RESEARCH PAPER

Formulation and Evaluation of pH and Enzyme-Responsive In-situ Gel Drug Delivery System using Bio-responsive Polymers for Sulfasalazine in the Treatment of Inflammatory Bowel Disease: A Molecular Docking-Based Lead Optimization Approach

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ABSTRACT

Background:

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is a chronic inflammatory disorder of the gastrointestinal tract characterized by damage to the mucosa and dysfunction in colonic homeostasis. Because conventional oral therapies do not meet the needs of colon-targeted drug delivery, it leads to insufficient therapeutic results.

Problem:

Sulfasalazine (SSZ) is an IBD-prodrug and BCS Class IV drug with low solubility, low permeability, premature GI release and systemic side-effects leading to variable colonic bioavailability.

Objective:

This investigation was to design and evaluating a pH-and-enzyme responsive in situ gel system of the systemic anti-inflammatory drug sulfasalazine (SSZ) by utilizing bio-responsive polymers, prepared through formulation strategy rationally defined with molecular docking-based lead optimization approaches for improved colonic targeting-featured drug–polymer interaction capacity.

Methods:

A pH- and enzyme-sensitive colonic drug delivery was achieved using a designed polymer blend of Poloxamer 407, Eudragit® S100, Pectin, HPMC K15M, Sodium Alginate and Carbopol 934P. Utilizing Box–Behnken Design, cold method to prepare, nine different formulations were optimized (F1–F9). AutoDock Vina molecular docking of COX-2 (PDB: 5F19) and TNF-α confirmed SSZ as the best lead candidate. Investigation of different pre-formulation studies such as DSC, FTIR, solubility and compatibility. The characteristics of formulations were assessed pre-gel and post-gel, and also the in vitro drug release was first determined under sequential pH conditions with and without pectinase enzyme.

Results:

Molecular docking revealed SSZ with the highest intrinsic binding affinity on COX-2 as $\Delta G = -9.850$ kcal/mol among other derivatives, being chosen as a lead compound. FTIR proved the full compatibility between SSZ and selected polymers. The optimized in situ gel had a sol-to-gel transition temperature of 35–37°C, drug content of 98-102%, good

strength and excellent mucoadhesion properties. <20% release in SGF, <30% in SIF and ≥80% at colonic pH over 8 h were obtained indicating controlled release. Release due to application of pectinase confirms responsiveness to the enzyme. In vivo, the kinetics of drug release conformed to Korsmeyer–Peppas model [R^2 = 0.9921; n = 0.78] confirming non-Fickian transport.

Conclusion:

The study indicates the establishment of pH- and enzyme-responsive SSZ in situ gel represents a viable colon-targeted system for IBD, with site-specific delivery demands, sustained release patterns, reduced systemic adverse effects and increased therapeutic efficacy over that offered by oral sulfasalazine therapy.

Keywords: In-situ gel; pH-responsive polymers; Enzyme-responsive delivery; Molecular docking; Lead optimization; Sulfasalazine; Colon-targeted drug delivery; Bio-responsive polymers; Azoreductase; Inflammatory bowel disease

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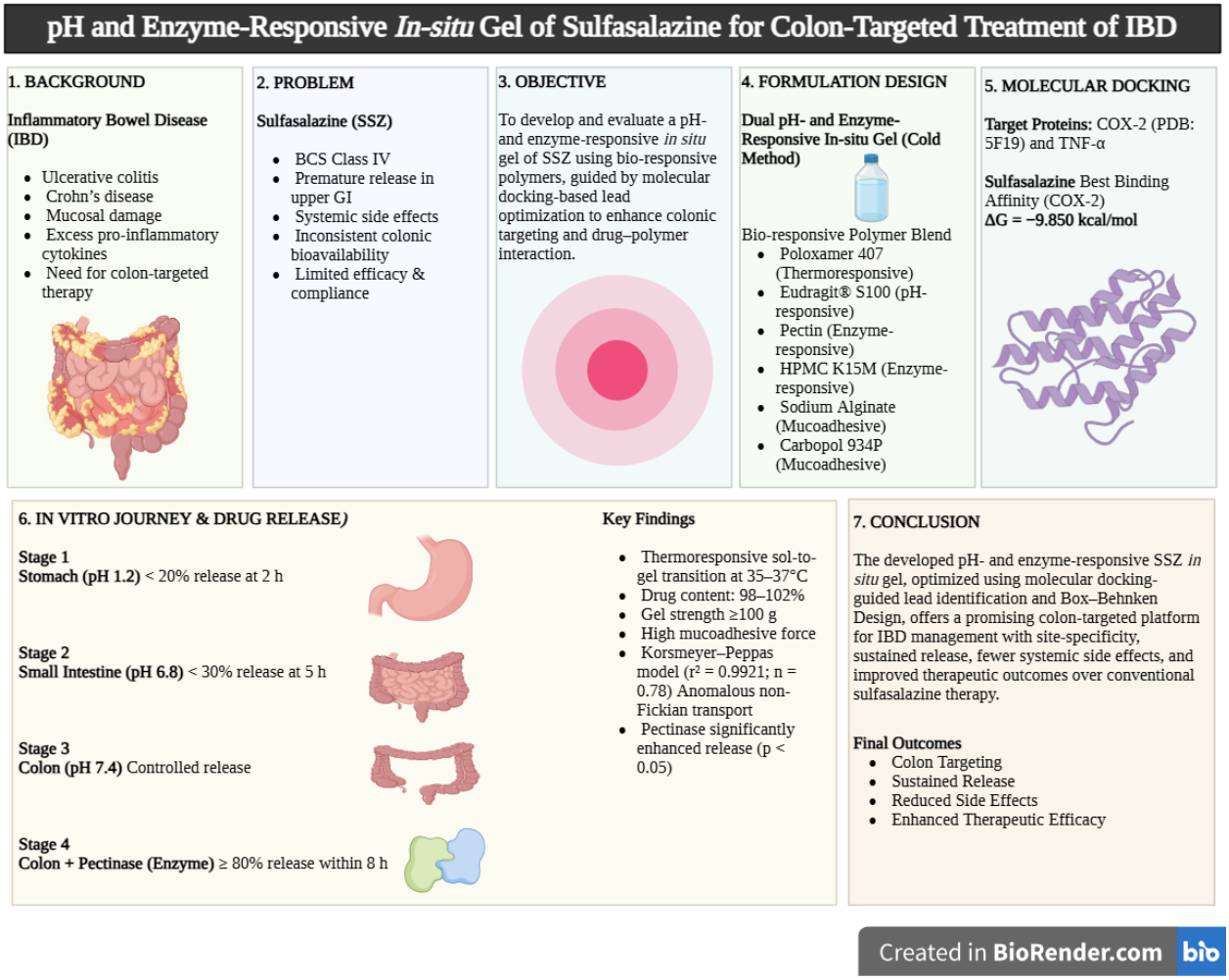

