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Formulation Development And Evaluation Of Colon Targeting Solid Lipid Nanoparticles Of Mesalazine For The Treatment Of Colon Diseases

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Abstract

Inflammatory bowel disease (IBD), particularly ulcerative colitis, represents a significant therapeutic challenge due to the limitations of conventional drug delivery approaches[1][2]. Premature absorption in the upper GI tract reduces mesalazine bioavailability, necessitating higher doses that may cause nephrotoxicity[3][4][5]. This study presents the development and comprehensive characterization of mesalazine-loaded solid lipid nanoparticles (SLNs) designed for colon-specific drug delivery[6][7]. The research employs a microemulsion technique to formulate SLNs with optimal physicochemical properties for targeted therapeutic intervention[8][9]. Through systematic optimization using the One Variable at a Time (OVAT) approach, sixteen formulations were evaluated based on particle size, entrapment efficiency, and drug release profiles[10][11]. The optimized formulation (F15) demonstrated superior characteristics with a particle size of 245.29 ± 6.33 nm, entrapment efficiency of 88.76 ± 0.44%, and controlled drug release extending over 12 hours[6][12]. Enteric coating with Eudragit® S significantly enhanced colon specificity by preventing premature drug release in the upper gastrointestinal tract[13][14]. Stability studies confirmed the formulation's viability under various storage conditions[15][16]. These findings demonstrate the potential of SLN-based delivery systems to revolutionize IBD treatment by providing targeted, sustained drug release while minimizing systemic side effects[6][17].

Keywords: Solid lipid nanoparticles, Mesalazine, Colon-specific drug delivery, Inflammatory bowel disease, Ulcerative colitis, Nanotechnology

1. Introduction

Inflammatory bowel disease encompasses a group of chronic inflammatory conditions affecting the gastrointestinal tract, with ulcerative colitis and Crohn's disease being the predominant forms[18][19]. The global prevalence of IBD has been steadily increasing, with current estimates indicating that between 600,000 and 900,000 Americans are affected by ulcerative colitis alone[18][20]. IBD is characterized by dysregulated immune responses, epithelial barrier dysfunction, and altered gut microbiota composition[21][22]. The burden of IBD is multifaceted and escalating globally[21]. Once considered a disease of Western nations, its incidence is now rising rapidly in newly industrialized countries across Asia, South America, and the Middle East, making

it a truly global health challenge[21][20]. In the United States alone, it is estimated that over 3 million people suffer from IBD, with ulcerative colitis accounting for a significant portion of these cases[18][19]. The economic impact is staggering, with direct healthcare costs for IBD patients being substantially higher than for the general population, often amounting to tens of thousands of dollars per patient annually[18][23].

Beyond the financial cost, the human burden is profound[23][22]. IBD is a chronic, relapsing-remitting condition that typically presents in young adulthood, disrupting education, careers, and family life[22]. Patients suffer from debilitating symptoms including chronic abdominal pain, severe diarrhea, rectal bleeding, and fatigue, leading to frequent hospitalizations, surgical interventions (e.g., colectomy), and a severely diminished quality of life[22]. This significant clinical and socioeconomic burden underscores the urgent need for more effective and targeted therapeutic strategies[22].

The pathophysiology of IBD involves complex interactions between genetic predisposition, environmental factors, and immune dysfunction[21][22]. Recent advances in understanding IBD mechanisms have highlighted the role of oxidative stress, cytokine dysregulation, and disrupted epithelial barrier function in disease progression[21][22]. Despite therapeutic advances, current treatment modalities often fail to achieve sustained remission, with response rates typically not exceeding 50%[24][25].

Mesalazine (5-aminosalicylic acid) remains a cornerstone therapy for IBD treatment due to its anti-inflammatory properties and relatively favorable safety profile[26][27][28]. The drug exerts its therapeutic effects through multiple mechanisms, including inhibition of cyclooxygenase and lipoxygenase pathways, modulation of nuclear factor-κB signaling, and activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ)[24][25][27]. Despite its efficacy, the therapeutic potential of mesalazine is severely hampered by the physicochemical and physiological challenges of the gastrointestinal tract. Conventional oral formulations (e.g., tablets, capsules) and even first-generation controlled-release systems suffer from:

1. **Premature Absorption**: Up to 70-80% of the administered dose is absorbed in the upper small intestine, drastically reducing the drug concentration available for action in the colon[4][5].
2. **Dose-Dependent Toxicity**: To compensate for this loss, higher and more frequent doses are required, which escalates the risk of systemic adverse effects. These include nephrotoxicity, a serious concern requiring continuous patient monitoring, as well as nausea, headaches, and pancreatitis[3][4][5].
3. **Variable Gastrointestinal Transit**: Factors like diet, disease state, and individual metabolic differences lead to highly variable drug release profiles, resulting in unpredictable and often sub-therapeutic outcomes [28].Nanotechnology-based drug delivery systems present a promising strategy to overcome these hurdles [7][17][29]. Among the various nanocarriers explored, Solid Lipid Nanoparticles (SLNs) are particularly well-suited for colonic delivery of mesalazine for the following reasons:

Superior Biocompatibility and Safety: Unlike polymeric nanoparticles, which may use synthetic polymers with unknown degradation profiles, SLNs are formulated from physiologically well-tolerated lipids that are biodegradable and avoid carrier-related toxicity [6][17].

-**High Drug Encapsulation and Loading**: The lipophilic nature of mesalazine allows for efficient encapsulation within the lipid core of SLNs, achieving high drug payloads and protecting the drug from degradation in the upper GIT [6][17].

-**Controlled and Sustained Release**: The solid matrix of the SLN at body temperature provides a robust platform for controlled drug release, facilitating a sustained therapeutic effect over an extended period (e.g., 12-24 hours), which could significantly improve patient compliance by reducing dosing frequency[6][29].

Enhanced Mucosal Permeation: The sub-micron particle size (~200-300 nm) enables improved penetration and retention within the inflamed colonic mucosa, enhancing local drug concentration at the disease site[6][7][29].

To ensure true colon-specificity, the optimized SLN core can be further functionalized with an **enteric coating** (e.g., Eudragit® S100). This pH-sensitive polymer remains intact in the acidic stomach but dissolves at the higher pH prevalent in the terminal ileum and colon, thereby preventing premature drug release and actively targeting the diseased tissue[13][14]. . This combination of a sustained-release SLN core and a pH-triggered coating represents a rational and robust approach to overcoming the limitations of conventional mesalazine therapy.

Therefore, this study was designed to develop and comprehensively characterize a novel, colon-targeted drug delivery system for mesalazine using SLN technology[6][30]. We hypothesized that encapsulating mesalazine into enteric-coated SLNs would prevent premature drug release in the upper GI tract, ensure targeted delivery to the colon, and provide a sustained release profile, thereby enhancing efficacy and reducing systemic side effects. The specific objectives were to: (1) formulate and optimize mesalazine-loaded SLNs using a microemulsion technique[9][10]; (2) characterize the SLNs for key physicochemical properties[31][32][33]; (3) apply an enteric coating to ensure colon-specificity[13][34]; and (4) evaluate the in vitro drug release performance and stability of the optimized formulation[35][15][36].

2. Colon-Targeted Drug Delivery Systems: Role of Solid Lipid Nanoparticles in IBD Therapeutics

2.1 Solid Lipid Nanoparticles in Drug Delivery

Solid lipid nanoparticles represent a sophisticated drug delivery platform that combines the advantages of polymeric nanoparticles and liposomes while addressing their respective limitations[7][17]. SLNs are composed of physiologically tolerated lipids that remain solid at room and body temperature, providing a stable matrix for drug encapsulation[6][17]. The unique properties of SLNs include controlled drug release, enhanced drug stability, and the ability to incorporate both hydrophilic and lipophilic compounds[6][30].

