RESEARCH PAPER

Formulation Of Eudragit Based Sulfasalazine Nanosuspension With Enhanced Drug Encapsulation

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Abstract

The present study focuses on the development and optimization of a novel sulfasalazine nanosuspension for the treatment of inflammatory bowel disease, particularly ulcerative colitis. Sulfasalazine-loaded nanoparticles were formulated using Eudragit RS 100 polymer and optimized through a 3-level factorial design employing Design-Expert software. The primary objective was to achieve nanoparticles with minimal particle size and polydispersity index (PDI), and maximum entrapment efficiency (%EE). The software predicted a particle size of 234 nm and %EE of 83.89% with a desirability of 0.476. The optimized formulation exhibited a particle size of 228 nm, PDI of 0.263, and %EE of 84.46%, closely matching the predicted values. Zeta potential analysis showed a high negative value of -40.8 mV, indicating excellent colloidal stability. Characterization studies including DSC, FTIR, and XRD confirmed good physicochemical compatibility and successful drug encapsulation. TEM analysis revealed uniform and well-defined nanoparticles in the first image, while the second image showed some aggregation, suggesting morphological variability. The formulation's viscosity and pH were within acceptable ranges, and drug content analysis confirmed uniform distribution in the hydrogel. In-vitro drug release studies demonstrated sustained release of sulfasalazine over 12–24 hours, attributed to the polymer's controlled-release properties. the developed sulfasalazine nanosuspension presents a promising and superior therapeutic strategy for the effective management of ulcerative colitis.

Keywords: Sulfasalazine, Nanosuspension, Ulcerative Colitis, Controlled Drug Release

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1.0. INTRODUCTION

Nanosuspensions are sub-micron colloidal dispersions composed of pure drug particles suspended in an aqueous medium, stabilized by surfactants and often polymers to ensure uniform distribution and prevent agglomeration. These formulations offer a promising strategy for enhancing the solubility and bioavailability of poorly water-soluble or environmentally unstable drugs. By reducing drug particles to the nanoscale, either through top-down approaches such as crystalline size reduction or bottom-up methods like precipitation from molecular solutions, nanosuspensions significantly increase the surface area available for dissolution, as supported by the Noyes Whitney equation.(1) This enhancement in dissolution rate directly correlates with improved oral absorption and therapeutic efficacy. Oral nanosuspension-based drug delivery systems provide site-specific delivery, minimizing systemic exposure and reducing adverse effects commonly associated with conventional therapies.(2) Recent research has shown particular promise in the application of nanosuspension platforms for the targeted treatment of ulcerative colitis (UC), enabling localized delivery to inflamed colonic regions and thereby optimizing therapeutic outcomes. These advanced delivery systems not only improve the pharmacokinetic profile of hydrophobic drugs but also represent a significant step forward in achieving efficient, patient-friendly, and effective treatment strategies in clinical practice. (3)

Sulfasalazine, an anti-inflammatory agent, finds applications in pharmaceutical formulations for treating inflammatory bowel disease (IBD) like ulcerative colitis and rheumatoid arthritis. It is often formulated into tablets, capsules, or enteric-coated formulations to improve drug delivery and patient compliance. Specific formulation techniques, like colon-targeted delivery using nanoparticles or microparticles, are

employed to enhance its efficacy in targeting the colon, the main site of inflammation in IBD.

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory bowel disease characterized by relapsing inflammation and ulceration of the colonic mucosa, leading to symptoms such as abdominal pain, diarrhea, rectal bleeding, and weight loss. Conventional therapeutic strategies rely heavily on aminosalicylates, corticosteroids, immunomodulators, and biologics, among which sulfasalazine remains a cornerstone in the treatment of mild to moderate UC [4,5]. However, sulfasalazine suffers from poor aqueous solubility and variable bioavailability, limiting its therapeutic efficacy and often resulting in systemic side effects due to nonspecific distribution. Recent advancements in nanotechnology have introduced nanosuspensions as a promising drug delivery approach to overcome the limitations of poorly water-soluble drugs. Nanosuspensions are colloidal dispersions of pure drug particles, typically below 1 μm in size, stabilized by surfactants and/or polymers to prevent aggregation and improve stability. By significantly increasing the surface area, nanosuspensions enhance the dissolution rate and absorption of hydrophobic drugs, as explained by the Noyes–Whitney equation. This approach is especially beneficial for oral drug delivery, where enhanced solubility translates to improved bioavailability and therapeutic outcomes. The targeted delivery of sulfasalazine in nanosuspension form directly to the colon can offer site-specific action, reduce systemic exposure, and minimize adverse effects, thereby optimizing the treatment of UC. Moreover, nanosuspension-based formulations offer the potential for sustained drug release, improved patient compliance, and reduced dosing frequency. Despite the known therapeutic value of sulfasalazine, its formulation into a nanosuspension specifically for colonic targeting remains underexplored. Therefore, the present study aims to formulate and evaluate an oral nanosuspension of sulfasalazine with the goal of enhancing its solubility, stability, and colonic targeting for the effective management of ulcerative colitis. The study focuses on the selection of suitable stabilizers, optimization of formulation parameters, and comprehensive evaluation of physicochemical characteristics, in vitro drug release, and potential for targeted drug delivery to the colon. [6,7]

