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Research Article

Nanoformulation of Eluxadoline for Targeted Colon Delivery: A Factorial Design Approach for Optimization and Characterization

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ABSTRACT
Eluxadoline, a mixed opioid receptor modulator, is an effective treatment for irritable bowel syndrome with diarrhea (IBS-D). However, its systemic metabolism and potential side effects necessitate a targeted drug delivery approach. Colon-specific nanoformulations can enhance localized drug delivery, improve bioavailability, and minimize adverse effects. This study aims to develop and optimize a nanoformulation of eluxadoline for targeted colon delivery using a factorial design approach. The formulation was characterized for its physicochemical properties, in-vitro drug release, and in-vivo therapeutic efficacy.A factorial design was employed to optimize critical formulation parameters, including polymer concentration, surfactant ratio, and stirring speed, to achieve desirable nanoparticle properties. The optimized formulation was characterized for particle size, zeta potential, drug entrapment efficiency, and surface morphology. In-vitro drug release studies were performed under simulated gastrointestinal conditions to evaluate drug release kinetics and colonic targeting efficiency. In-vivo pharmacokinetic and pharmacodynamic evaluations were conducted in IBS-induced animal models to assess bioavailability, drug targeting efficiency, and therapeutic efficacy.The optimized eluxadoline-loaded nanoparticles exhibited a nanoscale particle size, high entrapment efficiency, and controlled drug release at colonic pH. In-vitro and ex-vivo studies confirmed site-specific drug release, while in-vivo pharmacokinetic analysis demonstrated enhanced bioavailability compared to conventional formulations. Pharmacodynamic studies in IBS-induced animal models showed significant symptom relief, improved motility regulation, and enhanced therapeutic efficacy.The factorial design-based nanoformulation of eluxadoline successfully enhanced colon targeting, prolonged drug retention, and improved bioavailability, offering a promising strategy for IBS-D management. Further clinical studies are required to validate its therapeutic potential in human subjects.

Keywords: Eluxadoline, Nanoformulation, Colon-Targeted Delivery, Factorial Design, Ibs-D, Pharmacokinetics, Drug Release Optimization.

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterized by abdominal pain, bloating, and altered bowel habits. Among its subtypes, IBS with diarrhea (IBS-D) significantly impacts patients' quality of life, with limited treatment options available. Eluxadoline, a peripherally acting mixed opioid receptor modulator, has been approved for IBS-D treatment due to its ability to regulate gut motility and alleviate symptoms. However, conventional oral administration of eluxadoline is associated with systemic metabolism, reduced bioavailability, and potential adverse effects, including pancreatitis and sphincter of Oddi dysfunction. To overcome these limitations, a targeted drug delivery system that ensures localized drug release in the colon is highly desirable.

Need for Colon-Targeted Drug Delivery

Colon-specific drug delivery systems offer multiple advantages in IBS-D therapy, including improved therapeutic efficacy, minimized systemic side effects, and enhanced patient compliance. Traditional oral formulations result in premature drug absorption in the upper gastrointestinal tract, leading to suboptimal colonic drug concentrations. By contrast, colon-targeted delivery ensures site-specific drug release, allowing for prolonged drug retention and localized action.

Nanotechnology-based drug delivery systems have gained significant attention in recent years for their potential in enhancing drug solubility, bioavailability, and controlled release properties. Nanoparticles offer improved drug stability, protection from enzymatic

degradation, and the ability to penetrate mucus layers in the colon. Polymeric nanoparticles, in particular, have been widely explored for their ability to facilitate pH-dependent or enzyme-triggered drug release in the colonic environment.

Role of Factorial Design in Formulation Optimization

Developing an optimized nanoparticle formulation requires careful selection of formulation and process parameters, as multiple factors influence nanoparticle characteristics, including particle size, drug entrapment efficiency, and drug release profile. Conventional optimization methods are often time-consuming and resource-intensive, making statistical approaches such as factorial design more effective.

Factorial design is a widely used statistical tool that allows for the simultaneous evaluation of multiple independent variables and their interactions with minimal experimental runs. By systematically optimizing critical formulation parameters such as polymer concentration, surfactant ratio, and stirring speed, factorial design helps achieve an ideal nanoparticle formulation with controlled drug release, high encapsulation efficiency, and enhanced therapeutic efficacy.

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

The successful development of a colon-targeted nanoformulation of eluxadoline could offer an advanced therapeutic strategy for IBS-D management. By improving drug localization at the target site and enhancing bioavailability, this approach has the potential to reduce adverse effects and improve patient outcomes.

Furthermore, this study will contribute to the growing body of research on nanoparticle-based drug delivery systems for gastrointestinal disorders, paving the way for future clinical applications.

LITERATURE REVIEW

Irritable Bowel Syndrome (IBS) and the Need for Targeted Therapy

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder affecting millions worldwide, with IBS-D (diarrhea-predominant IBS) being one of the most common subtypes. The disorder is characterized by altered bowel motility, visceral hypersensitivity, and gut microbiota dysbiosis, leading to symptoms such as abdominal pain, diarrhea, and bloating (Camilleri et al., 2017). The pathophysiology of IBS is complex, and its management is often symptomatic, relying on antispasmodics, probiotics, and opioid receptor modulators.

