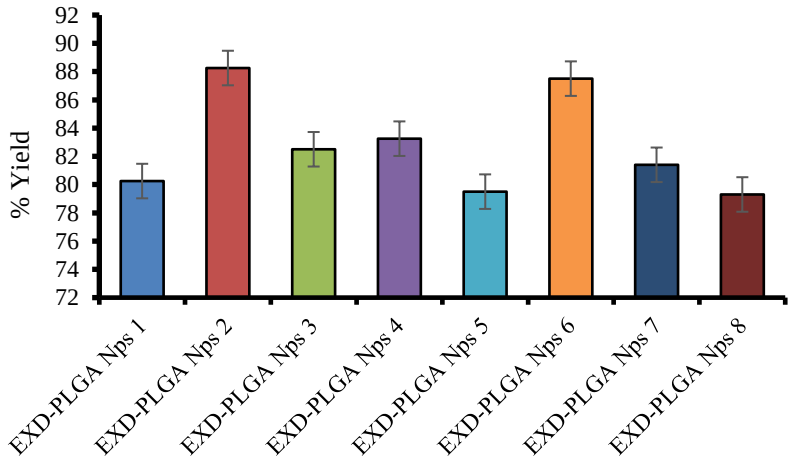
Std	Run	Qty of PLGA in mg (X1)	Qty of PVA in % (X2)	Qty of EXD (X3)
1	1	100	1.5	10
2	2	200	1.5	10
7	3	100	3	20
5	4	100	1.5	20

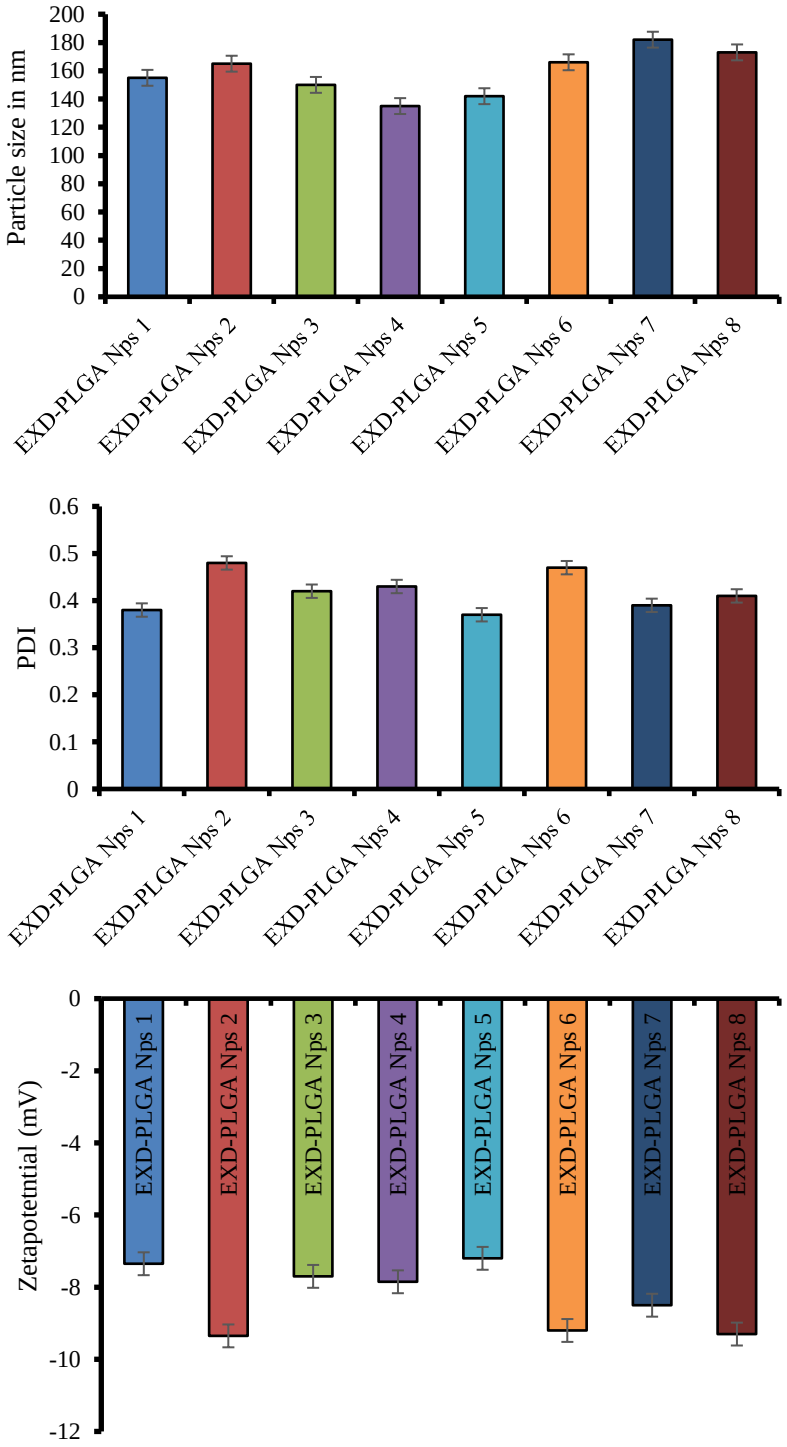
3	5	100	3	10
4	6	200	3	10
8	7	200	3	20
6	8	200	1.5	20