2.0 MATERIAL AND METHODS

Sulfasalazine was provided by Aspire Lifesciences, Poloxomer was received from BASF PVPK 30 was received from research lab and Ascorbic acid, tween 80, DMSO received from modern laboratories,

2.1. Preparation of nanosuspension

2.1.1. Antisolvent precipitation method: [8]

The nanosuspension of the drug was formulated using a combined precipitation and ultrasonication technique. Initially, the required amount of drug and polymer was dissolved in DMSO with the aid of sonication for about 5 minutes at room temperature to ensure complete solubilization. Meanwhile, various stabilizers were dissolved in distilled water to prepare different antisolvent systems. Both the drug solution and the antisolvent preparations were filtered using a 0.45 μm membrane filter to remove any particulates and ensure clarity. The antisolvent was then cooled to approximately 3 °C using an ice-water bath to facilitate rapid precipitation. The drug solution was then promptly introduced into 40 mL of the chilled antisolvent using a syringe, with the needle placed directly into the stabilizer solution. The mixing was performed under constant stirring at different speeds using an overhead stirrer. And the homogenise at 2000RPM also DMSO is removed by rotary evaporator leading to the precipitation of the drug.

Although DMSO has a relatively high boiling point of around 189 °C, it can still be effectively removed using a rotary evaporator by applying reduced pressure. Under vacuum conditions, the boiling point of DMSO decreases significantly, allowing it to evaporate at much lower temperatures—often well below 100 °C. This is particularly useful when working with temperature sensitive drugs like Sulfasalazine, as it helps prevent any thermal degradation during the solvent removal process. While DMSO does not evaporate as easily as solvents like ethanol or acetone, rotary evaporation offers a gentle and efficient way to eliminate it from the nanosuspension without compromising the stability of the drug. After Rotary evaporation, Dialysis membrane methods is used to eliminate the remain DMSO concentration in much extent. Cut the dialysis tubing and soak it into water for soften for 15 to 20 minutes. Tie one end properly with clamp or thread. Load the sample into tube using syring. Do not overfill leave some air space to allow expansion and tie another open end properly. Take distilled water in beaker (at least 100x volume of your sample). Pour the bag with nanosuspension into beaker ensure that the bag is fully submerged and suspended in the middle. Stir water in magnetic stirrer gently. Replace the Dialysis medium after every 1 to 2 hours upto 12 to 14 hours. After that remove dialyzed nanosuspension from the tubing.

Table No: 01 Time interval for solvent removal

Interval (Time)	Action
After 30 minutes	Replace the water
After 1 hour	Replace the water
After 2 hours	Replace the water
After 4 hours	Replace the water
After 8 hours	Replace the water
After 12 hours	Final replacement of water

2.2. Experimental Design: Optimization by Box-Behnken Design

To optimize the formulation parameters for the Sulfasalazine nanosuspension, a Box-Behnken design (BBD) was employed

using Design-Expert® software (or equivalent DOE software) as shown in table no. 02 and 03

Coded Levels of Independent Variables

Table No: 02 Coded Levels of Independent Variables

Factors	Symbol	Low (–1)	Medium (0)	High (+1)
Eudragit RS100 (mg)	X_1	600	750	900
Poloxamer 407 (% w/v)	X_2	1	1.5	2
PVP K30 (% w/v)	X_3	1	3	5