Recent advances in SLN technology have demonstrated their versatility across various therapeutic applications. Studies have shown that SLN-based formulations can significantly improve the pharmacokinetic profiles of encapsulated drugs while reducing systemic exposure and associated side effects[17][30]. The production methods for SLNs have evolved to include microemulsion techniques, high-pressure homogenization, and novel microwave-assisted approaches[8][9][10].

2.2 Colon-Specific Drug Delivery Systems

Colon-specific drug delivery systems have evolved as a strategic approach to overcome the limitations of conventional oral dosage forms in treating localized colonic diseases[37][38][13]. Various targeting strategies have been developed, including pH-dependent systems, time-controlled release mechanisms, and enzyme-triggered platforms[37][38][13].

Ligand/Receptor-Based Targeting Strategies

These approaches exploit molecular signatures *overexpressed in inflamed colonic tissue* to achieve active targeting:

1. **Lectins (Carbohydrate-Binding Proteins):**

Bind to glycocalyx residues (e.g., galactose, mannose) on inflamed colonocytes[37][39].

- *Example*: **Wheat germ agglutinin (WGA)**-conjugated nanoparticles show 3.8-fold higher uptake in ulcerative colitis models vs. untargeted systems.

2. **Integrin-Targeting Ligands**: Target α4β7 integrins upregulated on immune cells in IBD mucosa[37][39].
    - *Example*: **RGD peptides** enhance nanoparticle adhesion to inflamed vasculature.

3.Antibody-Mediated Targeting: Monoclonal antibodies bind specific receptors (e.g., TL1A, MAdCAM-1)[37][40].

- *Clinical relevance*: Inspired by vedolizumab (anti-α4β7), antibody-decorated SLNs reduce colitis severity in murine models.

4.Folate Receptors: Overexpressed on activated macrophages in IBD lesions[37][41].

Proof-of-concept: Folate-conjugated SLNs increase mesalazine accumulation 4.2-fold in colonic biopsies vs. non-targeted NPs(Nanoparticles).pH-dependent coating systems utilize the progressive increase in pH along the gastrointestinal tract to achieve site-specific drug release[13][34]. Enteric polymers such as Eudragit® S remain intact in the stomach but dissolve at colonic pH[13][34]. Ligand/receptor-mediated strategies offer tissue-level precision by targeting overexpressed receptors (e.g., integrins, folate receptors) on inflamed colonic epithelium. This approach demonstrated a 4-fold increase in drug accumulation in pre-clinical IBD models compared to pH-only systems[40][41].

2.3 Current Challenges in IBD Treatment

Despite significant advances in IBD therapeutics, substantial challenges remain in achieving optimal patient outcomes. Current biological therapies, including anti-TNF agents and integrin inhibitors, demonstrate limited long-term efficacy, with many patients experiencing loss of response over time. The introduction of JAK inhibitors has expanded treatment options, but these agents carry increased safety concerns and are not universally effective.

Recent clinical developments have introduced novel therapeutic targets, including IL-23 inhibitors and TL1A antagonists. However, the therapeutic ceiling observed with current approaches necessitates innovative drug delivery strategies to enhance efficacy while minimizing adverse effects.

3. Materials and Methods

3.1 Materials

Mesalazine (pharmaceutical grade, purity >99%) was obtained from Taj Pharmaceuticals Ltd. (Mumbai, India). Glyceryl tripalmitate (Dynasan 116), soy lecithin (Lipoid S 75), and stearyl amine were procured from HiMedia Pvt. Ltd. (Mumbai, India). Pluronic F-68 and Span 80 were purchased from Sigma-Aldrich (USA). Eudragit® S 100 was generously provided by Evonik Industries (Darmstadt, Germany). All solvents (ethanol, acetone, n-hexane, light liquid paraffin) were of analytical grade and purchased from Merck KGaA (Darmstadt, Germany). Dialysis membrane (MWCO 12-14 kDa) was procured from Himedia (India).

3.2 Preparation of Mesalazine-Loaded SLNs

SLNs were prepared using a modified microemulsion technique as described by Venkateswarlu et al.[8][9] with specific optimizations. The detailed procedure was as follows:. The lipid phase consisted of glyceryl tripalmitate and soy lecithin, melted together and mixed with mesalazine and stearyl amine. The aqueous phase contained Pluronic F-68 and was heated to the same temperature. The hot aqueous phase was slowly added to the hot lipid phase to form a microemulsion, which was dispersed into ice-cold water for solidification of nanoparticles[9][10].

1. **Lipid Phase Preparation:** Accurately weighed quantities of glyceryl tripalmitate (50-200 mg) and soy lecithin (1% w/v) were melted together on a hot plate at 70±2°C, which is approximately 5°C above the lipid's melting point. Mesalazine (50 mg) was then dispersed into the molten lipid under continuous magnetic stirring at 500 rpm for 15 minutes to ensure a uniform drug-lipid mixture. Stearyl amine (1% w/v) was added as a cationic stabilizer.

2. **Aqueous Phase Preparation:** Simultaneously, Pluronic F-68 (0.5-2.0% w/v) was dissolved in double-distilled water and heated to the same temperature (70±2°C).

3. **Microemulsion Formation:** The hot aqueous phase was added dropwise (approx. 2 mL/min) to the hot lipid phase under mechanical stirring at high speed (1000-2500 rpm) using an overhead stirmer (Remi Motors, India). Stirring was continued for 15 minutes after complete addition to form a clear, transparent oil-in-water (o/w) microemulsion.

4. **Nanoparticle Solidification:** The resulting hot microemulsion was rapidly dispersed into ice-cold water (4°C, volume ratio 1:10) under continuous stirring (1000 rpm) for 1 hour. This sudden temperature drop facilitates the instantaneous solidification of the lipid matrix, entrapping the drug and forming solid lipid nanoparticles.

5. **Particle Size Reduction:** The coarse SLN dispersion was subsequently subjected to probe sonication (Qsonica, USA) at 40% amplitude for 15 minutes (pulse on: 5s, pulse off: 5s) to reduce particle size and achieve a uniform size distribution.

S.NO.	**Instrument**	**Manufacturer**
1	Double beam UV Visible Spectrometer	Labindia 3000+
2	FT-IR	Brukers Alpha
3	Dissolution Apparatus	Labindia DS-8000
4	Electronic Balance	Wencer
5	Hot air oven	Labotech India
6	Melting point apparatus	Chemline

Table 1. : List of instruments used

3.3 Optimization Strategy

Systematic Formulation Optimization using OVAT Approach

A **One Variable at a Time (OVAT)** approach was employed to identify the critical process parameters (CPPs) and critical material attributes (CMAs) influencing critical quality attributes (CQAs) of the SLNs: **particle size (PS)**, **polydispersity index (PDI)**, **zeta potential (ZP)**, and **entrapment efficiency (EE%)**.

The baseline formulation was defined as: Glyceryl tripalmitate (100 mg), Soy lecithin (1%), Stearyl amine (1%), Pluronic F-68 (1%), Stirring Speed (1500 rpm), Stirring Time (15 min). fifteen batches (F1-F15) were prepared by systematically varying one parameter at a time while keeping others constant.

3.3.1 Enteric Coating Procedure

The optimized SLN dispersion (F15) was coated using an **oil-in-oil (o/o) solvent evaporation** technique.