A3. Characterization of EXD-PLGS-NPs

Table. Characterization of EXD-PLGA_Nps

Nps	% Yield	Size	PDI	Zeta potential	% Entrapment	% Drug loading
EXD-PLGA Nps 1	80.25 ± 3.31	155.00 ± 5.96	0.38 ± 0.02	-7.35 ± 0.36	79.30 ± 3.68	32.85 ± 1.20
EXD-PLGA Nps 2	88.25 ± 3.50	165.00 ± 5.62	0.48 ± 0.02	-9.35 ± 0.42	94.30 ± 3.79	37.85 ± 1.47
EXD-PLGA Nps 3	82.50 ± 3.41	150.00 ± 5.59	0.42 ± 0.01	-7.70 ± 0.27	83.68 ± 3.25	35.70 ± 1.34
EXD-PLGA Nps 4	83.25 ± 3.47	135.00 ± 5.18	0.43 ± 0.02	-7.85 ± 0.28	83.37 ± 4.06	35.85 ± 1.55
EXD-PLGA Nps 5	79.50 ± 2.61	142.00 ± 4.63	0.37 ± 0.02	-7.20 ± 0.34	79.60 ± 3.14	32.70 ± 1.11
EXD-PLGA Nps 6	87.50 ± 3.66	166.00 ± 6.37	0.47 ± 0.02	-9.20 ± 0.37	94.52 ± 4.22	37.70 ± 1.44
EXD-PLGA Nps 7	81.40 ± 3.39	182.00 ± 7.31	0.39 ± 0.02	-8.50 ± 0.41	83.71 ± 2.79	38.10 ± 1.85
EXD-PLGA Nps 8	79.30 ± 2.44	173.00 ± 7.08	0.41 ± 0.01	-9.30 ± 0.42	76.80 ± 3.23	35.60 ± 1.60




Particle size in nm
200
180
160
140
120
100
80
60
40
20
0
EXD-PLGA Nps 1
EXD-PLGA Nps 2
EXD-PLGA Nps 3
EXD-PLGA Nps 4
EXD-PLGA Nps 5
EXD-PLGA Nps 6
EXD-PLGA Nps 7
EXD-PLGA Nps 8
PDI
0.6
0.5
0.4
0.3
0.2
0.1
0
Zetapotetntial (mV)
0
-2
-4
-6
-8
-10
-12

