Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Chitosan-Modified SLNs for Enhanced Colonic Delivery of Mesalamine: Formulation and Statistical Optimization

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Cite this paper as: Hema Devi Tumma, Praveena Chinthala, (2025) Chitosan-Modified SLNs for Enhanced Colonic Delivery of Mesalamine: Formulation and Statistical Optimization. *Journal of Neonatal Surgery*, 14 (32s), 1944-1952.

ABSTRACT

The goal of the current study was to create and assess solid lipid nanoparticles coated with polysaccharides for the targeted delivery of Mesalamine, a medication used to treat Ulcerative colitis, a chronic inflammatory bowel disease. Three independent formulation variables—glyceryl monostearate content (X_1), Chitosan concentration (X_2), and Poloxamer concentration (X_3)—were optimized, and their effects on entrapment efficiency, particle size and drug release in colonic medium were assessed using a factorial design. Solid lipid nanoparticles were prepared by ultrasonication and hot homogenization. The optimized solid lipid nanoparticles F7 had a particle size of 100.1 nm, an entrapment efficiency of 78.01 %, and a sustained drug release of less than 20% at acidic pH and 90.9% in colonic medium after 24 h. The findings demonstrated that the formulation F7 showed controlled drug release in the colon and has the potential to enhance the results of Ulcerative colitis treatment. This research highlights that SLNs with a polysaccharide coating may be useful for delivering medications to the colon. In addition to targeted drug delivery, this work lays the groundwork for future investigations into novel interaction strategies in the manufacturing of biopharmaceuticals.

Keywords: *Mesalamine, colon delivery, optimization, factorial design, solid lipid nanoparticles, chitosan, ulcerative colitis*

1. INTRODUCTION

To lessen systemic side effects and increase treatment effectiveness, inflammatory bowel disease (IBD), which includes ulcerative colitis and crohn's disease, requires targeted drug delivery techniques [1, 2]. IBD primarily affects the colon and rectum. The first-line treatment for mild to moderate IBD, Mesalamine, has issues like poor water solubility and upper gastrointestinal tract (GIT) degradation that restrict its availability at the site of inflammation [3, 4]. By incorporating natural polysaccharides like Chitosan, which is biocompatible and biodegradable, solid lipid nanoparticles (SLNs) offer a workable solution to these problems by enabling site-specific drug administration [5, 6]. This work explores the use of polysaccharide-coated SLNs with Poloxamer, Chitosan, and Glyceryl monostearate (GMS) as formulation variables to enhance Mesalamine delivery and assesses their impact on particle size (PS), zeta potential (ZP), entrapment efficiency (EE), and colonic drug release [7-9].

MATERIALS AND METHODS

Materials

Mesalamine was procured from Sigma Aldrich, GMS, Chitosan from SD Fine-chem and Poloxamer acquired from Arati Chemicals for the formulation development. All secondary materials and reagents used are completely analytical and pharmaceutical grade.

Methodology

General method of preparation of SLNs

SLNs were made by hot homogenization and then ultrasonication was applied. GMS was melted, and Mesalamine was added to the lipid mixture. Poloxamer and Chitosan were mixed in water. Aqueous phase was added to the lipid phase and stirred vigorously at a 15,000 rpm for 10 min [10, 11]. The produced emulsion was kept in an ultrasonic bath for 5 minutes before

being chilled so SLNs could form. Then the nanoparticles were coated with Chitosan to make sure they are distributed mostly in the colon [12].

Experimental design and analysis

For examining polysaccharide-coated SLNs, the researchers used a statistical method. Eight formulations were prepared in a 2^3 factorial design, which an expert software analysed to examine the effects and interactions between three variables [13,14]. The variables used were the GMS amount X_1, Chitosan concentration X_2 and Poloxamer concentration X_3 and each was assigned a different level (low and high) and a corresponding code of -1 or +1. The analysis was performed one step at a time to find out which control factors make a major difference to the dependent variables PS Y_1, Zeta ZP Y_2 and EE Y_3 [15].