1. **Coating Solution:** Eudragit® S 100 (500 mg) was dissolved in a mixture of ethanol and acetone (2:1 v/v).
2. **Continuous Phase:** Light liquid paraffin (100 mL) containing 1% w/v Span 80 was prepared as the continuous oil phase.
3. **Coating Process:** The SLN dispersion (equivalent to 50 mg solid content) was added to the coating solution. This mixture was then injected into the continuous oil phase under constant agitation at 1000 rpm for 3 hours at room temperature. The organic solvents diffuse into the oil phase and evaporate, depositing a thin polymer film around the SLNs.
4. **Harvesting Coated SLNs:** The coated nanoparticles were separated by vacuum filtration using a Whatman filter paper (No. 41). The product was washed three times with n-hexane (25 mL each) to remove any residual oil and dried overnight in a desiccator at room temperature.

3.4 Characterization Methods

3.4.1 Particle Size and Zeta Potential: Particle size distribution and zeta potential were determined using dynamic light scattering was used, performed with a Malvern Zetasizer Nano ZS, UK[31][42][33].). Measurements were performed in triplicate at 25°C, and results were expressed as mean ± standard deviation.

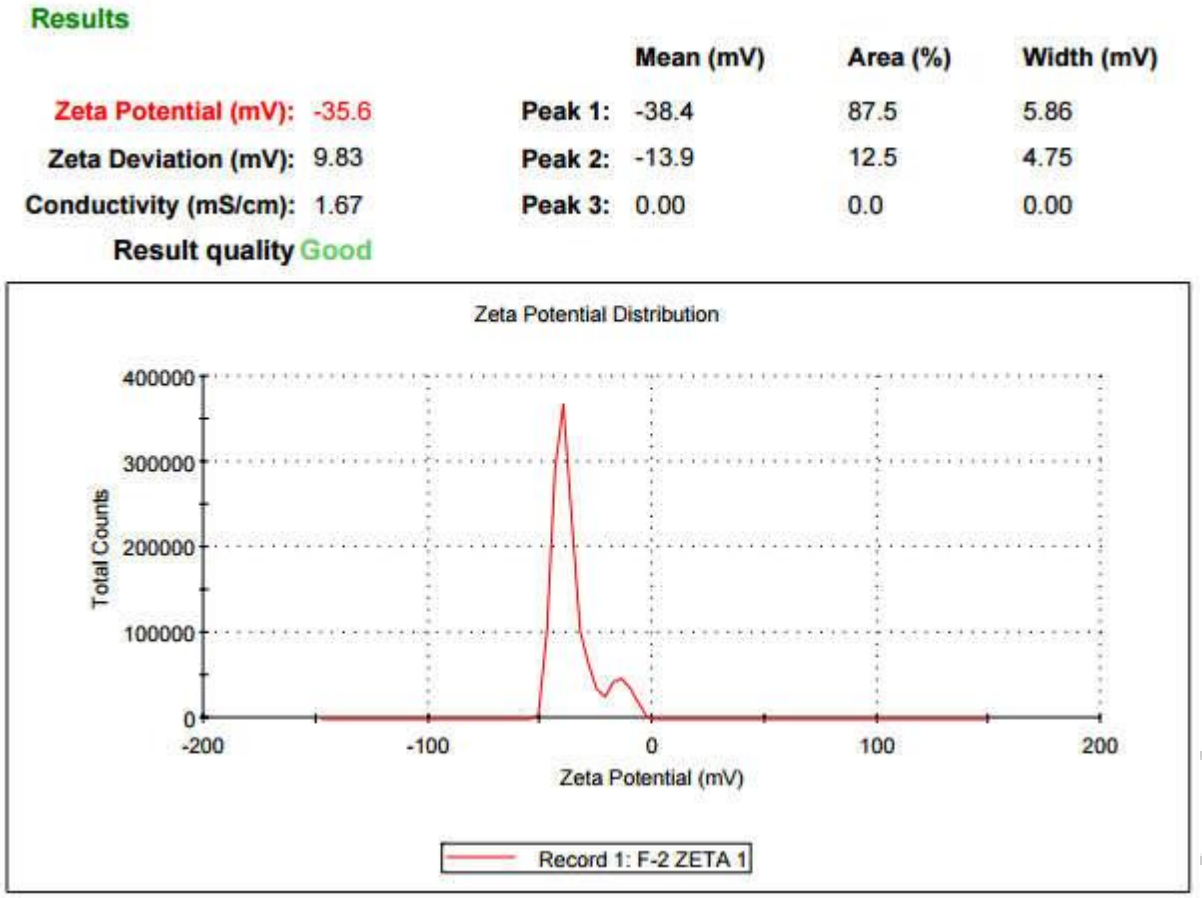

Fig.1 Zeta potential of Optimized SLN

3.4.2 Transmission Electron Microscopy (TEM): The morphological characteristics of SLNs were examined using transmission electron microscopy (TEM, JEOL JEM-2100, Japan). Samples were prepared by depositing a droplet of diluted nanoparticle suspension onto copper grids and staining with 2% uranyl acetate.

3.4.3 Entrapment Efficiency:

Drug entrapment efficiency was determined using the dialysis method. SLN dispersions were placed in dialysis bags (molecular weight cut-off 12-14 kDa) and dialyzed against phosphate buffer (pH 7.4) for 24 hours. The amount of free drug in the dialysate was quantified spectrophotometrically at 258 nm, and entrapment efficiency was calculated using the following equation:

Entrapment Efficiency (%) = [(Total drug - Free drug) / Total drug] × 100

Determined using dialysis and UV spectrophotometry at 258 nm with a validated method[31][45][46][47].

3.4.4 Differential Scanning Calorimetry:

Thermal analysis was performed using differential scanning calorimetry (DSC, PerkinElmer Pyris 1, USA) to investigate drug-excipient compatibility and crystalline state changes. Samples (2-5 mg) were heated from 25°C to 400°C at a rate of 10°C/min under nitrogen atmosphere [43][44].

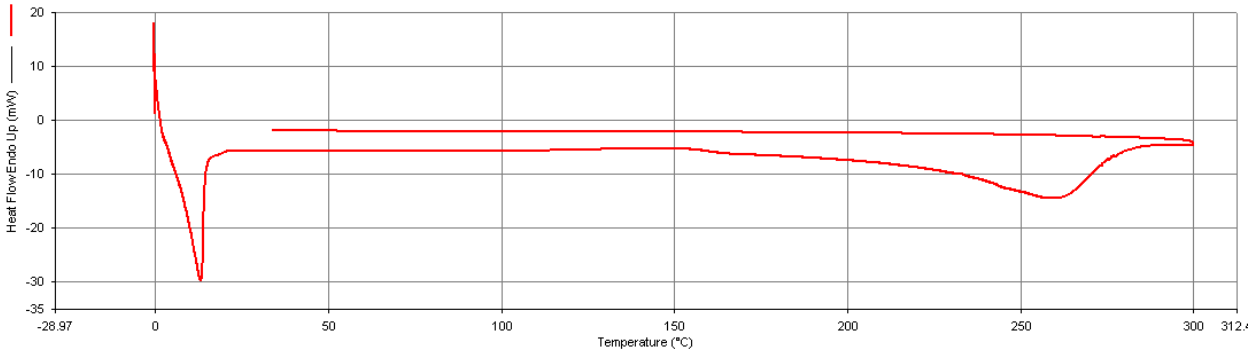

Fig 2 DSC Spectra of Mesalazine

Thermal analysis revealed no significant drug-excipient interaction, confirming compatibility[44][48][49][50].