Table No: 03 Responses from Box-Behnken Design

Run / Batch	X_1 (mg)	X_2 (% w/v)	X_3 (% w/v)	Y_1 (nm)	Y_2 (%)
F-1	(+1)	(0)	(-1)	275	89
F-2	(0)	(-1)	(-1)	240	83
F-3	(+1)	(+1)	(0)	280	91
F-4	(-1)	(-1)	(0)	200	78
F-5	(0)	(+1)	(+1)	260	86
F-6	(+1)	(-1)	(0)	270	90
F-7	(0)	(-1)	(+1)	250	85
F-8	(-1)	(+1)	(0)	230	81
F-9	(-1)	(0)	(-1)	190	78
F-10	(0)	(0)	(0)	245	84
F-11	(+1)	(0)	(+1)	278	90
F-12	(0)	(+1)	(-1)	255	87
F-13	(0)	(0)	(0)	250	85
F-14	(0)	(0)	(0)	250	85
F-15	(-1)	(0)	(+1)	220	80

3.0. CHARACTERIZATION OF NANOSUSPENSION

3.1. Particle Size Analysis and Polydispersity Index: Particle size and Polydispersity index are the two important parameters in the preparation of nanoformulations. The particle size and PDI value of nanosupension prepared by antisolvent precipitation method was determined. The particle size and Polydispersity index of the prepared nanosuspension was determined by Malvern zetasizer at 25ºC temperature condition. The prepared formulations were diluted to make the samples for the nano-formulations. properly prior to analysis.

3.2. Entrapment Efficiency (% EE): The prepared nanosuspension was placed for centrifugation at 5000 RPM for 60 minutes. After 60 minutes supernatant was collected and dilute the supernatant with suitable solvent and the entrapment efficiency was determined by UV-vis Spectrophotometer (LABINDIA UV 32000). Entrapment efficiency was determined by using following formula.

3.3. Zeta Potential: Zeta potential is one of the important parameter in the preparation of nanoformulations. The Zeta potential of nanosupension prepared by antisolvent precipitation method was determined. The Zeta potential of the prepared nanosuspension was determined by Malvern zetasizer at 25oC temperature condition. The prepared formulations were diluted to make the samples for the nano-formulations. properly prior to analysis**.**

3.4. Drug content evaluation[9, 10]

Drug content was evaluated by dissolving 1 ml of nanosuspension in DMSO under the magnetic stirring at 900 rpm. After the complete dissolution, appropriate dilutions were made and absorbance were recorded using UV spectrophotometer (Lab India UV 32000) at 359nm. Samples were taken. Calibration curve of the drug was already created between concentration range of 2 to 10 µg/ml.

3.5. Invitro drug release: The dialysis bag method served as the experimental platform to study drug release in vitro conditions. Dialysis membranes were soaked overnight in dissolution media for hydration and removal of preservatives. One end was sealed, filled with 2–5 mL of nanosuspension, and then tightly closed at the other end. The prepared bag was placed in the basket of the dissolution apparatus containing 900 mL of medium pre-heated to 37°C. The basket was rotated at 50 rpm, and 5 mL samples were withdrawn at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours, replacing each with fresh medium at 37°C. Drug concentration was analyzed using UV-VIS spectrophotometry.

3.6. X-ray Diffraction: X-ray Diffraction (XRD) analysis was carried out to by using X-Ray diffractometer (D8 Advance, Pune, India) having a voltage/current of 40 KV/40 mA at a scan speed of 4˚/min, at angle range of 5˚ to 50˚ and 0.5 s step time for 1 h to determine the crystalline or amorphous nature of the sample and to identify the polymorphic form(s) present in the drug substance/formulation.

3.7. Transmission Electron Microscopy (TEM): The analysis of morphological attributes used Transmission Electron Microscopy (TEM). A TEM grid contained nanosuspension samples which were observed through the microscope at suitable magnification levels. The assessment of particle morphology relied on capturing multiple images through the microscope.