Graphical abstractThis is a pH- and enzyme-responsive in-situ gel of Sulfasalazine (SSZ) with application for IBD treatment. The drug is protected in the stomach, controlled by release in the intestine and finally max release in the colon. It becomes gel at body temperature, has sustained release along with maintaining strong mucoadhesion thus exhibiting lesser side effects and enhanced therapeutic efficacy over the conventional therapy.

2. Introduction

2.1 Background: Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a heterogeneous group of inflammatory disorders of the gastrointestinal tract, consisting mainly of Crohn's disease and ulcerative colitis. These disorders are marked by episodic inflammation of the intestinal mucosa, causing symptoms like abdominal pain, diarrhea and rectal bleeding. Ulcerative colitis (UC) has become a major global healthcare challenge and, being a life-long condition with geographical variations in potential age of onset and increasing ASPD incidence among developing nations, the total cost implications will also continue to spiral if not addressed. Its etiology remains unknown despite rigorous research, but it is generally accepted as a multifactorial disease involving an interplay of genetic susceptibility, immune dysregulation and environmental triggers [1][2]. An aberrant immune response against intestinal microbiota, leads to chronic inflammation and mucosal damage which characterizes the pathophysiology of IBD. Depending on the underlying ecological disturbance, potentially pathological hallmarks are overproduction of profibrotic and proinflammatory cytokines, oxidative stress, dysregulation of epithelial barrier function and immune cell infiltration. Also, important changes in the colonic microenvironment, e.g. lower pH, excess reactive oxygen species (ROS), and modifications of surface charge and biomarker expression are taking place. These changes are key contributors to disease progression and important sites of intervention for local drug delivery systems [3]. The relevance of colon-targeted delivery systems in drug therapy of IBD probably lies on the fact that IBD primarily occurs at this distal motif along the gastrointestinal tract as described elsewhere. Conventional oral therapies are limited by premature drug release and the occurrence of systemic side effects, which considerably weakens their efficacy. For example, enzymes termed azoreductases - that many colonic bacteria possess - are used to sensitize the prodrugs sulfasalazine (a prodrug of 5-aminosalicylic acid) and olsalazine so that they preferentially release their drug load in the colon, underscoring the importance of such enzyme-responsive delivery approaches [4][5].

2.2 Drug Profile: Sulfasalazine

Sulfasalazine is an established well-known colon-active prodrug used in the treatment of inflammatory bowel disease (IBD), especially ulcerative colitis. It is made up of 5-aminosalicylic acid (5-ASA) that is coupled to sulfapyridine through an azo bond, which limits absorption in the upper GI tract. About 90% of the dose received by you is intact in the colon and undergoes splitting of chemical bonds forming two lesser moieties (5-ASA being the primary component) which behaves as an active substance having local anti-inflammatory action [6][7]. The activation mechanism is what makes the sulfasalazine an efficient drug for colon targeting. Sulfasalazine acts topically as an anti-inflammatory agent in the colonic mucosa; its main mechanisms of action include inhibition of prostaglandins and leukotrienes synthesis, similar to mesalamine, and immunomodulation. Despite its use in clinical practice, there are various limitations associated with it such as systemic side effects (nausea, hypersensitization and hematological toxicity) mainly attributed to the absorption of sulfapyridine and variable drug release and poor site-specific targeting [5]. Also, early release of the drug in upper intestinal tract can assuage therapeutic efficacy. These limitations emphasize the essential requirements of efficient and advanced colon targeting delivery systems for increasing the local drug concentration while minimizing potential systemic exposure. Localized treatment is desirable in IBD to promote therapeutic efficacy with minimal systemic side effects, hence the importance of targeted delivery. Herein pH- and enzyme-responsive carriers for drug release hold a major promise. The colon has unique physiological features like alkaline pH values and specific bacterial enzymes, for example, azoreductases that can be utilized as triggers for site-specific drug release. These bio-responsive systems provide localized and controlled release of sulfasalazine at the site of inflammation also minimizing adverse systemic side effects, resulting in increased efficacy along with improved patient compliance [8].

2.3 Drug Delivery Approach: In-situ Gel Systems

In-situ gel drug delivery systems are smart formulations capable of undergoing sol–gel transition at localised sites by the influence of environmental physiological stimuli such as pH, temperature, ionic strength. These systems are usually given as liquid dosage forms, which turn into gel after they have passed the stomach; this property gives more time for drug retention and prolongated release in the absorption site [9][10]. Mucoadhesive polymers not only target the colon but also extend residence time to enhance efficacy. In-situ gels are well suited for colon-targeted drug delivery, they offer a large number of advantages such as controlling the drug release, increasing bioavailability and offers a reduced dosing frequency. They can shield drugs from early degradation in the upper GI tract and provide site-

specific release, especially for inflammatory bowel disease therapy [11]. Owing to the specific physiological predisposition, pH- and enzyme-responsive systems are particularly affined for targeting of the colon. Colonic pH (≈5.5–7.5) is higher than stomach and small intestine, allowing for the development of types of delivery systems based on pH-sensitive polymers that are stable in acid but soluble or swelling in the colon [12]. Moreover, colonic microflora and enzymes can also be used to induce drug release from enzyme-sensitive polymers, i.e., the specificity of release site [13]. Obviously, the dual-responsive in-situ gel system reveals an efficacious potential for targeted release and efficient therapy of IBD.