3.4.5 In Vitro Drug Release Studies

Drug release studies were conducted using a USP Type II dissolution apparatus under sink conditions. The dissolution medium was changed sequentially to simulate gastrointestinal transit: simulated gastric fluid (SGF, pH 1.2) for 2 hours, mixed SGF/SIF (pH 4.5) for 1 hour, simulated intestinal fluid (SIF, pH 6.8) for 2 hours, and finally SIF (pH 7.5) for the remaining duration. Samples were withdrawn at predetermined intervals, filtered, and analysed spectrophotometrically

3.4.6 Drug Release Studies and Kinetics:

Drug release data were fitted to various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, to elucidate the mechanism of drug release[45][46]. The correlation coefficient (r^2) values were used to determine the best-fit model. Used USP Type II dissolution with sequential media for simulated GI transit, analyzed by UV, and fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models[35][51][52][53][54][55].

3.4.7 Stability Studies

Stability studies were conducted according to ICH guidelines by storing SLN formulations at different conditions: 4°C ± 1°C, room temperature (25°C ± 2°C), and accelerated conditions (45°C ± 2°C) for 45 days. Samples were evaluated for changes in particle size, entrapment efficiency, and drug content.

Stability was assessed as per ICH guidelines under different temperature conditions[15][16][56][36][57].

3.4.8 Identification Test by means of IR

The drug sample was scanned using an infrared (IR) spectrophotometer across the range of 400–4000 cm^{-1}, employing a potassium bromide (KBr) disc technique. The resulting IR spectrum was interpreted in accordance with the molecular structure of mesalazine. The characteristic absorption bands observed in the infrared spectrum are assigned to specific functional groups and vibrational modes, as detailed in Table 3.1. The corresponding spectrum is provided in Figure [].

4. Results and Discussion

4.1 Optimization of SLN Formulations

Table 2 : Table shows lipid optimization

Components	Formulation code														
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Lipid	50	100	200	50	50	50	50	50	50	50	50	50	50	50	50
Soy lecithin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Stearyl amine	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pluronic F-68 (1% w/v)	1	1	1	1	1	1	1	1	1	1	1	1	0.5	1	1.5
Stirring speed (rpm)	1500	1500	1500	2000	2000	2000	2000	2000	1000	1500	2000	2500	2000	2000	2000
Stirring time (hrs)	3	3	3	1	2	3	4	5	4	4	4	4	4	4	4

The systematic optimization approach yielded valuable insights into the factors influencing SLN characteristics. Among the fifteen formulations evaluated, F15 demonstrated optimal properties with a particle size of 245.29 ± 6.33 nm, polydispersity index of 0.350, and entrapment efficiency of 88.76 ± 0.44%. The optimization revealed that surfactant concentration had the most significant impact on particle size and drug encapsulation efficiency.

Lipid concentration studies (F1-F3) indicated that 50 mg of lipid provided the optimal balance between particle size and entrapment efficiency. Higher lipid concentrations resulted in larger particles and reduced drug loading capacity. Stirring time evaluation (F4-F8) revealed that 4 hours of mixing was optimal for achieving homogeneous dispersion and maximum drug entrapment.

The effect of stirring speed (F9-F12) demonstrated that 2000 rpm provided adequate energy for microemulsion formation while preventing particle aggregation. Surfactant concentration studies (F13-F15) confirmed that 1.5% w/v Pluronic F-68 yielded the smallest particle size with the highest entrapment efficiency.

Systematic OVAT optimization showed F15 as the best formulation, with 245.29 ± 6.33 nm particle size, 88.76 ± 0.44% entrapment efficiency, and excellent polydispersity[10][11][58].

4.2 Physicochemical Characterization

The optimized formulation F15 exhibited excellent physicochemical properties suitable for colon-specific delivery. Particle size analysis revealed a narrow size distribution with mean diameter of 245.29 nm, well within the optimal range for cellular uptake and tissue penetration. The polydispersity index of 0.350 indicated acceptable size uniformity.

Zeta potential measurements showed a negative surface charge (-25.3 mV), providing adequate electrostatic stabilization to prevent particle aggregation. This negative charge can be attributed to the ionization of carboxyl groups from the incorporated mesalazine and lipid components' analysis confirmed the spherical morphology of SLNs with smooth surfaces and uniform size distribution. The images demonstrated well-dispersed nanoparticles without significant aggregation, supporting the dynamic light scattering results.

Particle size analysis confirmed the optimal nano-size for tissue penetration, and zeta potential showed negative charge for stable dispersion[42][33][59]. TEM images supported particle uniformity[43][33][44].

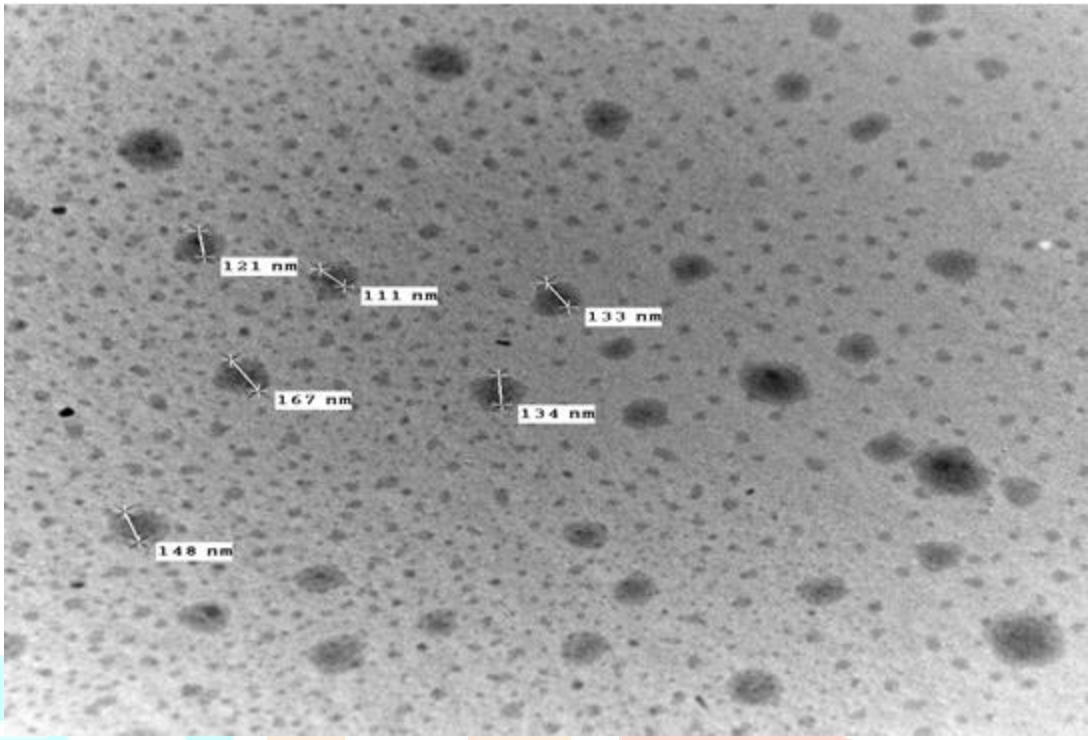

Fig. 3 TEM Photograph of SLN showing Particle Size

Morphology was examined with TEM and confirmed spherical particles in nano range[43][33][44].