3.8. Stability studies: The preparation filled in glass vial kept it for one month at conditions 30ºC/ 65%RH and formulation investigated for particle size PDI, zeta potential,

4.0. RESULTS AND DISCUSSION

4.1 Preformulation Studies

4.1.1. Organoleptic Properties

Sulfasalazine appeared as a yellowish fine powder with a slight sulfur-like odor and bitter metallic taste. The formulated nanosuspension showed a light orange, opalescent suspension with a consistent organoleptic profile, indicating no significant change post-formulation.[11]

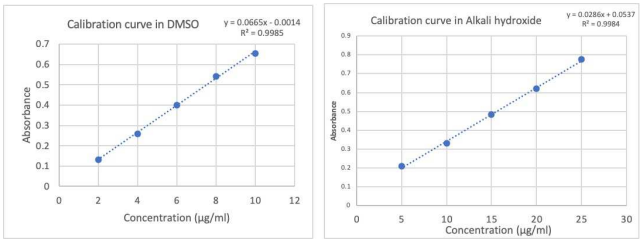
4.1.2. UV-Visible Spectroscopy

The maximum absorbance in DMSO was 359.3 nm, consistent with the standard value (359 nm) [12]. However, in alkali hydroxide, a blue shift was observed at 294 nm, suggesting solvent interaction effects. Both media displayed strong absorbance (4.4681), suitable for spectrophotometric analysis.

4.1.3. Calibration Curves

In DMSO, sulfasalazine showed linear absorbance (R^2 = 0.9985) between 2 $\mu g/ml$ to 10 $\mu g/ml$; in alkali hydroxide, linearity was maintained from 5 $\mu g/ml$ to 25 $\mu g/ml$ (R^2 = 0.9984). These results confirmed the method accuracy and sensitivity in both solvents.

A)



4.1.4. Solubility and Melting Point[11]

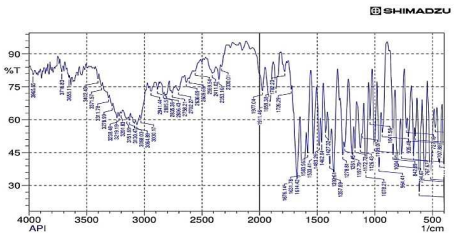
Solubility was highest in DMSO (99 mg/mL), moderate in alkali hydroxide (11.5 mg/mL), and poor in water (0.035 mg/mL). The melting point of sulfasalazine was found to be 270 ^{0}C by capillary tube method and 264.1^{0}C by DSC which is consistent with literature values.

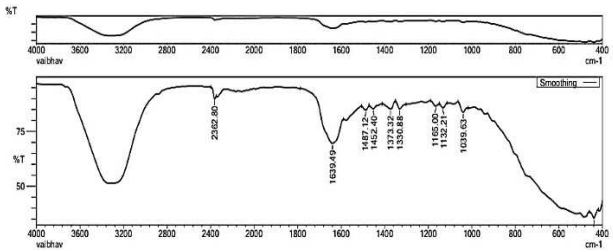
4.2. CHARACTERIZATION AND COMPATIBILITY

4.2.1. FTIR Analysis [13]

The spectra confirmed functional groups such as OH, NH, C=O, N=N, and S=O in the drug and nanosuspension. A new peak at 1730 cm^{-1} indicated ester formation with Eudragit. No major shifts or missing peaks suggested chemical compatibility between drug and excipients.

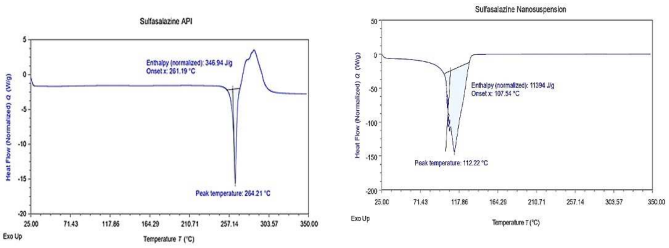
A)





4.2.2 Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD)

DSC thermograms displayed an endothermic peak at 112.22 ^{0}C for nanosuspension, indicating reduced crystallinity and a shift from the pure API profile. This transformation is desirable for improved solubility and bioavailability. The pure drug showed sharp peaks between 10° and 30°, 2θ confirming crystallinity, whereas the nanosuspension exhibited broadened, low-intensity peaks, indicating a transition to an amorphous form.