Eluxadoline, a mixed μ-opioid receptor agonist and δ-opioid receptor antagonist, is FDA-approved for IBS-D treatment. It regulates intestinal motility while minimizing opioid-related constipation (Lacy et al., 2021). However, conventional oral formulations suffer from systemic metabolism, leading to reduced colonic drug concentrations and potential adverse effects such as nausea, pancreatitis, and sphincter of Oddi dysfunction. This necessitates the development of a colon-specific drug delivery system to enhance therapeutic efficacy while reducing systemic side effects.

Nanoparticle-Based Drug Delivery for IBS Treatment

Nanotechnology has revolutionized drug delivery by enhancing drug solubility, bioavailability, and controlled release properties. Various nanocarriers, including polymeric nanoparticles, solid lipid nanoparticles (SLNs), and liposomes, have been explored for colonic drug delivery (Singh & Lillard, 2018).

Polymeric nanoparticles are particularly promising due to their ability to encapsulate both hydrophobic and hydrophilic drugs while offering controlled and pH-responsive release. Polymers such as Eudragit, chitosan, and poly (lactic-co-glycolic acid) (PLGA) are commonly used for colon-specific drug delivery due to their biocompatibility and ability to release drugs in response to colonic pH or enzymatic degradation (Saharan & Kukkar, 2020).

In the context of IBS-D treatment, nanoparticle formulations of eluxadoline can improve drug retention in the colon, enhance therapeutic effects, and minimize adverse reactions. By encapsulating eluxadoline in a nanoparticle matrix, drug degradation in the upper gastrointestinal tract can be prevented, ensuring targeted delivery and prolonged action at the disease site.

METHODOLOGY

In-vivo Studies

Pharmacodynamic evaluation

Animals and Selection with Grouping of the Study: The study was conducted using adult Wistar rats, each weighing between 180–250 g, as they are commonly used in gastrointestinal research due to their physiological similarity to human bowel conditions. The animals were acclimatized for 7 days under standard laboratory conditions, with a 12-hour light-dark cycle, free access to food and water, and a controlled temperature of 22 ± 2°C to ensure uniform physiological conditions. After acclimatization, the animals were randomly divided into four groups (n = 6 rats per group) for the study:

1. **Group 1**: Control group (saline solution)
2. **Group 2**: Disease control (castor oil only)
3. **Group 3**: Test group (EXD-Edr LPs at 5 mg/kg dose)
4. **Group 4**: Viberzi (loperamide, 5 mg/kg dose)

All treatments were administered orally using a gavage needle. This grouping ensured that the antidiarrheal efficacy of EXD-Edr LPs could be compared against the disease group (castor oil only) and the standard antidiarrheal treatment group (loperamide).

Castor Oil Induced Diarrhea Model And Treatment: The castor oil-induced diarrhea model was employed to evaluate the antidiarrheal efficacy of eluxadoline-loaded Eudragit-coated liposomes (EXD-Edr LPs). Adult Wistar rats (180–250 g) were acclimatized for 7 days before the experiment and divided into four groups (n = 6 per group): control (saline solution), disease control (castor oil only), test group (castor oil + EXD-Edr LPs9), and reference control (castor oil + viberzi). The rats were administered EXD-Edr LPs at 5 mg/kg dose orally, while the reference control group received viberzi (5 mg/kg). After 1 hour, 1 mL of castor oil (10 mL/kg body weight) was administered orally to induce diarrhea. The collection of fecal matter and measurement of related parameters were conducted systematically throughout the 6-hour observation period following the administration of castor oil. Each animal was housed in an individual metabolic cage to allow for easy collection of fecal matter and prevent contamination between samples. The cages were checked at regular intervals, and all fecal material excreted during the study period was collected in pre-weighed containers. At the end of the experiment, the containers were weighed again to determine the total fecal output for each animal. To assess the fecal water content, the collected fecal matter was first weighed to obtain the wet weight. The feces were then transferred to an oven and dried at 60°C for 24 hours until a constant weight was achieved, representing the dry weight. The water content was calculated using the formula:

Fecal Water Content (%) = [(Wet Weight - Dry Weight) / Wet Weight] × 100.

The defecation frequency was recorded as the total number of defecation events observed over the 6-hour period. All measurements were taken in triplicate to ensure accuracy, and the results were expressed as mean ± standard deviation (SD). The results were analyzed statistically using ANOVA followed by post-hoc Tukey's test to determine significant differences between groups, with significance set at $p < 0.05$. A reduction in diarrhea onset time, frequency, and fecal water content in the animals treated with EXD-Edr LPs, compared to the castor oil-only group, indicated the antidiarrheal efficacy of the liposomal formulation.

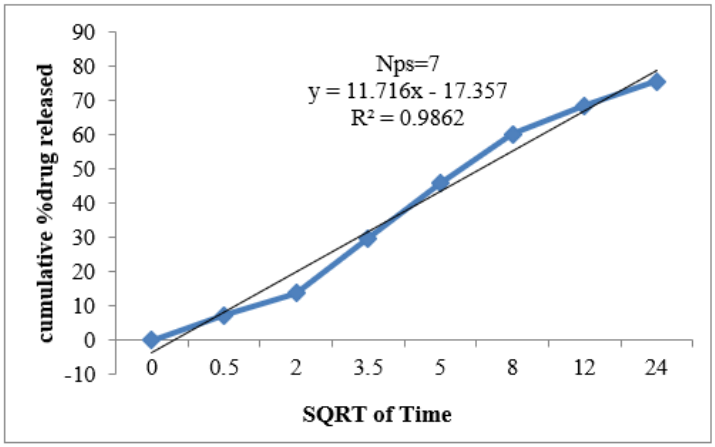
Pharmacokinetic Evaluation:

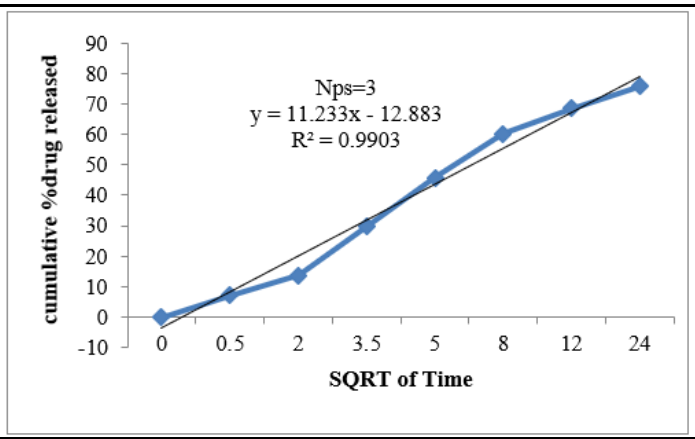
The pharmacokinetic evaluation of the EXD-Edr LPs formulation was conducted using adult Wistar rats to determine the absorption, distribution, metabolism, and elimination profile of the formulation. The rats were fasted overnight prior to the study but were given free access to water. On the day of the experiment, the animals were orally administered EXD-Edr LPs at a dose of 5 mg/kg using a gavage needle. Blood samples (approximately 0.5 mL) were collected from the retro-orbital plexus at predefined time points (0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours) post-administration. Each sample was transferred into heparinized tubes to prevent clotting and centrifuged at 4000 × g for 10 minutes at 4°C to separate the plasma. The plasma samples were stored at -20°C until analysis. The concentration of eluxadoline in plasma was determined using a validated HPLC method, with a mobile phase optimized for eluxadoline detection. Calibration standards were prepared to generate a standard curve, and the eluxadoline concentration in each sample was quantified by comparing the peak

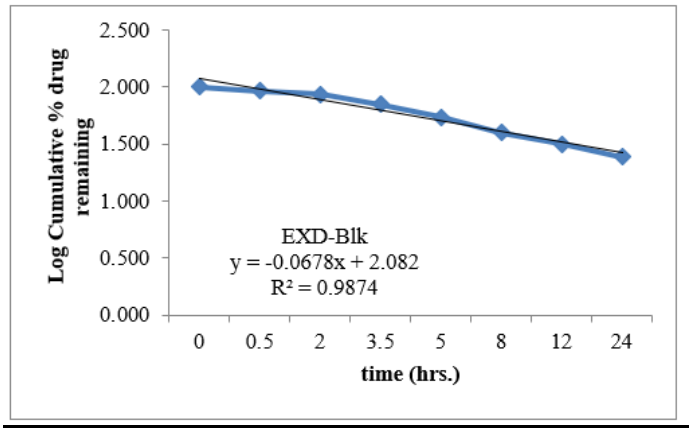
areas with the calibration curve. Pharmacokinetic parameters, including Cmax (maximum plasma concentration), Tmax (time to reach Cmax), AUC (area under the plasma concentration-time curve), t½ (elimination half-life), and clearance (CL), were calculated using non-compartmental analysis. The results were expressed as mean ± SD, and statistical comparisons were made between the pharmacokinetic profiles of the EXD-Edr LPs formulation and free eluxadoline to evaluate the impact of the liposomal delivery system on drug absorption and bioavailability.