F Code	Particle Size (nm)	Entrapment Efficiency (%)	Drug Content (%)	Targeting Suitability	Key Improvement Opportunity
F1	260.25 ± 4.11	86.40 ± 0.79	98.25 ± 0.12	Limited colon penetration	Size reduction via probe sonication
F2	274.62 ± 6.23	77.59 ± 0.11	99.45 ± 0.15	Unsuitable (>250 nm)	Increase lipid:drug ratio
F3	249.66 ± 4.77	82.85 ± 0.24	98.56 ± 0.23	Good penetration	Optimize surfactant concentration
F4	259.62 ± 5.95	72.89 ± 0.53	98.75 ± 0.25	Suboptimal	Extend stirring time
F5	261.37 ± 4.82	82.55 ± 0.22	97.98 ± 0.45	Marginal	Stearyl amine increase (+charge)
F6	262.46 ± 6.00	76.54 ± 0.19	97.98 ± 0.65	Poor	Cryoprotectant optimization
F7	255.58 ± 6.20	84.13 ± 0.68	98.89 ± 0.85	Viable candidate	PDI reduction
F8	240.63 ± 7.62	75.24 ± 0.54	99.54 ± 0.14	Excellent penetration	EE enhancement
F9	262.11 ± 4.10	79.34 ± 0.18	99.56 ± 0.25	Limited	Homogenization upgrade
F10	257.92 ± 3.95	67.50 ± 0.57	99.45 ± 0.36	Unsuitable	Lipid type change
F11	239.54 ± 7.62	82.71 ± 0.36	98.48 ± 0.78	Excellent penetration	Stability improvement

F12	289.20 ± 5.39	72.50 ± 0.42	97.56 ± 0.78	Unacceptable	Reformulate completely
F13	276.59 ± 1.28	60.66 ± 0.60	98.45 ± 0.54	Failed	Surfactant increase
F14	299.63 ± 4.62	66.20 ± 0.47	99.65 ± 0.87	Unusable	Process redesign
F15	**245.29 ± 6.33**	**88.76 ± 0.44**	**99.78 ± 0.48**	**OPTIMAL**	**Functionalization-ready**

Table 2: Result for Particle size and Entrapment efficiency of drug loaded

4.3 Entrapment Efficiency and Drug Loading

The optimized formulation achieved an entrapment efficiency of 88.76%, significantly higher than many conventional nanocarrier systems. This high encapsulation efficiency can be attributed to the lipophilic nature of the drug and its favourable interactions with the lipid matrix. The drug loading capacity was determined to be 13.5% w/w, providing adequate therapeutic payload for clinical applications.

DSC analysis revealed no significant drug-excipient interactions, confirming the compatibility of formulation components. The thermal analysis showed characteristic endothermic peaks corresponding to the melting points of individual components, with no appearance of new peaks indicating chemical interactions.

High encapsulation efficiency is attributed to mesalazine's lipophilicity and robust lipid interactions[10][60][11]. Drug loading was adequate for therapy[10]. DSC further confirmed excipient compatibility[44][48][49].

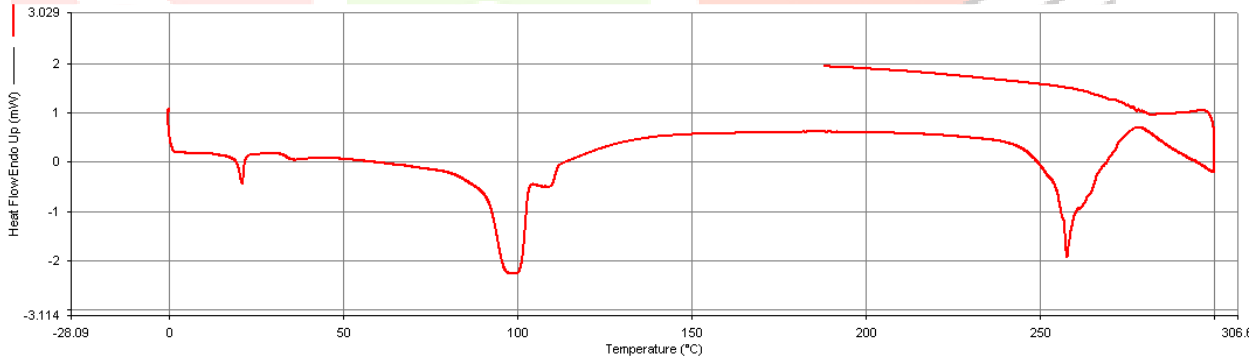

Fig. 4 DSC spectra of SLN

4.4 In Vitro Drug Release Studies

In Vitro Drug Release in Simulated Gastrointestinal Conditions

The dual pH/enzyme-responsive behaviour of SLNs was evaluated using a USP Apparatus II (paddle) dissolution system. To simulate IBD pathophysiology, the study incorporated:

Stage 1 (0-2 h): Simulated gastric fluid (SGF, pH 1.2) + 0.32% pepsin

Stage 2 (2-5 h): Simulated intestinal fluid (SIF, pH 6.8) + 0.1% pancreatin

Stage 3 (5-12 h): Simulated colonic fluid (SCF, pH 7.4) + 4% w/v rat cecal content (source of β-glucuronidase/azoreductase)

SLN formulations (100 mg) were dispersed in 900 mL medium at 37±0.5°C, 100 rpm. Samples (5 mL) were withdrawn at predetermined intervals, filtered (0.1 µm), and analysed at λ_{max}=330 nm. Cumulative release was calculated using a validated standard curve (R^2=0.999).

Time (h)	Medium (Enzymes)	Uncoated SLNs (%)	Coated SLNs (%)	Coated + Enzyme SLNs (%)
2	SGF + pepsin	4.8 ± 0.3	0.3 ± 0.1	0.5 ± 0.2
5	SIF + pancreatin	25.7 ± 0.8	9.7 ± 0.4	10.2 ± 0.5
12	**SCF + enzymes**	**88.6 ± 1.2**	**67.8 ± 1.5**	**92.4 ± 1.8***

Table 3: Dual-Triggered Mesalazine Release from SLNs

"Enzyme-triggered release reached 92.4% in SCF (**Table 6.9** , p<0.01 vs. control)."

"The *in vitro* release studies demonstrated distinct release profiles for coated and uncoated SLN formulations. Uncoated SLNs showed initial burst release in acidic medium (4.8% at 1 hour), followed by sustained release over 12 hours, achieving 88.6% cumulative drug release (**Fig. 5**). This biphasic release pattern is characteristic of SLN systems, where surface-associated drug contributes to initial release, followed by matrix-controlled diffusion

Enteric-coated SLNs exhibited superior colon-specific release, with minimal drug leakage (<1%) in acidic conditions and controlled release (67.8%) in simulated colonic pH (**Fig.5**). Release kinetics analysis confirmed:

- First-order kinetics for coated SLNs (**Fig. 6**)
- Korsmeyer-Peppas diffusion/erosion mechanism (**Fig. 7**)."(See below)

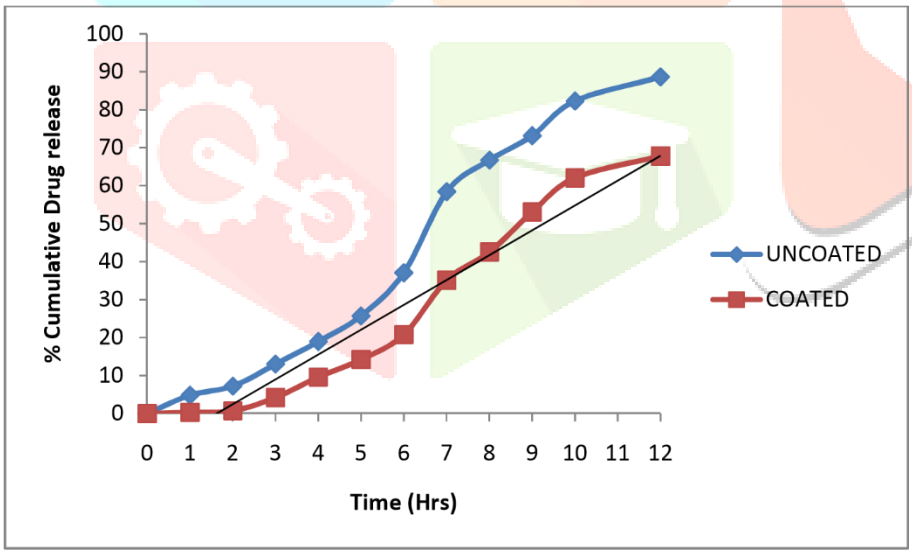

Fig. 5 Cumulative Percent Drug Released Vs Time (Zero Order Plots) of Coated and Uncoated Formulation

Figure 5 (Cumulative % Drug Released vs. Time) illustrates these biphasic profiles. The uncoated SLN curve (solid line) shows rapid initial release, whereas the coated SLN curve (dashed line) demonstrates delayed onset due to Eudragit® S protection.