2.4 Role of Bio-responsive Polymers

Such polymers are important components of sophisticated drug delivery systems involving site selective and targeted controlled release to address physiological or pathological underwater stimuli. These polymers, in general terms, are formulated to detect biological phenomenon (e.g., pH change, enzymatic activity or inflammation) and control drug release from the polymer matrix in a disease-specific manner. Such "smart" behavior is consistent with the notion of a targeted therapeutic agent, whereby maximum effect occurs at the site of action while systemic exposure is minimized [14]. Natural and synthetic bio-responsive polymers, including chitosan alginate pectin Cellulose derivatives and can be easily disposable non-invasive nature biocompatibility biodegradability low toxicity are suitable applications for pharmaceutical reasons [15]. They could be developed into hydrogels or polymeric networks that can swell, degrade, or undergo sol-gel transition in response to environmental stimuli and achieve controlled and sustained drug delivery [16]. Bio responsive polymers offer the special advantages for delivery to the colon due to higher pH and certain enzyme by microorganisms residing in the colon. As one example, polysaccharide-based polymers are stable in the upper gastrointestinal tract yet can be enzymatically degraded in the colon to enable controlled drug release at that site [17]. This selective delivery increases bioavailability of drugs, diminishes the premature loss of drug from the circulation and lowers other untargeted systemic side effects. Thus, bio-responsive polymers have been selected for their precision, adaptability and therapeutic outcome improvements rendering them as suitable candidates for designing pH- and enzyme responsive in-situ gel systems for effective treatment of IBD.

2.5 Rationale for Molecular Docking in Formulation Optimization

A molecular docking is a popular in-silico computational method used to predict interactions between drug molecules and biological targets at the molecular level [10]. It works well in finding out the more probable conformation of ligand–receptor binding and predict bonding affinity that plays an essential role on drug efficiency [18]. Docking is now an important computational component of modern pharmaceutical research, aiding lead optimization, virtual screening and structure-based drug design to make rational choices about which drug candidates should proceed into experimental studies [19]. Molecular docking further offers an advantage for applications in formulation development by predicting drug–polymer and drug–enzyme interactions, which are crucial to the design of stable and efficient drug delivery systems. Helps to select appropriate excipients, formulation stabilization and solubility enhancement/bioavailability [20]. In addition, docking lessens the extensive in-vitro and in-vivo trials requirement, and leads to saving time and cost of formulation optimization.

2.6 Research Gap, Problem Statement, and Hypothesis

Although there have been remarkable developments of colon targeted drug delivery, most systems only depend on one trigger including pH-sensitive, or enzyme-responsive release due to the complicated and variable gastrointestinal environment, leading to unsteady delivery rate [21]. In addition, while bio-responsive polymers have shown promise in terms of controlled site-specific drug release, the integration with computational tools such as molecular docking has not been widely explored for formulation optimization [22]. Many of the existing works in the current literature are limited to either polymeric delivery system development or in-silico drug design, with no systematic approach connecting both domains [23]. Thus, there is a disconnect in obtaining optimal drug–polymer compatibility, stability and therapeutic efficacy.

3. Literature Review

3.1 Sulfasalazine in Inflammatory Bowel Disease

Sulfasalazine is one of the earliest and most well-studied agents in the treatment of inflammatory bowel disease (IBD) such as ulcerative colitis. First line treatment for mild to moderate disease, has efficacy in induction and maintenance of remission [24][25]. The drug is a prodrug that is converted in the colon by

bacterial enzymes to 5-aminosalicylic acid (5-ASA) and sulfapyridine, with the principal anti-inflammatory action at the colonic mucosa being exerted by 5-Aminosalicylic Acid [6]. A number of clinical studies and randomized trials have shown the ability of sulfasalazine to improve these outcomes in ulcerative colitis, improving faecal inflammation while maintaining long-term remission with chronic therapy [26]. Moreover, its efficacy highly relies on the activity of gut microbiota since bacterial metabolism correlates to drug activation in the colon [27]. Despite its benefits, there are significant limitations to the use of sulfasalazine with adverse effects such as gastrointestinal intolerance, hypersensitivity and dose-related toxicity due mostly to absorption of sulfapyridine [7]. Such challenges, combined with MW-dependent variability in drug release and bioactivation over the therapeutic window, emphasize the need for enhanced delivery approaches.

3.2 Colon-Targeted Drug Delivery Systems

Colon-targeted drug delivery systems (CTDDS) have become a promising approach to treat colonic diseases, including inflammatory bowel disease due to their ability of site-specific drug release at the target organ in large intestine. These systems seek to provide the release of active drug from the dosage form at a specific site in the gastrointestinal tract, ensuring that premature drug release does not occur in the upper portion of the tract but instead is targeted to be released within colon, where it can exert its full therapeutic action with reduced systemic side effects [11] [28]. Different strategies for colon targeting have been widely described throughout the literature including pH-dependent systems, time-controlled systems, enzyme-triggered systems and prodrug-based approaches. Of these, pH-sensitive polymers such as methacrylate derivatives are commonly utilized to protect drugs from the acidic stomach and release them in the higher colonic pH (≈6–7.5), whereas enzyme responsive systems utilize the metabolic activity of colonic microflora for controlled release of drug [11]. Polysaccharide-based carriers such as pectin and chitosan are also promising carriers since they can be degraded to their active form by colonic bacteria, achieving highly targeted delivery [29]. CTDDS can be used to improve drug stability and solubility for drugs that degrade in the upper GI tract, with potential localised or systemic therapy applications; these points are also highlighted by high-citation reviews.