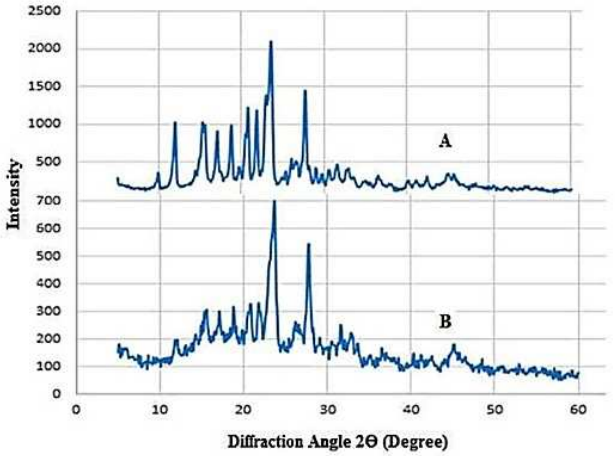


Figure No 03: DSC thermo grams of (A) Sulfasalazine API and (B)Sulfasalazine nanosuspension. C) XRD spectra of A Sulfasalazine API , B Sulfasalazine nanosuspension

4.2.3. TEM Imaging

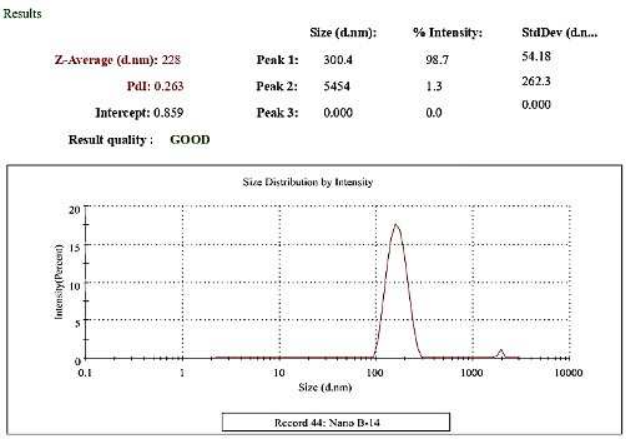
TEM revealed nanoparticles with sizes between 200nm to 250 nm and spherical morphology. A distinct core shell structure confirmed encapsulation within the Eudragit matrix.

Figure No 04: TEM of sulfasalazine nanosuspension

4.3. OPTIMIZATION AND EVALUATION

4.3.1. Particle Size, PDI, Zeta potential and Entrapment Efficiency

The optimized nanosuspension had a particle size of 228 nm, PDI 0.263, and %EE of 84.46%, closely matching predicted values. Higher polymer concentration led to increased particle size and entrapment efficiency, while poloxomer had a lesser impact. Zeta potential was -40.8 mV, indicating strong repulsive forces and excellent colloidal stability. The formulation was uniformly charged, ensuring minimal aggregation.



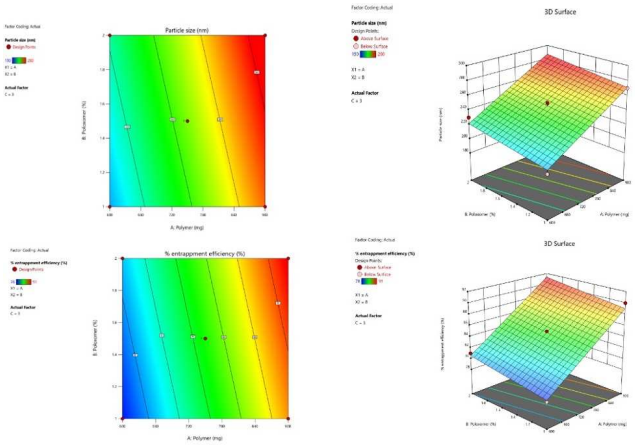


Table No 04: Drug content evaluation of formulation

4.3.2. Drug Content and pH

Across various concentrations (10, 15, 20 $\mu g/ml$)), % label claim ranged between 96.6% to 99.4%, confirming accuracy and repeatability. The pH remained stable (7.2 to 7.5pH) across all batches.

$$y = 0.0665x - 0.0014$$

Concentration recovery: $x = \frac{y+0.0014}{0.0665}$ where y = Absorbance.

Label claim	Label claim	Conc. Prepared ($\mu g/ml$)	Absorbance (A)	Conc. Recovered ($\mu g/ml$)	% label claim	Avg % label $\pm$ SD (n=3)
250mg/5ml	50mg/ml	10	0.6569	9.9	99	97.86 $\pm$0.1208
			0.6503	9.80	98	
			0.6409	9.66	96.6	
		15	0.9881	14.88	99.2	98.73 $\pm$0.2554
			0.9728	14.65	97.6	
			0.9901	14.91	99.4	
		20	1.3166	19.82	99.1	98.46 $\pm$ 0.1115
			1.3015	19.65	98.25	

			1.3026	19.61	98.05	

4.3.3. Viscosity and Stability

The viscosity ranged from 58.2cP to 62.1 cP, suitable for nanosuspension delivery. One-month stability data showed negligible changes in particle size, PDI, viscosity, pH, and drug content, confirming formulation stability.