Table 1: Composition of polysaccharide coated SLNs

F. Code	Mesalamine (mg)	GMS (%)	Chitosan (% w/v)	Poloxamer (%)
F1	10.0	2	0.5	1
F2	10.0	1	0.5	1
F3	10.0	1	1.5	1
F4	10.0	1	0.5	0.5
F5	10.0	1	1.5	0.5
F6	10.0	2	1.5	1
F7	10.0	2	1.5	0.5
F8	10.0	2	0.5	0.5

Characterization of SLNs

Particle size (PS), zeta potential (ZP) and poly dispersity index (PDI)

A Malvern Zetasizer laser diffraction analyzer was used to measure the PS [11]. To analyse PS, suspended SLNs were used in the portion of the machine containing distilled water and measured by the software supplied by the manufacturer. Using the zeta seizer, the PDI was checked for formulation homogeneity, while ZP was examined due to the association of a large amount of particles being kept apart in SLNs.

Entrapment efficiency (EE)

To find the EE, the dispersion containing the SLNs were centrifuged to separate the supernatant which was analysed for free drug using RP-HPLC at 232 nm and the concentration was determined from the standard curve [10].

Particle morphology

Transmission electron microscopy (TEM): TEM was a significant method to know more about the shape, structural elucidation of very small samples. Samples were made by applying a drop of emulsion to a grid and observed using TEM (TECNAI T 12 G2, Japan), and recorded [6].

***In vitro* drug release**

An *in vitro* drug release study was carried out using a modified dissolution method based on dialysis membrane. Initially, the dialysis bags were soaked in water for 12 h before being used. Firstly, the known amount of SLNs were enclosed in a dialysis bag and placed in a beaker containing HCl (pH 1.2 buffer), which was maintained at 37 ± 0.5°C and agitated continuously for 2 h at 100 rpm. Later the buffer was changed to pH 6.8 (simulating small intestine conditions) and after 2 h, to pH 7.4 (simulating the colon conditions). At regular intervals, 1.0 ml samples were withdrawn, and the buffer is replaced with fresh medium. [16, 17]. All samples were analyzed using RP-HPLC at 232 nm.

Stability studies

The study was carried out according to the rules set by the International Council on Harmonisation (ICH). Mesalamine-loaded F7 SLNs were stored at 4°C, 25°C and 37°C for 60 days during the study. The stored samples were tested for their PS, ZP and EE as well as the drug release [7].

***In-vivo* studies**

All animal procedures in this study were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats of either sex were randomly selected and divided into four groups, with six animals in each group. They were housed under controlled conditions of 12-hour light/12-hour dark cycles at 25 ± 2°C and 60 ± 10% relative humidity (RH) to allow acclimatization [18]. After acclimatization, colitis was induced in groups 2, 3, and 4 to mimic inflammatory bowel disease (IBD) conditions. Group 1 served as the healthy control. Group 2 remained untreated following colitis induction. Group 3 received oral administration of the pure drug in suspension form, while group 4 was treated with Mesalamine-loaded SLNs (F7). Following induction and oral administration, the animals were monitored for changes in body weight, diarrhea symptoms, and colon length. These parameters were compared with those of the control and untreated groups for analysis [19].

2. RESULTS AND DISCUSSION

SLNs were synthesized using the hot homogenization method. Varying compositions of GMS and Chitosan, along with Poloxamer (Table 1), were homogenized in the hot method to produce SLNs, and these were then assessed using *in vitro* tests. SLNs were characterized using various in vitro physicochemical parameters.

Characterization of SLNs

The polysaccharide-coated mesalamine SLNs were evaluated for PS, surface morphology, EE, PDI, and drug release. A 3-factor factorial design was employed to evaluate the influence of GMS, Chitosan, and Poloxamer concentrations on the critical physicochemical features of mesalamine-loaded polysaccharide coated SLNs. Response surface plots and interaction plots were utilized to evaluate the individual and interaction impacts on PS, ZP, and EE (20-25).

Particle size

The SLNs were measured using the Malvern Zetasizer, and the findings are displayed in Table 2. The PS of the SLNs measured were in the range of 98 nm to 187 nm The results (Figure 1) illustrate that increasing GMS concentration leads to a greater PS value, most likely because the higher percentage of lipids in the mixture enhance the viscosity of internal phase, resulting in larger droplets during emulsification. Most likely, the rise in PS was due to Chitosan being deposited on the surface. In contrast, Poloxamer had a PS-reducing effect. Higher Poloxamer concentrations reduced PS because of the emulsifying and stabilizing effects of its surfactant nature. Results showed that when GMS was used at high levels and Poloxamer at low, the particles resulted in large size, and more Poloxamer led to significantly decreased PS, demonstrating a synergistic effect in managing particle dimensions. The PDI values were in the range of 0.165 to 0.249, displaying a narrow distribution of PS. notably, a PDI of 0.165 was obtained for F7.