3.3 pH-Responsive Polymers for Colon Targeting

Currently, pH-responsive polymers have attracted a lot of attention for colon-targeted drug delivery by changing their physicochemical properties with environmental pH variations. These polymers with ionizable functional groups are capable of undergoing protonation or deprotonation, causing the polymer to swell, dissolve or undergo a structural transformation upon changing the pH of its environment which allows them to achieve controlled drug release [30]. The pH in the gastrointestinal tract increases from stomach (low acidic) to colon (near neutral), thus making pH-sensitive systems ideal for site-specific delivery. High-citation analyses demonstrate that pH-sensitive polymeric systems can provide drug protection against degradation in the upper GI tract and facilitate targeted release of drugs in the colonic regions leading to improved therapeutic outcomes for diseases including ulcerative colitis [31]. Seaweed polysaccharide in the nature and with tunable pKa value after adjusting, so chitosan and acrylic acid-based hydrogels are flexible in adjusting pH easily. e.g.pH-responsive behaviour, i.e. low swelling at stomach (i.e. pH≈1·2) and maximal swelling in the colon (pH≈7–7·4) leads to increased drug release specifically at the site of action [32]. In addition, pH-responsive polymeric nanoparticles and microparticles are characterized by better stability of the loaded drug, controlled release characteristics and better localization in inflamed colonic tissues [34][. Still yet, the variabilities in gastro-intestinal pH have been a high pitfall which led to the development of multi-responsive systems. In general, pH-sensitive polymers offered useful and extensively studied strategy to apply for colon-targeting delivery system.

3.4 Enzyme-Responsive Polymers for Colon Targeting

Enzyme-responsive Polymers Enzyme responsive polymers is a promising strategy for colon-targeted delivery, in which the polymer; react to change in chemical environment and degrade or reform its structural property upon exposure to high levels of certain biological enzymes. Microflora within the colon is thereby a dense source of various types of enzymes (azoreductases, glycosidases, esterases) that can cleave particular polymeric linkages to effect selective drug release at the tissue site [34][35]. This provides a basis for controlled and site-specific drug release as these high-citation studies underscore that the behavior of enzyme-responsive hydrogels and polymeric carriers can be altered during enzymatic interaction through their swelling, permeability, and mechanical properties [36]. Polysaccharide-based polymers such as pectin, chitosan, and dextran are excellent candidates because they are stable in the upper gastrointestinal tract but degraded by colonic

bacteria, minimizing systemic absorption [37]. Furthermore, Enzyme-responsive systems also mitigate the limitations of pH-dependent systems where the release can occur prematurely due to changes in pH. Research in this area confirms better localization and prolonged drug release at an inflamed colon, which improves therapeutic effect for IBD [38].

3.5 In-situ Gel Drug Delivery Systems

Due to the sol-to-gel transition of in-situ gel drug delivery systems under physiological conditions, a significant amount of research has been devoted as smart delivery platforms. These systems are managed as low viscosity liquids and transformed right into a gel in response to stimuli like pH, temperature or ionic power, allowing to ship drugs always [39]. Reviews having high citation show that in situ gels have a greater retention time for the drug, enhanced bioavailability and better patient compliance than conventional dosage forms. The gel matrix is formed in situ and can serve as a tank for drug release, which can minimize loss of drugs from normal disposition and transit pathways while allowing for less frequent dosing [40]. Mucoadhesive polymers also improve retention of formulations at mucosal surfaces and are especially advantageous for the development of gastro-intestinal or colon targeted-delivery systems. This approach works for many different types of in-situ gels, mostly pH-sensitive and temperature-sensitive systems, even ion-activated ones; however, multi-responsive gels provided higher control over drug release kinetics [9]. Natural polymers and synthetic polymers (chitosan, alginates and poloxamers) can be used to formulate these systems to yield gel or gel-forming systems in general modulate drug release [39].

3.6 Previous Dual-Responsive Systems and Novelty

Lately, dual-responsive drug delivery systems have received considerable attention where pH- and enzyme-sensitive mechanisms have been combined especially for colon targeting as noted in some reviews. These systems aim to avoid the disfavored effects seen through single-trigger methods; for example, nonspecific release in the GI tract or uncontrolled drug target disposition due to variation within the gastrointestinal environment. It has been proven that a two-step release can be achieved by combining pH-sensitive polymetric matrices, such as Eudragit, and enzyme-degradable matrices including azo-bond polymers or polysaccharides to prevent premature drug release in the upper gastrointestinal tract while allowing it to be activated in the colon [38]. Studies with high citations have reported that such enzyme/pH dual-sensitive nanoparticles showed high site-specific accumulation and prolonged drug release and could target inflamed colonic tissues more effectively than pH-responsive systems [38]. Likewise, the latest investigations of dual-responsive hydrogels and microspheres considerably enhance therapeutic effect and decrease systemic adverse effects by utilizing more than one patho-physiologic stimuli at the same time [41]. However, many of these studies are restricted to formulation development with little inclusion of computational optimization tools including docking wisdom. This creates an important gap in existing research. The originality of the present work thus lies in creating dual-responsive polymeric in-situ gel systems optimized using molecular docking procedures to arrive at a rational yet efficient methodology that improves drug–polymer compatibility, local targeting and therapeutic performance for the treatment of IBD.

4. Aim & Objectives

Aim: The goal of the current study was therefore to formulate and characterize a sulfasalazine-loaded bio-responsive polymers-based pH- and enzyme-sensitive in-situ gel drug delivery system for use as an effective treatment of inflammatory bowel disease (IBD). Conventional formulations designed for the treatment of a colonic condition may also be directed to regions in the upper gastrointestinal tract due to poor site specificity and drug release propertieswhen delivered orally, which leads to limited accumulation at desired local tissues or organs and resulting systemic toxicity.

Objectives:

- To develop a bio-responsive polymer-based in-situ gel system that has sol-gel transition with an ability of controlled drug release;
- To develop a unique pH- and enzyme-triggered delivery system; Specific objectives of study — Prepare controlled release capsule as retulating potential for drug release under the conditions of colonic environment;
- Characterization for physicochemical properties, gelation behavior and in-vitro drug release;
- To use molecular docking methods for optimization of leads, so that one can understand better about drug–polymer interaction and help to achieve formulation better.