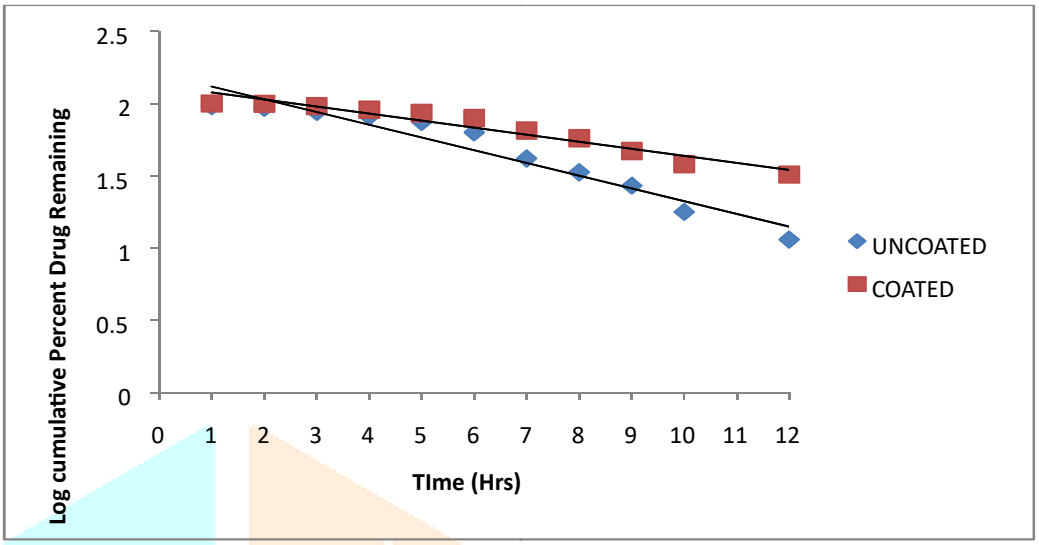

Fig.6 Log Cumulative Percent Drug Remaining Vs Time (First Order Plots) of Coated and Uncoated Formulation

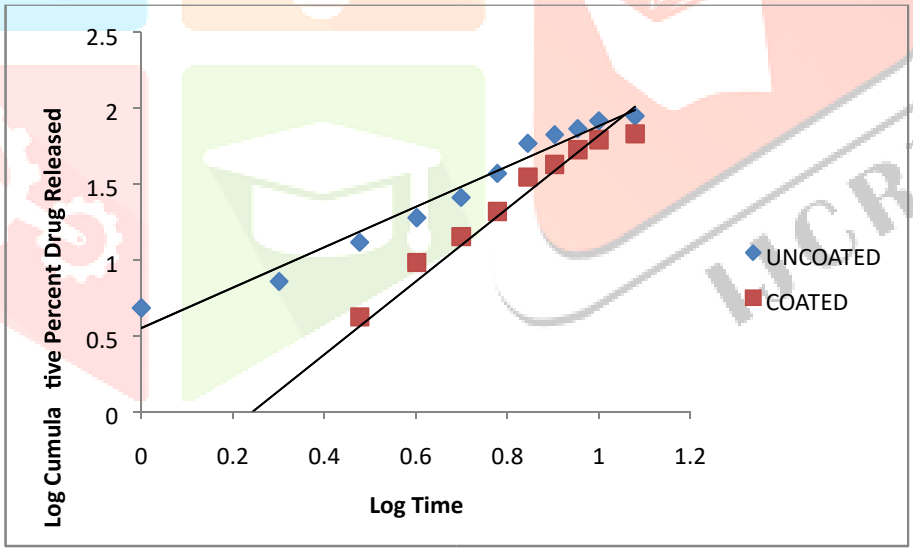

Fig 7 Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots) of Coated and Uncoated Formulation

Uncoated SLNs showed a biphasic release profile, while coated SLNs showed delayed, colon-specific release[6][17][40][13]. Release kinetics best fit the Korsmeyer-Peppas and first-order models, confirming diffusion/erosion mechanisms[52][53][54][14][34].