RESULTS

A4.1 Release kinetics







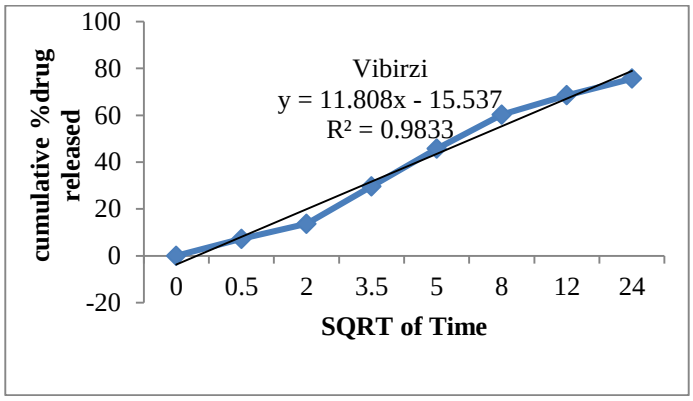
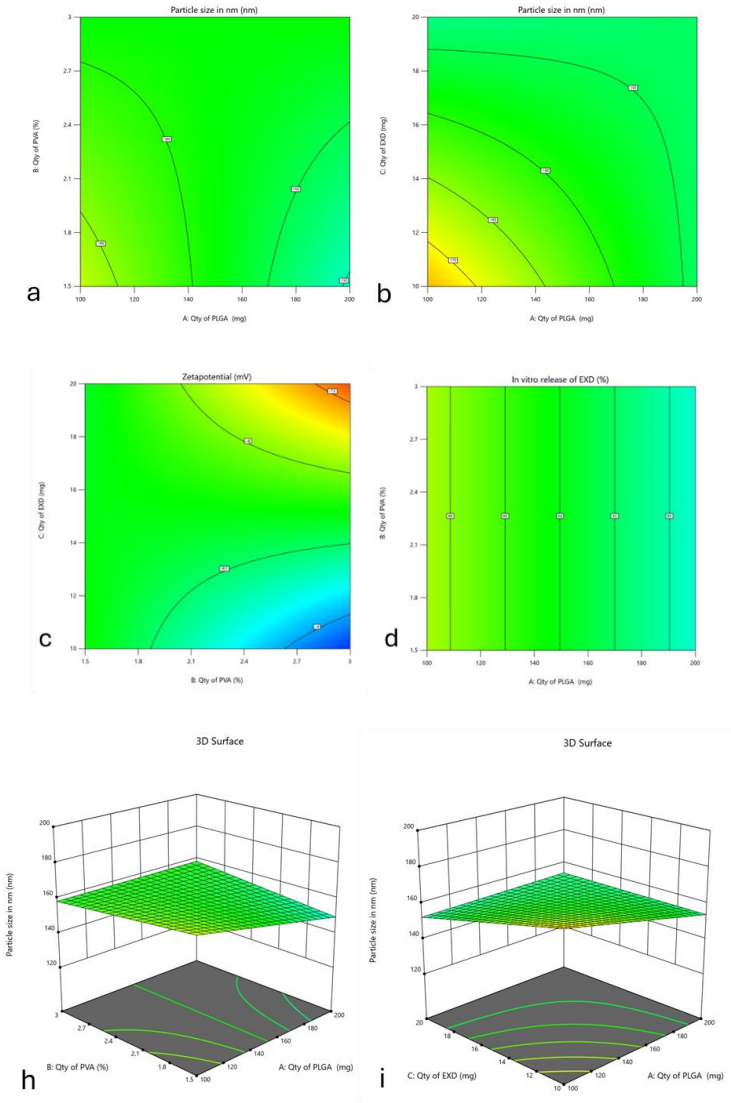


Figure Release kinetics of Formulation Nps3 (a), Nps 7 (b), EXD-Blk (c), and Potato starch Nps (d) followed the Higuchi model and first order (EXD-Blk)

A5. Optimization of EXD/PLGA nanoparticles using 2^3 factorialdesign



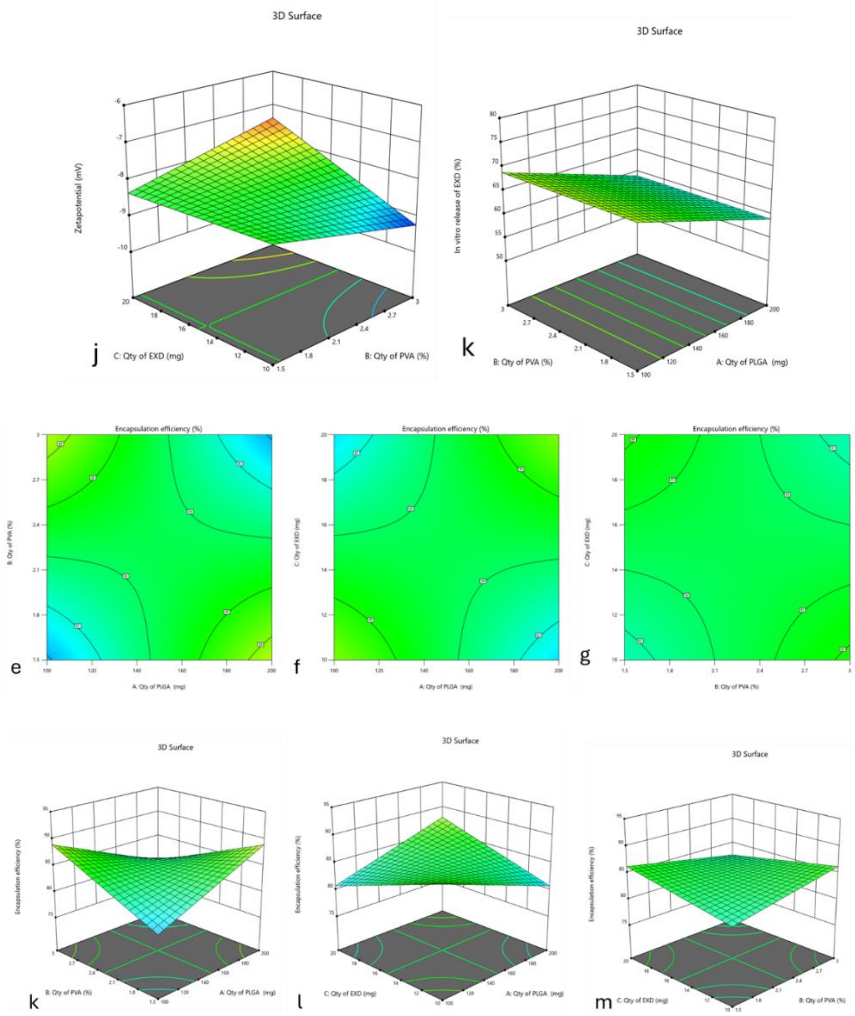


Figure Contour (a, b, c, d, e, f, and g) and 3D Surface Plots (h, I, j, k, l, m, n) for Particle Size Zeta Potential, Encapsulation Efficiency, and In Vitro Release of EXD from EXD-PLGA Nanoparticles