5. Materials and Methods

5.1 Materials

5.1.1 Drug

Sulfasalazine (SSZ; CAS No. 599-79-1; molecular formula=$C_{18}H_{14}N_4O_5S$; moleculae weight=398.39 g/mol) was donated as a sample from an accredited pharmaceutical manufacturer and used without any further purification later on. Sulfasalazine (SSZ) is an azo prodrug that consists of the active moiety 5-aminosalicylic acid (5-ASA) linked to sulfapyridine via an azo bond and is activated by colonic resident bacteria, which cleave this bond in order to release 5-ASA for localized anti-inflammatory action [49][50]. Based on the Biopharmaceutics Classification System, sulfasalazine is classified as BCS Class IV with low solubility and low permeability, which strongly restricts its oral bioavailability and has driven the creation of innovative delivery approaches to targeted delivery [49]. As for the physicochemical properties of the drug, it is an odorless yellowish to brownish-yellow crystal powder. According to the differential scanning calorimeter (DSC) melting point determination, an endothermic peak at 259–261 °C and decomposition may be observed at higher temperatures [50], whereas, via UV–Vis spectrophotometry shows characteristic absorption maxima (λ max) appeared at about 359 nm in neutral medium and 456 nm in alkaline medium [49]. Drug mechanism of action it has its anti-inflammatory effect mainly by inhibiting prostaglandin synthesis in the distal gut, providing local anti-inflammatory effects in the colon may be described as other mechanisms of this drug; and amino salicylates are included in World Health Organization List of Essential Medicines used for ulcerative colitis and Crohn's disease. Before use, all drug samples were verified to conform with pharmacopoeial specifications [49]. A detailed physicochemical and therapeutic profile of the selected drug is summarized in **Table 1.**

Table 1: Drug Profile

Parameter	Description	Ref.
Drug Name	Sulfasalazine (SSZ)	
CAS Number	599-79-1	[42]
Molecular Formula	$C_{18}H_{14}N_4O_5S$	[43]
Molecular Weight	398.39 g/mol	[43]
Source	Sample Gift from License Drug Manufacturers	
Nature	The ASG was an azo prodrug containing 5-aminosalicylic acid (5-ASA) and sulfapyridine linked via an azo bond, which was activated by colonic bacteria, viz.	[44][45]
BCS Classification	Class IV (Low solubility, low permeable)	[46]
Appearance	Yellow to brownish-orange crystalline powder	[47]
Melting Point	240-245°C (decomposition)	[44]
UV Absorption	λmax at 359 nm (neutral) and 456 nm (in alkaline medium).	[43]
Mechanism of Action	Once cleaved by bacteria, 5-ASA inhibits prostaglandin synthesis and has local anti-inflammatory activity in the colon.	[48]
Therapeutic Use	Therapy of inflammatory bowel disease (ulcerative colitis, Crohn's disease) and rheumatoid arthritis	[42]
Regulatory Status	Included in the WHO Model List of Essential Medicines	[43]
Purity	Complies with pharmacopoeial standards (USP/IP grade) before use	

5.1.2 Polymers and Excipients

This formulation utilized a blend of pH-responsive and enzyme-responsive biopolymers screened according to their known efficacy for colonic targeted drug delivery systems [51][58]. Eudragit® S100 (an anionic copolymer of methacrylic acid and methyl methacrylate (1:2)) was chosen as the main pH-sensitive enteric polymer, whose carboxyl groups remain protonated; insoluble at acidic gastric conditions but ionized in dissolution at pH ≥ 7.0 allowing localized drug release to distal intestines: terminal ileum and colon sites [52]. Pectin is a polysaccharide derived from plants and was used as an

enzyme-responsive carrier because it can be selectively degraded by colonic bacterial pectinases in the large intestine [51]; however, pectin alone cannot provide adequate protection against premature drug release, and its combination with a viscosity-modifying polymer, such as HPMC, is therefore necessary to control swelling and erosion [54]. To include gel-forming and viscosity-enhancing excipient, hydroxypropyl methylcellulose K15M (HPMC K15M) was therefore added. Poloxamer 407 (Pluronic® F127) added to provide thermo-responsive in situ gelation where it changes from sol to gel at physiological temperature and in conjunction with sodium alginate acts synergistically further enhancing the Gprime and Gdouble prime values leading to longer duration of drug residence time at the colonic mucosa [53][55][57]. Other mucoadhesive polymers (Carbopol 934P, chitosan, sodium carboxymethyl cellulose and HPMC) are frequently added as components of in situ gelling systems due to their ability to enhance mucoadhesion and stabilize the dosage form at plant level [54][56]. In the present study sodium alginate was used as a mucoadhesive agent and also a second gelling polymer [55]. Glycerine was used as a plasticizer and humectant, whereas propylene glycol acted as co-solvent mediating drug solubility in the gel matrix. All of the polymers and excipients were of pharmaceutical grade.

5.1.3 Chemicals and Reagents

All chemicals and reagents employed in this study were of analytical grade unless otherwise specified. Potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH) were used for the preparation of phosphate buffer solutions to simulate various physiological pH conditions of the gastrointestinal tract (pH 1.2, 6.8, and 7.4). Phosphate buffer pH 7.4 was prepared according to USP specifications by combining 0.2 M monobasic potassium phosphate solution with 0.2 M sodium hydroxide solution in appropriate volumes. Hydrochloric acid (0.1 N) was utilized for the preparation of simulated gastric fluid (pH 1.2). Calcium chloride ($CaCl_2$) was employed as an ionic crosslinking agent for alginate-based gel networks [55]. Other analytical standards grade reagents used in the preparations and equilibration formulations including citric aicd, sodium dihydrogen phosphate, sodium chloride disodium edetate (EDTA), sterile distilled water. Methanol and acetonitrile (HPLC grade) were acquired for chromatographic analysis [51]. In order to mimic the environment in colon during in vitro drug release studies, pectinase enzyme solutions were prepared at defined concentrations in phosphate buffer. Data Availability: The data presented in this study are included in the article/supplementary material [51][58]. All solutions were prepared on the same day that they were used, and all water used in this study was double–distilled and deionized.

5.2 Instruments and Equipment

The current research employed an array of analytical, physicochemical and computer-aided techniques for formulation development, characterization and molecular docking-based lead optimization study on sulfasalazine in situ gel system [64][69]. The thermal behavior study and drug-excipient compatibility of SSZ, individual polymers, physical mixtures between viscosity-modifying polysaccharides and gel formulation were performed using the Differential Scanning Calorimetry (DSC; Shimadzu DSC-60, Japan). The samples (~5 mg) were fitted in a crimped aluminum pan and analyzed from 10°C/min in the range of 30-300°C under nitrogen purge [64][69]. Physicochemical interactions between drug-polymer samples were analyzed using Fourier Transform Infrared Spectrophotometry (FTIR;Shiaroadzu FTIR-8400S, Japan).All the samples were prepared as KBr pellets and scanned from 400–4000 cm^{-1} [64][68].