Zeta potential

The ZP of an emulsion frequently falls in the range of +/− 30 mV, making it very stable. Table 2 demonstrates that over the whole design area, the values for SLNs ranged from around -22 mV to -33 mV, indicating moderate to strong electrostatic stability. As Chitosan has a cationic effect, increasing it made the ZP less negative, partially neutralizing the lipid particles negative charge. On the other hand, Poloxamer helped to stabilize the surface of the particles and increase their negative charge, which improved the overall colloidal stability. The results showed that Chitosan and Poloxamer could be used for optimizing the surface charge.

Entrapment efficiency

The amount of EE was indirectly found by estimating the amount of the drug that remained un-trapped. Between 40% and 78% EE was observed, and the results were best at the highest GMS and Chitosan concentrations. Increasing GMS increased drug entrapment because of more availability of lipid to encapsulate Mesalamine. Higher doses of Chitosan permitted more entrapping, but less with moderate amounts because the material became more porous. Moreover, the addition of large amounts of Poloxamer resulted in a decline in EE, probably because the drug dissolved more in the water during the formulation process. Based on the data available, the highest entrapment efficiency is observed for F7, 78.01 %.

Surface morphology

The surface morphology of the SLNs was determined using TEM. Figure 2 shows the TEM results for F7. According to the test results, the SLN particles contain a hydrophobic core and a surfactant monolayer layer. The drug was dispersed in a lipid matrix inside the core. A TEM picture was used to investigate the morphological characteristics of the SLNs, which were virtually spherical with a smooth surface.

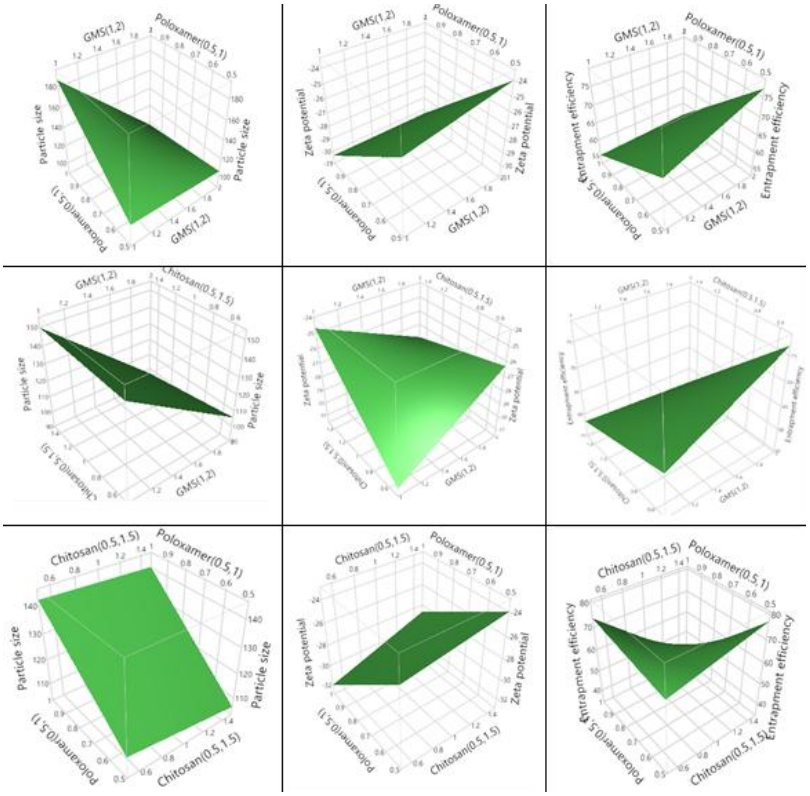

Figure 1: Response surface graphs – 3D representation of the interactions between the two factors and their effects on PS, ZP and EE