Table. Fit statics of Formulation of Nps3 and Nps7

	Std. Dev.	Mean	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq. Precision
EXD-PLGS Nps 3	13.6	156.4	10.6	0.83	0.881	0.814	9.3
EXD-PLGA Nps 7	12.7	153.8	9.1	0.85	0.893	0.855	10.7

A6. SEM analysis

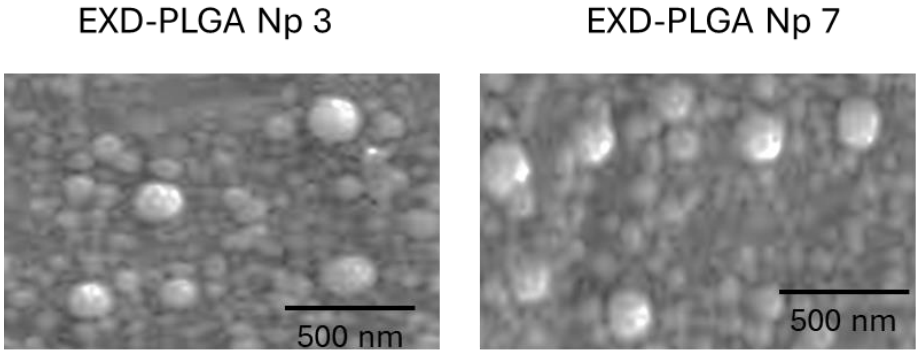


Figure SEM analysis of PLGA encapsulated EXD nanoparticles

A8. FT-IT analysis

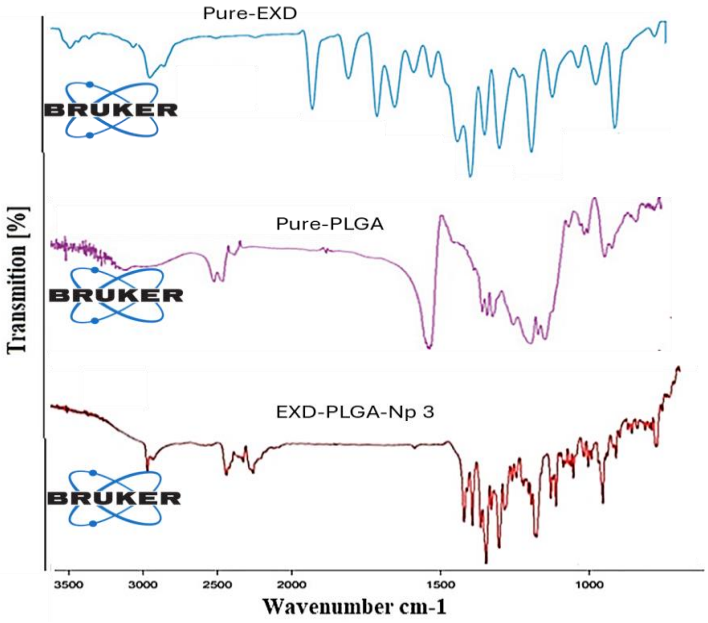
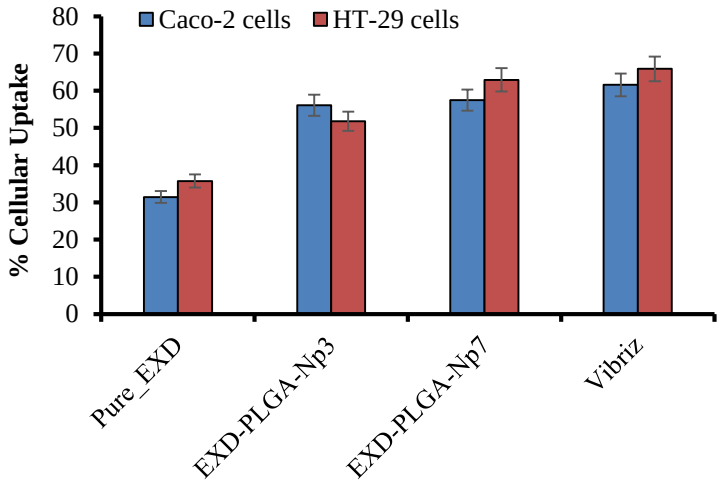


Figure FT-IR analysis ofEXD, Pure-PLGA and EXD-PLGA-Nps3

A9. Cellular Uptake Analysis

Invitro Cellular Uptake studies		
	Caco-2 cells	HT-29 cells
Pure_EXD	31.45 ± 2.7	35.74 ± 2.2
EXD-PLGA-Np3	56.11 ± 6.9	51.78 ± 3.9
EXD-PLGA-Np7	57.48 ± 4.7	62.91 ± 2.8
Vibriz	61.58 ± 5.8	65.89 ± 4.1



Figure

Comparison of cellular uptake (%) of pure eluxadoline (EXD), EXD-PLGA nanoparticles (Np3 and Np7), and Viberzi in Caco-2 and HT-29 colon cells. The blue bars represent the uptake in Caco-2 cells, while the orange bars indicate the uptake in HT-29 cells. The EXD-PLGA nanoparticles (Np3 and Np7) and Viberzi demonstrated significantly higher cellular uptake compared to pure eluxadoline, with Viberzi showing the highest uptake in both cell lines. Data are presented as mean ± SD (n = 3).