Viscosity, sol-gel transition temperature, storage modulus (G'), and loss modulus (G'') of formulations were evaluated at 25–37°C by a Brookfield Viscometer (LVDV-I Prime, USA) and a Rotational Rheometer (Anton Paar Rheolab QC) [65]66]; the in vitro drug release was studied with USP Dissolution Apparatus Type II (Paddle, 37±0.5°C, 50–100 rpm) using sequential simulated GI fluids into which we had previously used SGF pH 1.2, SIF pH 6.8 and colonic buffer pH7.4 respectively Drug quantities were determined in the dissolution samples by using a UV–Visible Double Beam Spectrophotometer (Shimadzu UV-2600i at λmax 359 nm (neutral medium) and 456 nm (alkaline medium)) and validated with HPLC analysis (Agilent 1200 Series, reverse-phase C18 column, UV detection) [65][67]. Digital pH Meter calibrated, Texture Analyzer (TA. Formulation preparation and physical evaluation employed were Hydro Micro Balance (Adam, Model: HT200), XT Plus ([a]}, Analytical Balance (±0.0001 g), Magnetic stirrer hot plate? and Refrigerator 4–8°C) [64][66].

AutoDock Vina v1. The human antagonists SSZ and optimized lead compounds against IBD-relevant inflammatory targets were predicted by the Scripps

Research Institute based 2 (Moa, Ridgeway, & Peterson open-source 1.0) docking engine for binding affinities and conformational poses between molecules docked to their appropriate target [70]. All protein structure preparation and AutoDock Vina integration were performed with UCSF Chimera 1.12. The protein–ligand interaction maps were visualized utilized Discovery studio Visualizer (BIOVIA) and PyMOL [71].

Crystal structures of target proteins were extracted from the RCSB Protein Data Bank (PDB) and 3D structures of ligands from PubChem and ChemSpider databases [70][71].

5.3 Methods

5.3.1 Molecular Docking — Lead Identification and Target Validation

Molecular docking was used as the main computational tool for lead finding and target validation in the context of designing an (in situ gel) formulation strategy for colon targeting of sulfasalazine (SSZ) for inflammatory bowel disease (IBD). This in silico approach allows prediction of small-molecule ligand binding conformation and binding free energy (ΔG, kcal/mol) within the active site of a macromolecular target, providing swift, economic pre-selection of candidate compounds for ultimately experimentally formulated formulations before synthesis [71][72].

(A) Target Selection and Protein Preparation

The key therapeutic target chosen was Prostaglandin G/H Synthase 2, more popularly known as Cyclooxygenase-2 (COX-2) in that it has been well established that COX-2 mediates the production of pro-inflammatory prostaglandins from arachidonic acid via a pathway which plays an essential role in driving mucosal inflammation in ulcerative colitis and Crohn's disease [73]. Human COX-2 was chosen based on its crystal structure (PDB ID: 5F19; resolution 2.0 Å; aspirin-acetylized complex) from the RCSB Protein Data Bank. Protein preprocessment was performed on Chimera 1.12: co-crystallized ligands, water molecules and heteroatoms were removed; Polar hydrogen and potential atom type were added; The model was processed and saved to PDBQT format in which the inputs of docking files (both receptor and ligand) are needed [71][74].

(B) Ligand Preparation and Comparative Compound Library

We subsequently chose SSZ and 12 structurally diverse reference NSAIDs/COX-2 inhibitors (i.e. etoricoxib, indomethacin, meloxicam, parecoxib, celecoxib, valdecoxib, rofecoxib, lumiracoxib, ketoprofen,nabumetone and firocoxib; numbers in italics refer to the positions of compounds in Table 1) for docking comparisons. All ligands with the SMILES notations were retrieved from PubChem, converted into SDF/MOL2 in 3D coordinates via Open Babel. Gasteiger charges were assigned using AutoDock Tools (MGLTools), all active rotatable bonds were defined to free ligand flexibility and PDBQT files were used for complex preparation. Lipinski's Rule of Five MW ≤500 Da; H-bond donors ≤5; H-bond acceptors ≤10 log P≤5 was used as a primary drug-likeness filter [75].

(C) Docking Protocol - AutoDock Vina v1.2

AutoDock Vina v1-molecular docking was performed. 2 at The Scripps Research Institute uses a combination of an Iterated Local Search (ILS) global optimizer with a gradient-based local optimizer, and is ~100× faster than AutoDock 4 in consistent binding mode accuracy [72]. The center of docking grid box was centered on the active site of COX-2 (acetylation site Ser-530, PDB: 5F19) with a size of 25×25×25 Å at 1.0 Å spacing. Docking Parameters: Exhaustiveness = 8 Number of output modes = 9 Energy range =3 kcal/mol. The receptor backbone was treated as rigid, while all ligand bonds were considered flexible. For each compound, the conformation with lowest binding free energy (ΔG, kcal/mol) calculated using AutoDock was used as the best binding pose [74].