Table No 05: Stability study of final formulation

Evaluation Tests	Initial result	After one month Results
Particle size	228	230.2
Polydispersity Index (PDI)	0.26	0.31
pH	7.4	7.4
Viscosity	62.1	61.4
Drug content	98.3	98.01

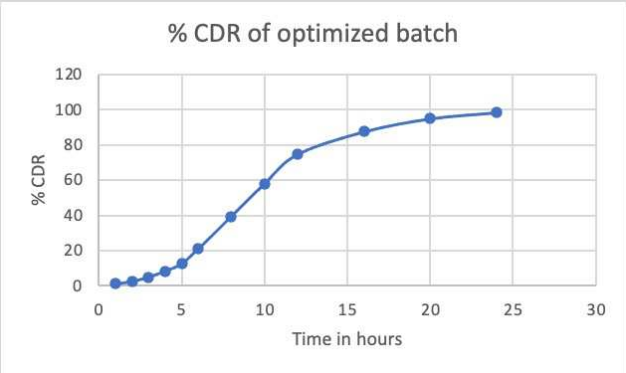
4.4. IN VITRO RELEASE AND KINETICS [150]

4.4.1. Drug Release Profile and Kinetic Modeling

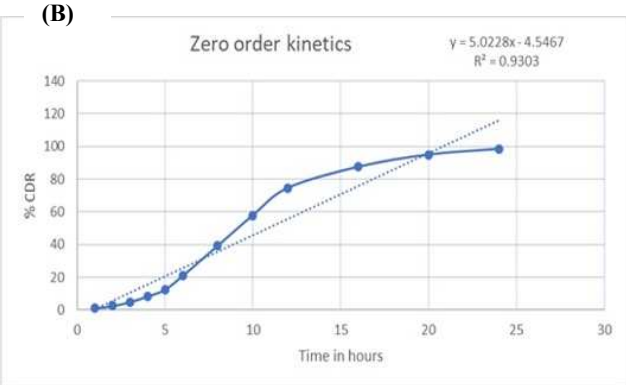
The nanosuspension demonstrated sustained drug release up to 24 hours (98.35%), unlike the marketed uncoated product which completed release within 1.5 hours (99%).

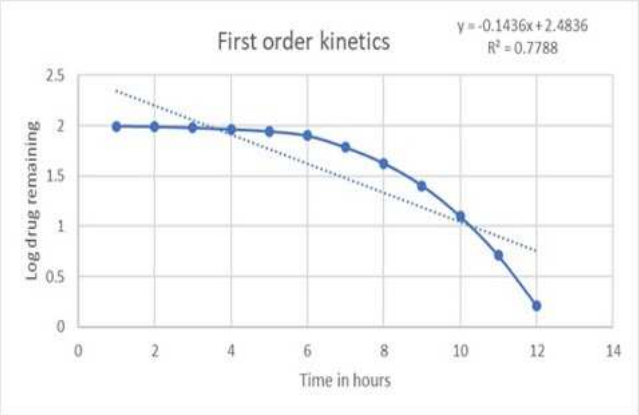
Table No 06: Cumulative percent drug release of optimized batch and marketed preparation

For optimized batch		For Marketed preparation	
Time in hours	%CDR	Time in hours	%CDR
1	1.2456	0.08 (5 minutes)	17
2	2.5462	0.16 (10 minutes)	38
3	5.0012	0.25 (15 minutes)	54
4	8.3658	0.5 (30 minutes)	78
5	12.5468	0.75 (45 minutes)	83
6	20.8745	1 (60 minutes)	95
8	39.29863	1.5 (90 minutes)	99
10	57.8325	-	-
12	74.6105	-	-
16	87.4302	-	-
20	94.8625	-	-
24	98.3564	-	-



(B)