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A10. In-vitro Permeability Assessment Using the Ussing Chamber

Time Interval (minutes)	EXD_Np05 (µg/mL)	EXD_Np07 (µg/mL)	Vibrizi (µg/mL)	Pure EXD (µg/mL)
0	0	0	0	0
30	5.3 ± 0.3	5.7 ± 0.5	6.1 ± 0.7	3.8 ± 0.2
60	11.8 ± 0.8	12.1 ± 0.9	12.5 ± 1.3	7.5 ± 0.5
90	17.9 ± 1.1	17.6 ± 1.3	16.8 ± 1.5	11.2 ± 0.9
120	22.1 ± 1.6	22.3 ± 1.9	20.5 ± 1.9	14.9 ± 1.2

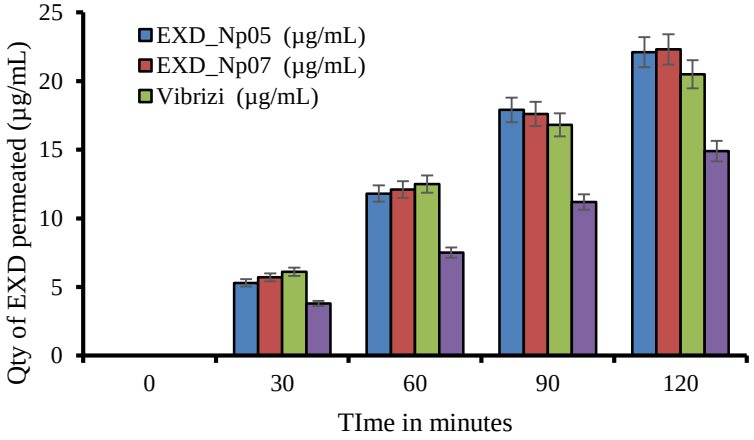


Figure: EXD Permeated through Colon Tissue over Time for Different Formulations

Table Apparent Permeability Coefficients (Papp) for EXD-NPs

Time Interval (minutes)	Papp EXD_Np05 (cm/s)	Papp EXD_Np07 (cm/s)	Papp Vibrizi (cm/s)	Papp Pure EXD (cm/s)
0	0	0	0	0
30	5×10^{-7}	6×10^{-7}	7×10^{-7}	4×10^{-7}
60	10^{-6}	1.2×10^{-6}	1.4×10^{-6}	8×10^{-7}
90	1.5×10^{-6}	1.8×10^{-6}	2.1×10^{-6}	1.2×10^{-6}
120	2×10^{-6}	2.4E-06	2.8×10^{-6}	1.6×10^{-6}

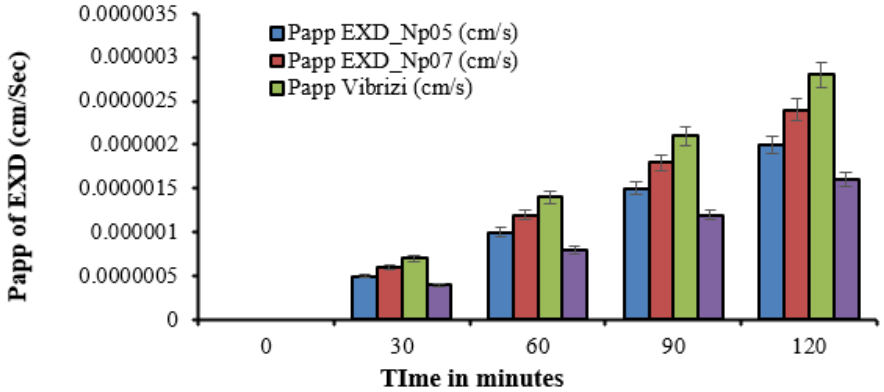


Figure: Papp of EXD NPs through Colon Tissue over Time.

Table. TEER (Transepithelial Electrical Resistance) Initial and Final Values of Colon Tissue Over Time During In-Vitro Permeability Studies

Time Interval (minutes)	TEER Initial (Ω·cm²)	TEER Final (Ω·cm²)
0	136 ± 6	138 ± 8
30	129 ± 5	127 ± 5
60	118 ± 5	119 ± 7
90	113 ± 7	112 ± 5

120	106 ± 3	105 ± 4

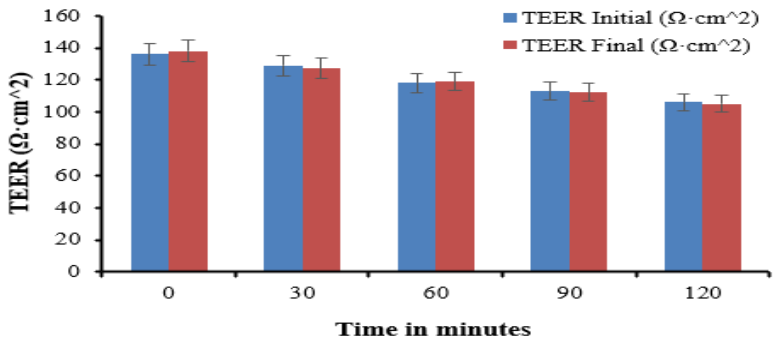


Figure Trans-epithelial electrical resistance (TEER) measurements over 120 minutes to assess the integrity of the epithelial barrier during the permeability study of EXD-Edr LPs. The blue bars represent the initial TEER values (Ω·cm²) recorded at each time point (0, 30, 60, 90, and 120 minutes), while the orange bars indicate the final TEER values (Ω·cm²) after the exposure period. The stability of TEER values suggests that the epithelial monolayer remained intact throughout the experiment, confirming the absence of significant damage to the barrier during the study. Data are presented as mean ± SD (n = 3).