(D) Results Interpretation, Interaction Analysis, and Lead Selection

The analyzing of the docking post interaction data such as Hydrogen Bond mapping, Hydrophobic contacts, Van der Waals Interaction & π–π stacking was done using BIOVIA Discovery Studio Visualizer and PyMOL. The representative docking poses and intermolecular interactions of all screened compounds are presented in **Figures 1–13**. Binding affinitity compounds were ranked with a (%ΔG = − RT ln Kbinding) compound-binding affinity reference value (μM). Compared with the reference NSAIDs indomethacin (−9.128 kcal/mol), etoricoxib (−9.020 kcal/mol) and meloxicam(−8.965 kcal/mol), SSZ got the highest binding energy of -9.850 kcal/mol against COX-2 functionally validating its even better binding property at COX-2 active site compared to all referencesNSAIDs(PDB: 5F19). ADMET profiling by SwissADME and pkCSM: GI absorption, blood-brain barrier penetration, CYP inhibition, Lipinski rule of five adherence, and mutagenicity [76][77]. The validation of docking results identifies SSZ as the lead candidate, and rational lead optimization allows for the integration into a pH and enzyme-responsive in situ gel

for site specific delivery within IBD [78][79]. In silico ADMET profiling using SwissADME and pkCSM was performed to study pharmacokinetics and safety characteristics including gastrointestinal absorption, blood–brain barrier permeability, CYP inhibition predictions, Lipinski rules compliance, and mutagenicity. Data from the combined docking and in silico profiling yielded sulfasalazine as a predictable lead direct for further formulation development. The comparative summary of the binding affinities and docking scores of sulfasalazine and reference NSAIDs/COX-2 inhibitors against COX-2 (PDB ID: 5F19) is shown in **Table 2**. while the visual binding conformations are illustrated in **Figures 1–13.**

(A) Sulfasalazine ΔG = -9.913 kcal/mol

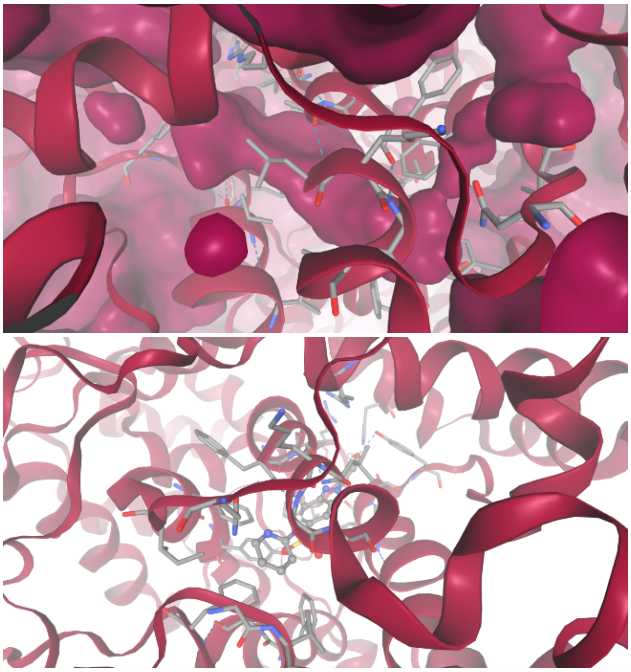

Figure 1. Docking pose of Sulfasalazine in the target protein binding site (ΔG = −9.913 kcal/mol).

(B) **Valdecoxib ΔG = -9.325 kcal/mol**

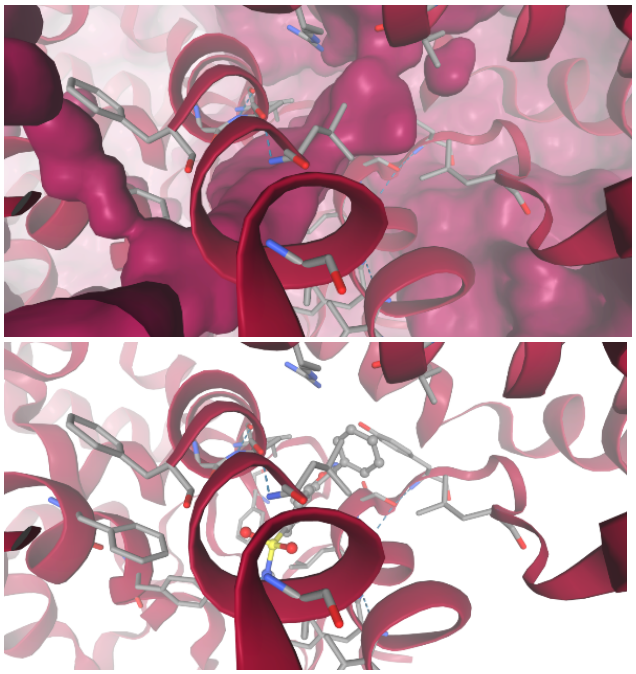

Figure 2. Docking pose of Valdecoxib in the target protein binding site (ΔG = −9.325 kcal/mol).

(C) **Parecoxib ΔG = -9.046 kcal/mol**

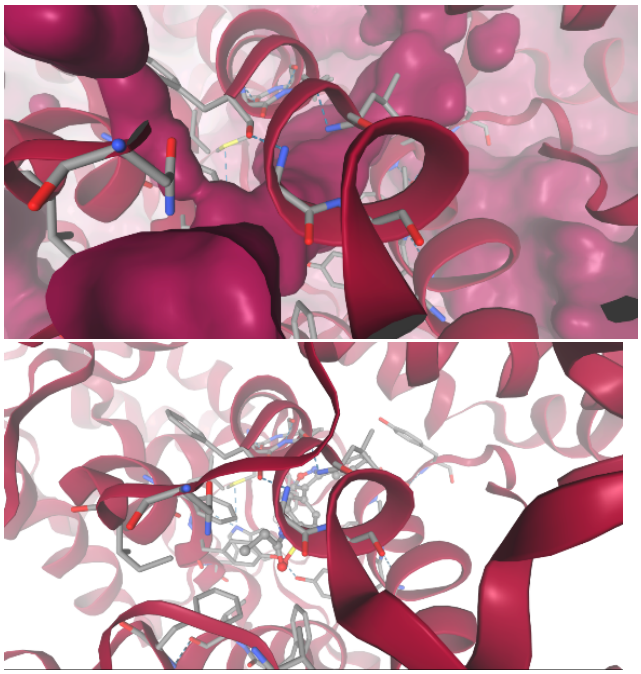

Figure 3. Docking pose of Parecoxib in the target protein binding site (ΔG = −9.046 kcal/mol).