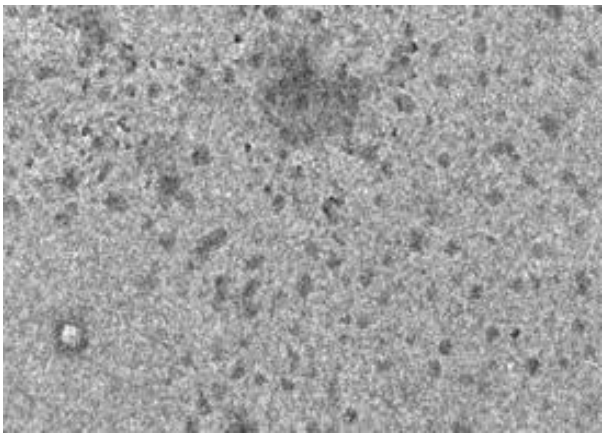

Figure 2: TEM of F7

Table 2: Particle size, zeta potential and entrapment efficiency of SLNs

F. Code	Coded pattern	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
F1	+ – +	101.4	-30.1	77.12
F2	– – +	184.6	-33.9	70.15
F3	- + +	187.1	-26.8	40.31
F4	- - -	110.5	-28.1	58.56

F5	- + -	112.1	-22.6	76.24
F6	+ + +	98.42	-30.6	35.13
F7	+ + -	100.1	-25.4	78.01
F8	+ - -	112.1	-22.6	76.24

***In-vitro* release**

Buffer solutions comprising pH levels of 1.2, 6.8, and 7.4 were utilized to investigate the *in vitro* release of Mesalamine SLNs. The drug release for each formulation was observed for 24 h. The SGF and SIF both showed little drug release, which indicates that the drug remained protected in the upper gastrointestinal system whereas SCF showed significantly more drug release because the enzymes in the intestine break down the polysaccharide coating. During the first two hours, every formulation showed a rapid exponential release rate in acidic solution. This may be due to the amount of free drug present in the formulation. In the next phase, which lasted up to 4 h, the rate of drug release from each type of formulation gradually improved. Around the third phase, the rate of drug release significantly started delaying. Drugs formulated with a larger concentration of Chitosan showed delayed release controlled by diffusion processes. It was also shown that the quantity of drug released from SLNs was not changed by varying the amount of surfactant used [26].

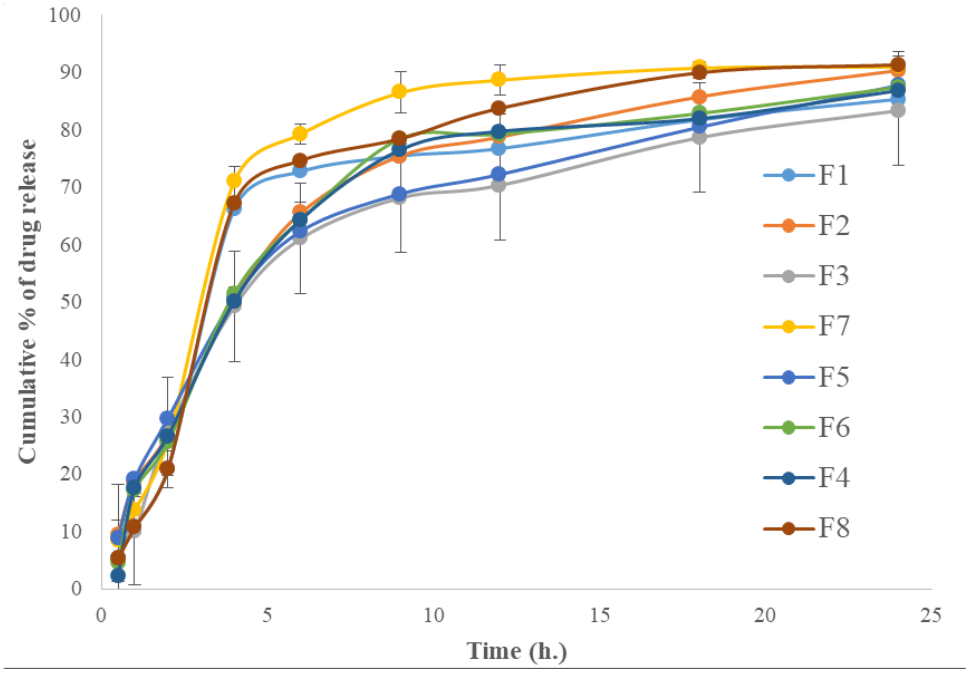

Figure 3: Cumulative drug release of F1 – F8 SLNs

Stability studies

Two-month stability studies were carried out at 4°C, 25°C and 37°C as part of the current investigation using the F7 formulation. After 60 days, the formulation was tested for PS, ZP, and EE and found no significant difference in SLNs. The optimized formula F7 was shown to remain stable when kept in storage for two months.