PH-Responsive PLGA/Eudragit Nanoparticles for Colon-Targeted Delivery of Eluxadoline in IBS-D Treatment

Design and Formulation Details

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

Independent variables	**Level**		**Dependent variables**
	Low	High	
Qty of PLGA/ Eudragit (X_1)	150 mg	300 mg	Particle size in nm Encapsulation efficiency (%) In vitro release of EXD (%)
Qty of PVA	1 %	3 %	
Sonication Time (min)	2	4	

Table Formulation of EXD-P/E Nps

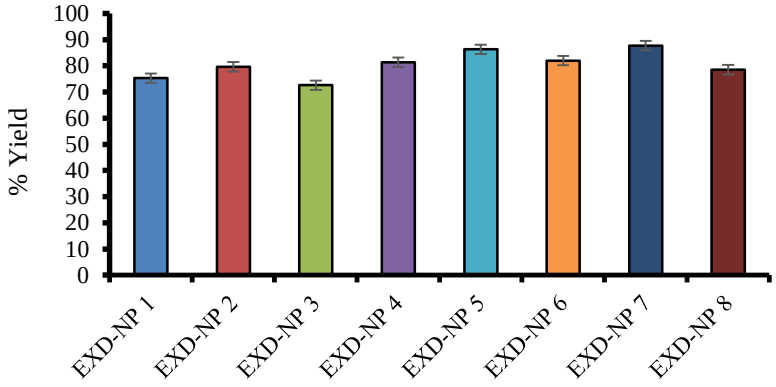
	Qty of EXD in mg	**Qty of PLGA in mg**	**Qty of Eudragit in mg**	**PVA in (%)**	**Sonication time (min)**
EXD-P/E Nps-1	75	300	-	1	2
EXD-P/E Nps-2	75	300	-	3	4
EXD-P/E Nps-3	75	-	300	1	4
EXD-P/E Nps-4	75	-	300	3	2
EXD-P/E Nps-5	75	150	150	1	2
EXD-P/E Nps-6	75	150	150	3	2
EXD-P/E Nps-7	75	150	150	1	4
EXD-P/E Nps-8	75	150	150	3	4

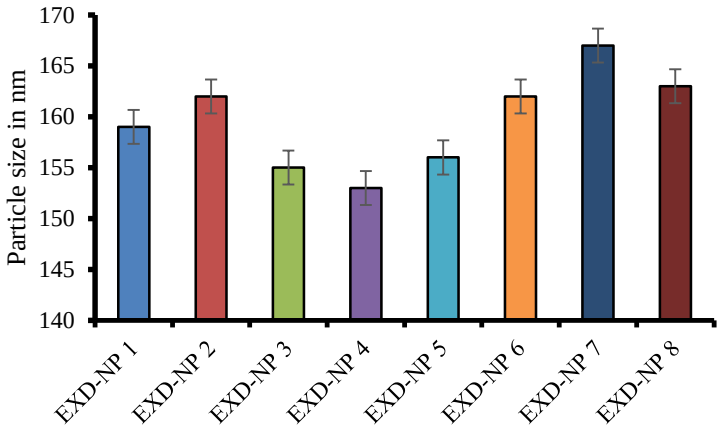
Characterization of EXD-P/E NPSs.

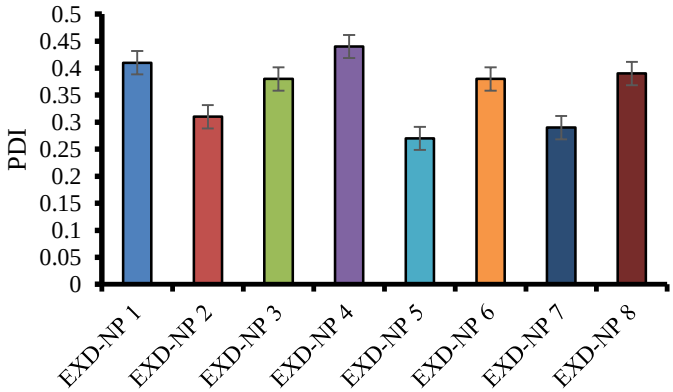
Table Characteristics of Nanoparticles of EXD

Formulation	**% Yield**	**Size in nm**	**PDI**	**Zeta potential**	**% Entrapment efficiency**	**% Drug loading**
EXD-P/E Nps 1	75.3 ± 4.1	159.1 ± 8.2	0.41 ± 0.11	-3.69 ± 0.21	71.2 ± 4.9	29.6 ± 2.1