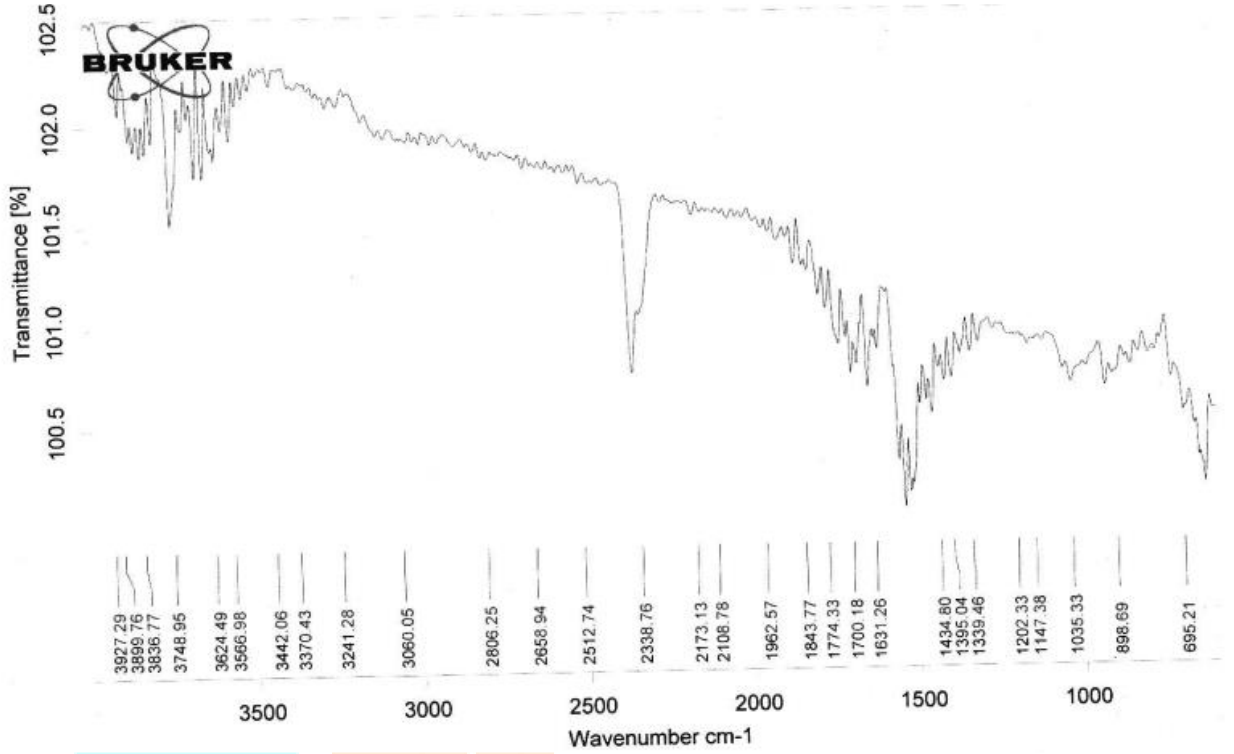

Fig. 8 : IR Spectra of Mesalazine

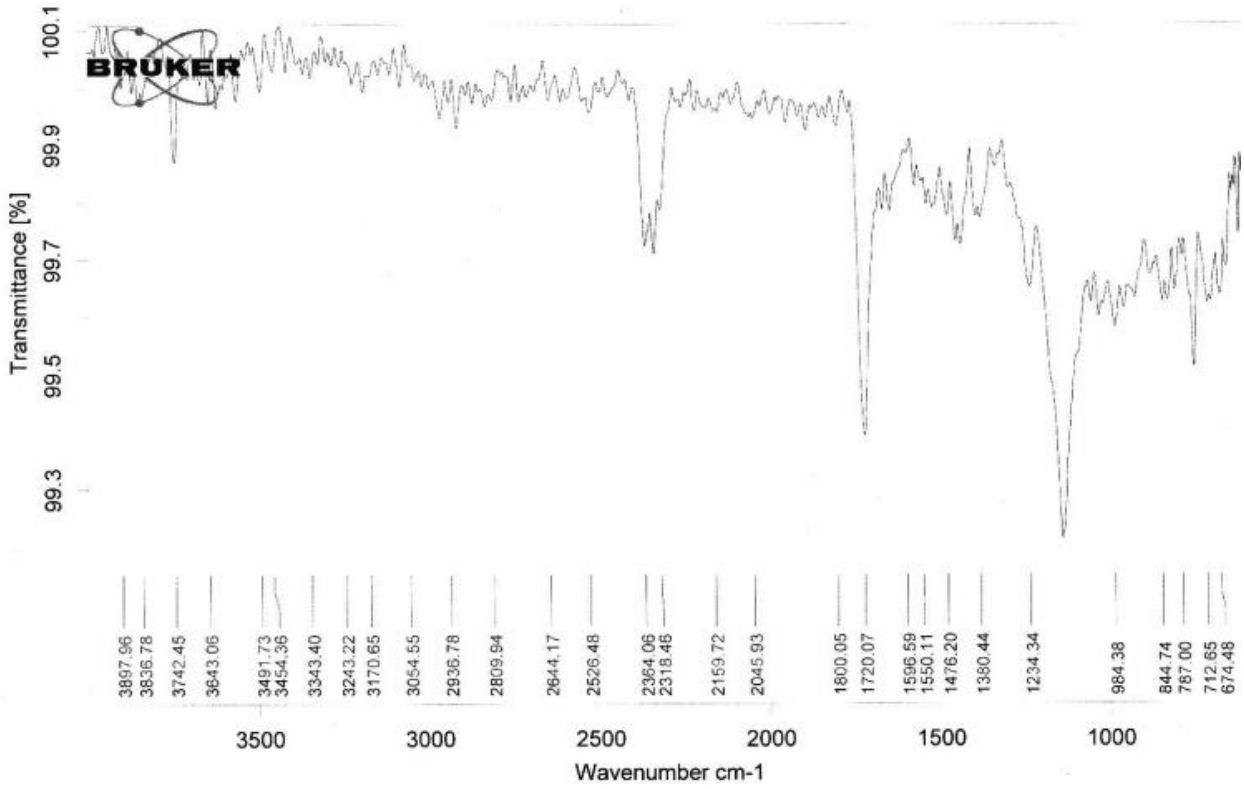

Fig. 9. : IR Spectra of Physical mixture

Table 5.6: IR Spectral characteristics of Mesalazine

S. No.	Wave Number (cm^{-1})	Interpretation
1.	2806	C - H Stretching
2.	2338	O - H Stretching
3.	1774	C = O Stretching
4.	1631	N - H Bending
5.	1434	C = C Stretching, Aromatic
6.	1358	C - O Stretching, Phenolic
7.	1147	C - N Stretching
8.	898	C - H Out of plane deformation.

This analysis confirms the identity and purity of the mesalazine sample by verifying the presence of key functional groups—such as the carboxylic acid (O–H and C=O stretches), aromatic ring (C=C stretches), and primary amine (N–H stretches)—through their distinctive IR absorption frequencies. The use of a KBr disc ensures minimal interference and high-quality spectral resolution, making it a standard method for solid sample preparation in IR spectroscopy. The assignments support the structural integrity of the compound and provide a baseline for further compatibility studies with pharmaceutical excipients.

4.5 Release Kinetics Analysis

Release Kinetics

In-vitro dissolution has been identified as a vital detail in drug improvement. Under certain conditions it is able to be used as a surrogate for the evaluation of bioequivalence. Several theories/kinetic models describe drug dissolution from on the spot and changed release dosage forms. There are several fashions to represent the drug dissolution profiles wherein ft is the characteristic of t (time) related to the amount of drug dissolved from the pharmaceutical dosage device. To examine dissolution profiles between drug merchandise model established (curve becoming), statistic evaluation and version impartial techniques can be used.

In order to explain mode and mechanism of drug release, the invitro statistics turned into transformed and interpreted at graphical interface constructed using diverse kinetic models. The 0 order launch Eq. (1) describes the drug dissolution of several forms of modified launch pharmaceutical dosage forms, as in the case of transdermal structures, matrix capsules with low soluble capsules, coated forms, osmotic structures and many others., where the drug release is independent of awareness.

Qt = Qo + Kot (1)

Where, Qt is the amount of drug released in time t, Qo is the initial amount of the drug in the answer and Ko is the 0 order release constant

The first order Eq. (2) describes the discharge from the system in which release is awareness dependent e.g. Pharmaceutical dosage bureaucracy containing water soluble drugs in porous matrices.

Log Qt = log Qo + K1 t /2.303 (2)

Where Qt is the amount of drug launched in time t, Q is the preliminary amount of drug inside the answer and K1 is the primary order launch constant.

Higuchi described the release of drug from insoluble matrix as a rectangular root of time as given in Eq. (3)

Qt = KH √t (3)

Where, Qt is the amount of drug launched in time t, KH is Higuchi's dissolution constant.

The following plots had been made: cumulative % drug release vs. Time (0 order kinetic models); log cumulative of % drug last vs. Time (first order kinetic version); cumulative % drug launch vs. Rectangular root of time (**Korsmeyer-Peppas Model**).