***In vivo* studies**

For six days, animals that had colitis were given drug-loaded SLNs orally. When the treatment was over, they were evaluated for colitis by looking at diarrhea, observing their weight, and measuring their colon length. The untreated colitis group had severe diarrhea, with signs of blood in the stool and much less body weight than the healthy animals. Before colitis was introduced, body weight did not differ between the treatment and placebo groups. The body weights of the animals given MES-SLNs recovered faster than the control group and other treated groups (Figure 4). The average length of the colon was much lower in animals with colitis caused by acetic acid, a main feature of the disease. Initially, when there was no colitis, the colon measured 58 cm, but inducing colitis reduced the colon length to 36 cm. The average colon length went up in the animals administered with the pure drug compared to those who were untreated (Figure 5).

As a consequence, treatment using MES-SLNs greatly improved the length of the colon, and a clear distinction in colon length was seen.

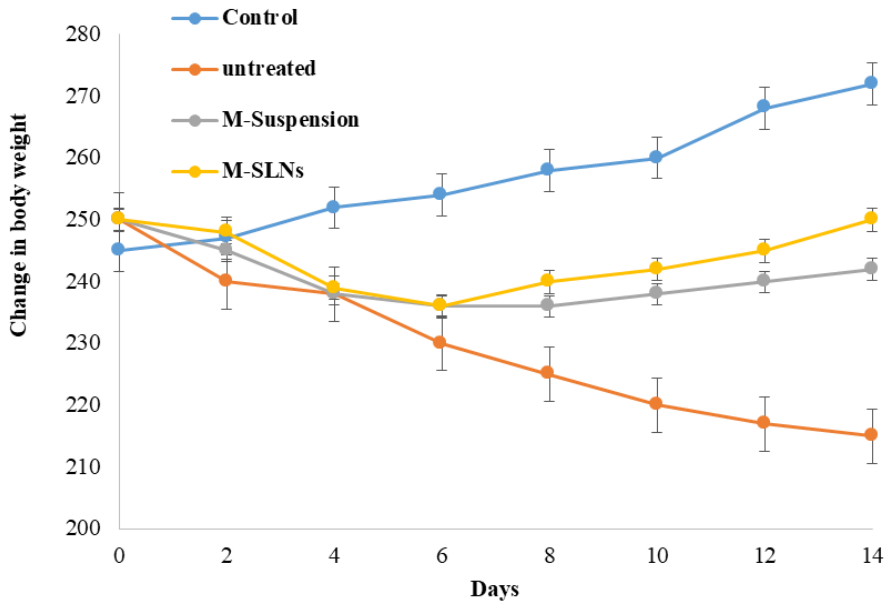

Figure 4: Body weight changes of control, untreated, drug treated group and SLN treated group during the experimental period