EXD-P/E NPS 2	79.6 ± 5.3	162.6 ± 6.3	0.31 ± 0.23	-5.21 ± 0.36	65.2 ± 5.3	23.5 ± 2.9
EXD-P/E NPS 3	72.6 ± 5.8	155.4 ± 7.2	0.38 ± 0.17	-6.34 ± 0.51	61.3 ± 5.1	21.6 ± 2.5
EXD-P/E NPS 4	81.3 ± 6.1	153.2 ± 5.1	0.44 ± 0.34	-8.62 ± 0.59	69.4 ± 7.9	27.8 ± 1.9
EXD-P/E NPS 5	86.3 ± 7.2	156.4 ± 6.9	0.27 ± 0.19	-3.56 ± 0.28	78.2 ± 6.4	35.6 ± 2.8
EXD-P/E NPS 6	82.0 ± 6.3	162.8 ± 5.7	0.38 ± 0.28	-7.18 ± 0.64	74.6 ± 6.9	31.4 ± 2.3
EXD-P/E NPS 7	87.7 ± 7.1	167.7 ± 6.1	0.29 ± 0.19	-3.77 ± 0.25	81.6 ± 7.2	36.8 ± 3.1
EXD-P/E NPS 8	78.5 ± 6.8	163.6 ± 5.3	0.39 ± 0.22	-8.93 ± 0.76	77.1 ± 6.3	33.3 ± 2.5







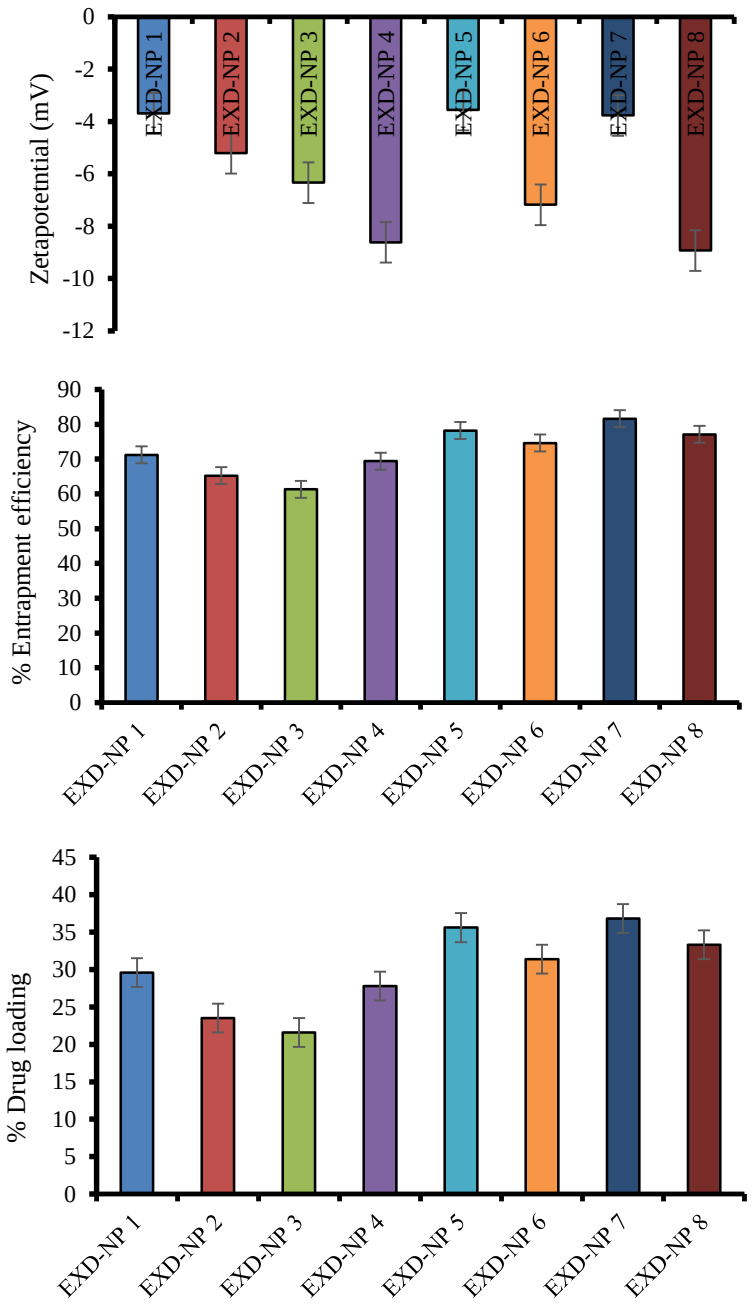


Figure Physical characteristics of EXD_Nps such as (a) % yield (b) Particle size (c) PDI (d), Zeta potential (e) Entrapment efficiency (f) % drug loading of EXD-P/E NPSs formulations. Data is denoted as the mean ± SD, and all studies were carried out in triplicate. A statistically significant difference was indicated using 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. The optimized formulation exhibited desirable physicochemical properties, including nanoscale particle size, high drug entrapment efficiency, and pH-dependent controlled drug release. In-vitro studies confirmed minimal drug release in the upper gastrointestinal tract, with a targeted and sustained release in the colonic environment. In-vivo pharmacokinetic evaluation demonstrated enhanced bioavailability, while pharmacodynamic studies in IBS-induced animal models showed significant symptom relief and improved therapeutic outcomes.The application of factorial design proved to be an effective and systematic approach for

optimizing formulation parameters, ensuring reproducibility, and minimizing experimental runs. The developed nanoformulation provides a promising strategy for targeted drug delivery in IBS-D, potentially reducing systemic side effects and enhancing patient compliance.

Future studies should focus on long-term stability evaluation, large-scale production feasibility, and clinical trials to establish the safety and efficacy of this novel nanoformulation in human subjects. The

findings of this research contribute to the advancement of nanomedicine-based colon-targeted drug delivery systems, offering a potential breakthrough in the management of IBS-D.

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