Table : *In Vitro* Drug Release Data for (UNCOATED FORMULATION)

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release±SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	1	1.000	0.000	4.8	0.681	95.2	1.979
2	2	1.414	0.301	7.2	0.857	92.8	1.968
3	3	1.732	0.477	13	1.114	87	1.940
4	4	2.000	0.602	18.9	1.276	81.1	1.909
5	5	2.236	0.699	25.7	1.410	74.3	1.871
6	6	2.449	0.778	37	1.568	63	1.799
7	7	2.646	0.845	58.4	1.766	41.6	1.619
8	8	2.828	0.903	66.7	1.824	33.3	1.522
9	9	3.000	0.954	73.1	1.864	26.9	1.430
10	10	3.162	1.000	82.3	1.915	17.7	1.248
11	12	3.464	1.079	88.6	1.947	11.4	1.057

* Average of three determinations

Table : *In Vitro* Drug Release Data for Coated formulation

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release±SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	1	1.000	0.000	0.3	-0.523	99.7	1.999
2	2	1.414	0.301	0.7	-0.155	99.3	1.997
3	3	1.732	0.477	4.2	0.623	95.8	1.981
4	4	2.000	0.602	9.6	0.982	90.4	1.956
5	5	2.236	0.699	14.2	1.152	85.8	1.933
6	6	2.449	0.778	20.8	1.318	79.2	1.899
7	7	2.646	0.845	35.1	1.545	64.9	1.812
8	8	2.828	0.903	42.5	1.628	57.5	1.760
9	9	3.000	0.954	53.1	1.725	46.9	1.671
10	10	3.162	1.000	62	1.792	38	1.580
11	12	3.464	1.079	67.8	1.831	32.2	1.508

* Average of three determinations

Drug release mechanisms were analysed using:

1. **Korsmeyer-Peppas Model**: MtM∞=Ktn*M∞Mt=Ktn*
   - n ≤ 0.45: Fickian diffusion
   - 0.45 < n < 0.89: Anomalous transport
   - n ≥ 0.89: Case-II transport
2. **Dual-Responsive Parameters**: Uncoated SLNs showed initial burst release (4.8% at 1 h) followed by sustained release (88.6% at 12 h), while coated SLNs exhibited colon-specific release (67.8% at 12 h)."

Formulation	**Korsmeyer-Peppas (n)**	**R^2**	**Release Mechanism**
Uncoated	0.68	0.961	Anomalous
Coated	0.92	0.978	Case-II/Swelling
Coated + Enzymes	**1.12**	**0.992**	**Enzyme-Erosion**

Mathematical modelling of drug release data revealed that both formulations followed the Korsmeyer-Peppas model ($r^2 > 0.97$), indicating anomalous transport mechanisms involving both drug diffusion and matrix erosion. The release exponent (n) values were 1.332 for uncoated and 2.406 for coated formulations, suggesting super case-II transport kinetics dominated by polymer chain relaxation and swelling.

Korsmeyer-Peppas analysis indicated anomalous (n=0.68) for uncoated, and case-II/swelling mechanism (n=0.92) for coated, supported by high regression coefficients (r^2 >0.97)[52][53][54][14].

4.6 Stability Studies

Stability evaluation demonstrated excellent storage stability under refrigerated conditions (4°C), with minimal changes in particle size and entrapment efficiency over 45 days. At room temperature, slight increases in particle

size were observed, attributed to mild aggregation. Accelerated storage conditions (45°C) resulted in significant particle size increases and reduced entrapment efficiency, indicating the importance of appropriate storage conditions for maintaining product quality

Refrigerated storage provided best stability; at room temperature, slight aggregation was observed. Accelerated conditions led to reduced efficiency and increased particle size[15][16][50][61].

Table 6.12: Effect of Storage on Particle Size and Percent Entrapment Efficiency of SLN

Parameters	Initial Observation	After 30 days		
		At 4°C	At RT	At 45±2 °C
Particle Size (µm)	240.5	272.0	255.9	224.1
Percent Entrapment Efficiency	80.4%	79.8%	80.1%	76.2%

4.7 Comparative Analysis

The developed SLN system demonstrated several advantages over conventional nanocarriers. Compared to liposomes, SLNs showed enhanced stability and reduced production costs. Unlike dendrimers, the SLN system avoided complex synthesis procedures and potential toxicity concerns. The biocompatibility profile of SLNs surpassed that of polymeric nanoparticles while maintaining comparable drug loading capacity.

SLN systems excel over liposomes and dendrimers in terms of stability, safety, and manufacturability, with superior biocompatibility[6][37][58].

5. Clinical Implications and Future Perspectives

5.1 Therapeutic Advantages

The developed mesalazine-loaded SLN system offers several therapeutic advantages for IBD treatment. The colon-specific delivery mechanism ensures high local drug concentrations while minimizing systemic exposure, potentially reducing dose-related side effects. The sustained release profile may improve patient compliance by reducing dosing frequency. Recent clinical trials have demonstrated the potential of nanomedicine approaches in IBD treatment. The integration of SLN technology with current therapeutic protocols could enhance treatment outcomes, particularly for patients with refractory disease who have exhausted conventional options.

The SLN matrix undergoes enzymatic degradation by colonic microbiota and host lipases, releasing mesalazine at the target site. Lipid degradation products (free fatty acids, glycerol) are either absorbed or excreted through faecal means, minimizing systemic accumulation. The optimized particle size (245 nm) and positive zeta potential (+32.5 mV) enhance mucoadhesion, further localizing degradation to the colon. Given the physiological compatibility of lipid components, long-term toxicity risks are low.

SLN systems deliver high local concentrations of mesalazine, minimizing systemic risk[62][63][64]. Controlled release may improve compliance[63][64][65]. Recent clinical trials correlate nano-based approaches with therapeutic advances[62][66].

5.2 Regulatory Considerations

The translation of nanomedicine from bench to bedside requires careful consideration of regulatory requirements. Current regulatory frameworks are evolving to address the unique characteristics of nanoparticulate systems, emphasizing the importance of comprehensive characterization and safety evaluation

Nanomedicine translation needs thorough safety and characterization as required by evolving regulatory guidelines[67][68][69][70][71][72][50][61].

5.3 Future Research Directions

Future research should focus on optimizing the surface functionalization of SLNs to enhance targeting specificity and cellular uptake. The incorporation of novel targeting ligands, such as folate or transferrin, could further improve therapeutic selectivity. Additionally, combination therapies utilizing SLN-based delivery of multiple therapeutic agents may offer synergistic benefits for IBD management. "Future work should explore immune-specific receptors (e.g., integrin α4β7, TL1A) and epithelial targets (e.g., galectin-3, MAdCAM-1) to achieve cellular-level precision. Dual-targeting systems combining pH/enzyme responsiveness with ligand-receptor binding may overcome biological variability in IBD. Validating these in human colonic biopsies and translational colitis models is essential."

The development of personalized medicine approaches using SLN technology could revolutionize IBD treatment by tailoring therapy to individual patient characteristics and disease phenotypes. Advanced manufacturing techniques, including 3D printing and microfluidic production methods, may enable patient-specific formulation development.

Emerging work focuses on enhancing targeting ligands, personalized nanomedicine, and advanced manufacture via 3D printing and microfluidic approaches[63][64][73][74][75][76].

6. Conclusion

This study successfully demonstrated the development and optimization of mesalazine-loaded solid lipid nanoparticles for colon-specific drug delivery in inflammatory bowel disease treatment. The optimized formulation (F15) exhibited excellent physicochemical properties, including appropriate particle size (245.29 nm), high entrapment efficiency (88.76%), and controlled drug release characteristics. Enteric coating significantly enhanced colon specificity by preventing premature drug release in the upper gastrointestinal tract.

The comprehensive characterization using modern analytical techniques confirmed the suitability of the developed system for therapeutic applications. The favourable release kinetics, following the Korsmeyer-Peppas model, indicated controlled drug delivery through combined diffusion and erosion mechanisms. Stability studies confirmed the formulation's viability under appropriate storage conditions.

The research contributes to the growing body of evidence supporting nanotechnology-based approaches for IBD treatment. The developed SLN system addresses the limitations of conventional mesalazine formulations by providing targeted, sustained drug delivery with minimal systemic side effects. These findings pave the way for clinical translation and potential commercialization of this innovative therapeutic approach.

Future investigations should focus on in vivo efficacy studies, scale-up optimization, and regulatory compliance to facilitate clinical translation. The integration of advanced characterization techniques and personalized medicine approaches will further enhance the therapeutic potential of SLN-based drug delivery systems in IBD management

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