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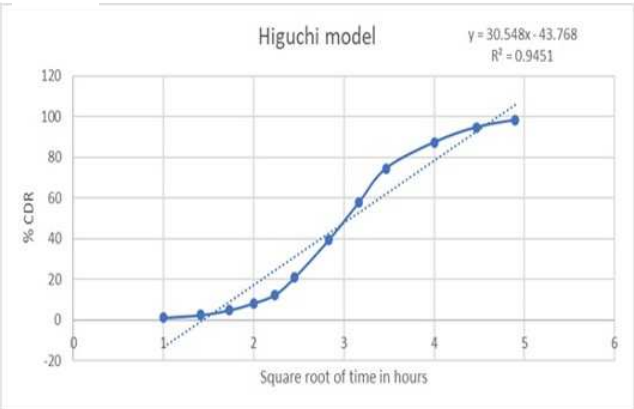

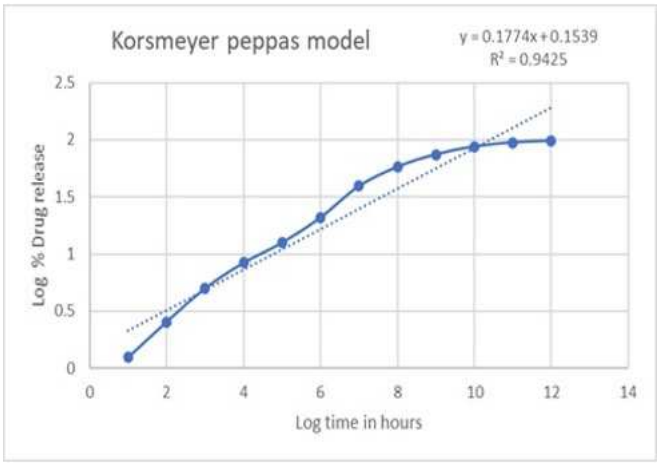

Figure No 7: (A) Cumulative percent drug release of optimized batch and marketed preparation (B) Zero order drug order kinetics, (C) First order drug order kinetics, (D) Higuchi model, (E) Korsmeyer peppas model

Higuchi model: R^2 = 0.9451; Best fit, indicating diffusion-based release.

Korsmeyer-Peppas: R^2 = 0.9425, n = 0.1774; Indicates Fickian diffusion mechanism.

5.0. CONCLUSION

To summarize, this study aimed on the development of novel sulfasalazine nanosuspension for treatment Inflammatory bowel disease specially ulcerative colitis. The sulfasalazine nanosuspension were formulated using Eudragit RS 100 polymer and was optimized using 3 Level Factorial design employing the Design-Expert software. The nano formulations were prepared with an objective to have nanoparticles of minimum particle size and PDI, with maximum %EE. Design-Expert software predicted the values of the particle size 234nm and %EE 83.89% with a desirability of 0.476. The optimized formulation developed had particle size of 228 nm, PDI 0.263 with %EE of 84.46% which was found to be very close to the predicted values. The optimized NPs showed particle size 228 nm, PDI 0.263 with %EE of 84.46%. The zeta potential analysis showed a strong negative value of -40.8 mV, indicating excellent colloidal stability of the nanoformulation. DSC, FTIR, XRD analysis revealed good physiochemical compatibility and confirmed the encapsulation of sulfasalazine nanosuspension. TEM analysis showed that the nanoparticles in the first image were uniform, well-defined, and minimally agglomerated, indicating successful synthesis. In contrast, the second image revealed irregular shapes and some aggregation, suggesting incomplete stabilization. These differences highlight variability in particle morphology, which can influence the stability and effectiveness of the formulation. Viscosity and pH was found between the range. Drug content evaluation showed that the drug was uniformly distributed in formulated hydrogel. In-vitro drug release study showed that sulfasalazine was released from the from the polymeric nanosuspension in a sustained manner for 12 to 24 hours due to the controlled release property of the polymer. Based on the findings of this study, it can be concluded that the developed formulation was found to be more effective and superior approach for developing an sulfasalazine nanosuspension for the treatment of ulcerative colitis.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Suryawanshi Kunal Anil: Investigation, Conceptualization, Literature search, Data curation, writing original draft, Kiran Bhausaheb Erande: Supervision, Writing review & editing, Final approval of manuscript, Mangesh Popat Ubale, Shubham Prashant bagad and Yuvraj Sanjay Jadhav Dinesh P. Patil, Yash

CONFLICTS OF INTEREST

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