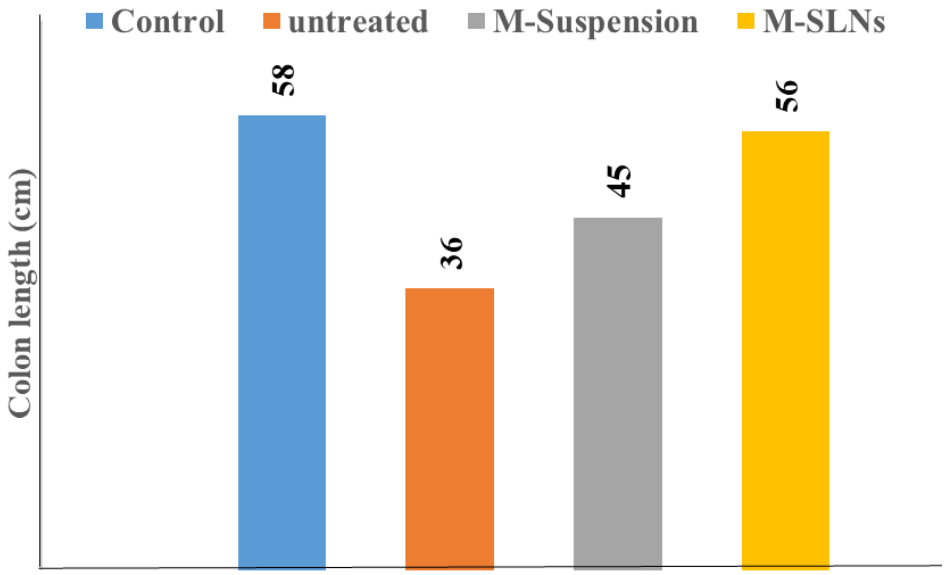

Figure 5: Macroscopic evaluation of colitis. (Colon length) n=6

Statistical analysis

From the factorial design, it can be observed how independent variables influence the outcomes and the overall performance of the formulation. GMS, a significant lipid made both encapsulation and release more successful. In the formula, Poloxamer performed as a stabilizer as well as improved the level of permeation. It was found that PS and EE depended mainly on the amount of Chitosan and GMS. Poloxamer added into SLNs did not affect their ability to release the drug. *In vitro* studies suggested that polysaccharide-coated SLNs protect the drug in the upper gut and release in the colon to treat IBD. Because F7 showed better PS and sustained drug release, it is ideal for targeting in the colon. Using ANOVA, it was possible to confirm that making changes to the drug formulation significantly affected the results of drug release.

The actual results of particle sizes were close to those predicted values. The Figure 6 reveals that the theoretical and observed particle sizes were very close. P-value is found to be 0.0336 which is < 0.05 proves the results are significant. ZP model R^2 0.99945 values are very close to 1 and a P-value (0.0441) is under 0.05 both support statistical significance.

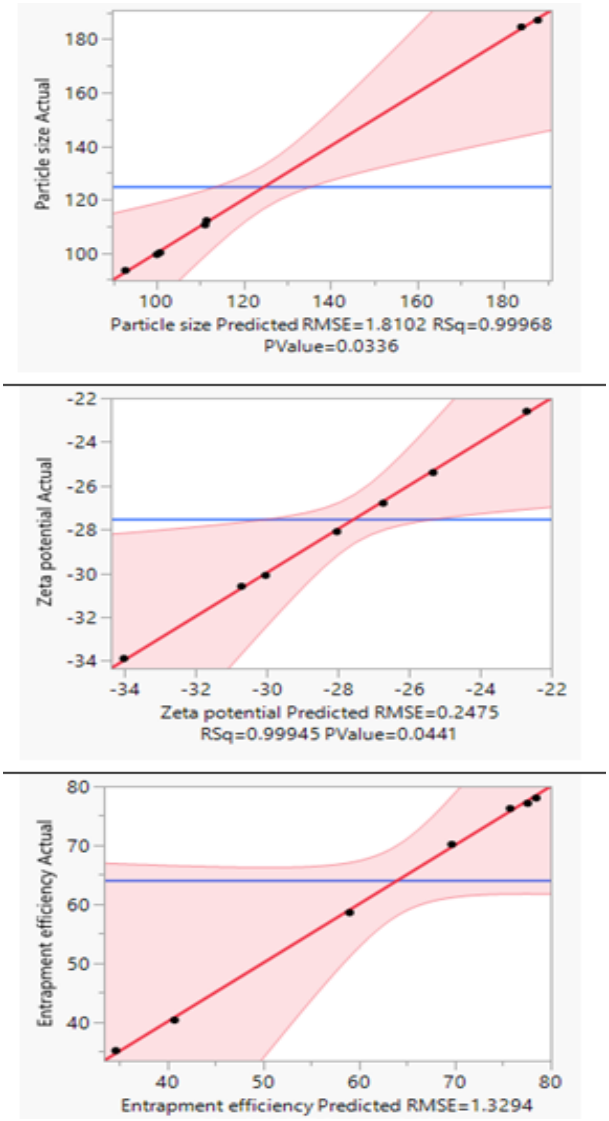

Figure 6: Plots of actual and predicted values

3. CONCLUSION

The present study illustrates the potential delivery of polysaccharide-coated SLNs to the colon. The factorial design method allowed for better physicochemical properties and drug release in the formulation development. According to the evaluation parameters, F7 showed a small amount of drug release at the beginning and larger amounts closer to the colon. SLNs are a potential technique for delivering mesalamine directly to the colon. Owing to the appropriate PS, high EE, and sustainable drug release in colonic medium, optimized SLNs F7 seem to be a perfect choice for treating colitis.

4. ACKNOWLEDGMENT

The authors gratefully acknowledge the secretary and correspondent of haitanya deemed to be university, hyderabad, Telangana, for their generous support, encouragement, and for providing the essential research facilities.

5. CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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