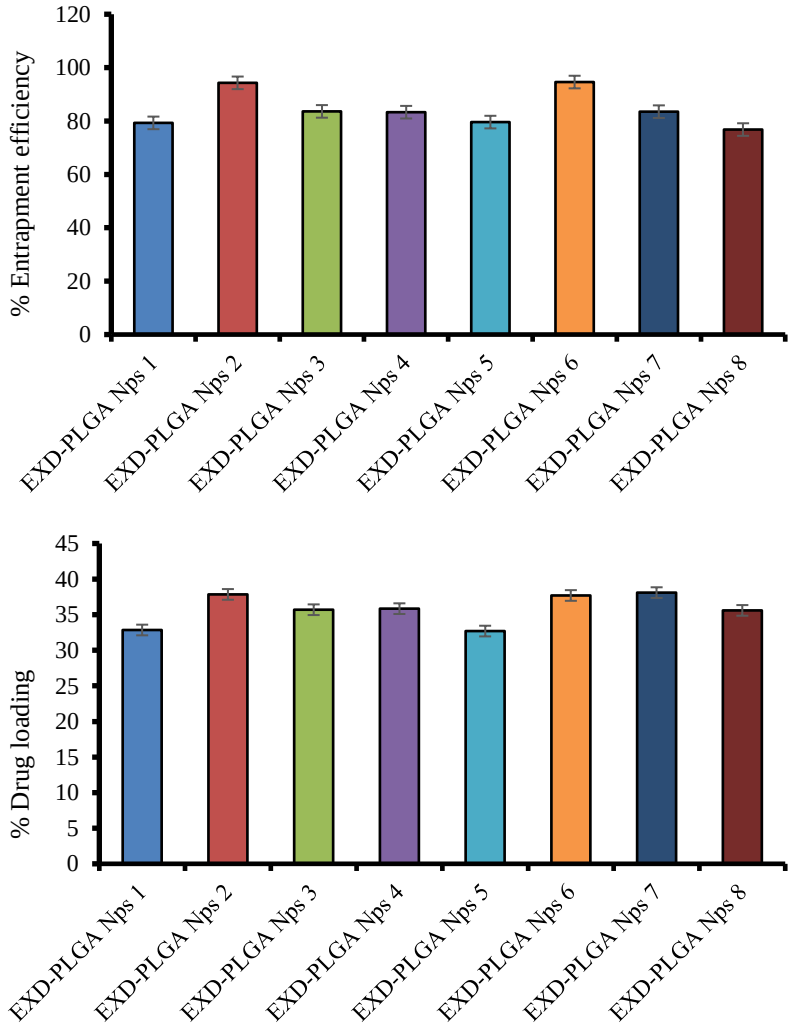


Figure 4. Physical characteristics of EXD_Nps such as (a) % yield (b) Particle size (c) PDI (d), Zeta potential (e) Entrapment efficiency (f) % drug loading of EXD-PLGA-NPs formulations. Data is denoted as the mean ± SD, and all studies were carried out in triplicate. A statistically significant difference was indicated using a t-test of variance with GraphPad Prism 6.0 program. p-value less than 0.05 was deemed statistically significant.

CONCLUSION

This study successfully developed and optimized a colon-targeted nanoformulation of eluxadoline using a factorial design-based approach to enhance its therapeutic efficacy for irritable bowel syndrome with diarrhea (IBS-D). The optimized nanoparticles exhibited desirable physicochemical properties, including nanoscale particle size, high drug entrapment efficiency, and sustained colonic drug release. In-vitro release studies confirmed controlled and pH-dependent drug release, while ex-vivo permeability studies demonstrated enhanced colonic absorption. Furthermore, in-vivo pharmacokinetic evaluation revealed improved bioavailability compared to conventional formulations, and pharmacodynamic studies in IBS-induced animal models confirmed significant symptom relief and therapeutic efficacy.

Future studies should focus on scaling up the formulation process, conducting long-term stability studies, and performing clinical trials to validate the therapeutic potential of this novel nanoformulation in human subjects. This research contributes to the advancement of colon-targeted nanomedicine and offers a promising approach for improving IBS management through precision drug delivery.

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