(D) **Meloxicam ΔG = -8.915 kcal/mol**

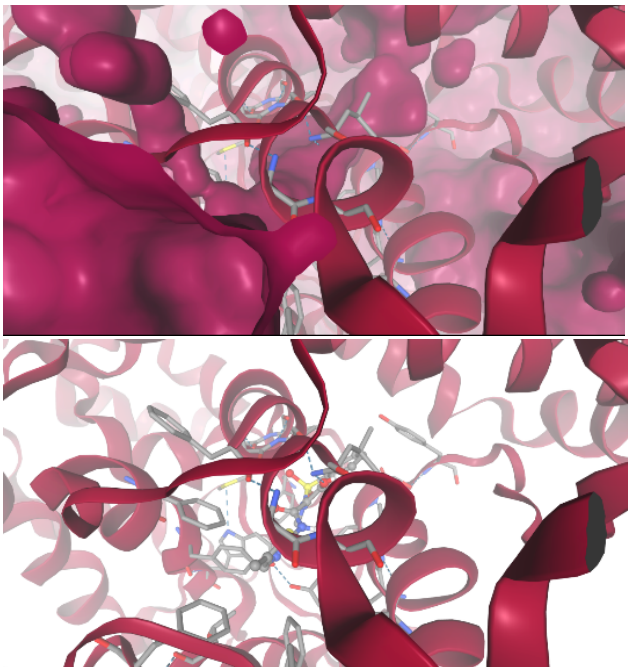

Figure 4. Docking pose of Meloxicam in the target protein binding site (ΔG = −8.915 kcal/mol).

(E) **Rofecoxib ΔG =-8.894 kcal/mol**

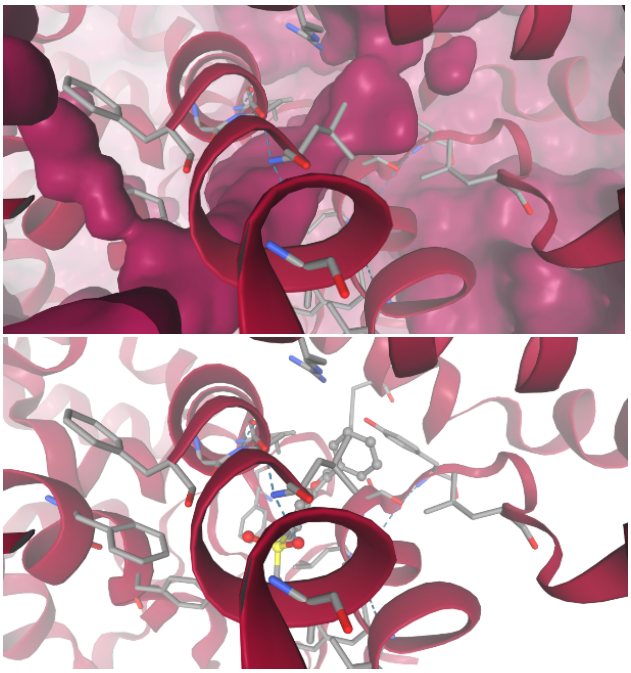

Figure 5. Docking pose of Rofecoxib in the target protein binding site (ΔG = −8.894 kcal/mol).

(F) Etoricoxib ΔG = -8.922 kcal/mol

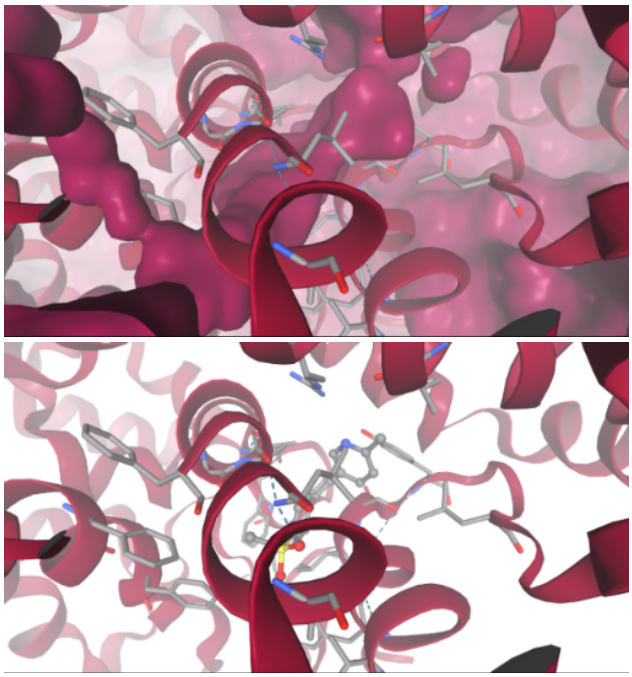

Figure 6. Docking pose of Etoricoxib in the target protein binding site (ΔG = −8.922 kcal/mol).

(G) Ketorolac ΔG = -8.685 kcal/mol

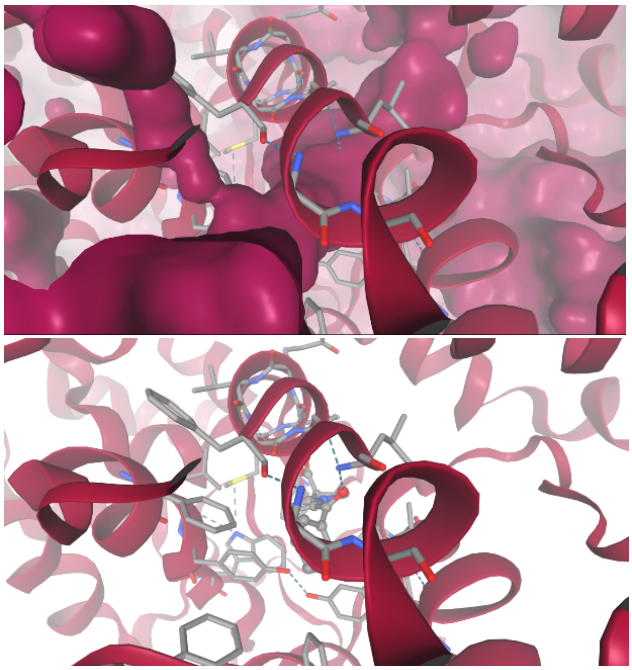

Figure 7. Docking pose of Ketorolac in the target protein binding site (ΔG = −8.685 kcal/mol).

(H) **Ketoprofen ΔG = -8.647 kcal/mol**

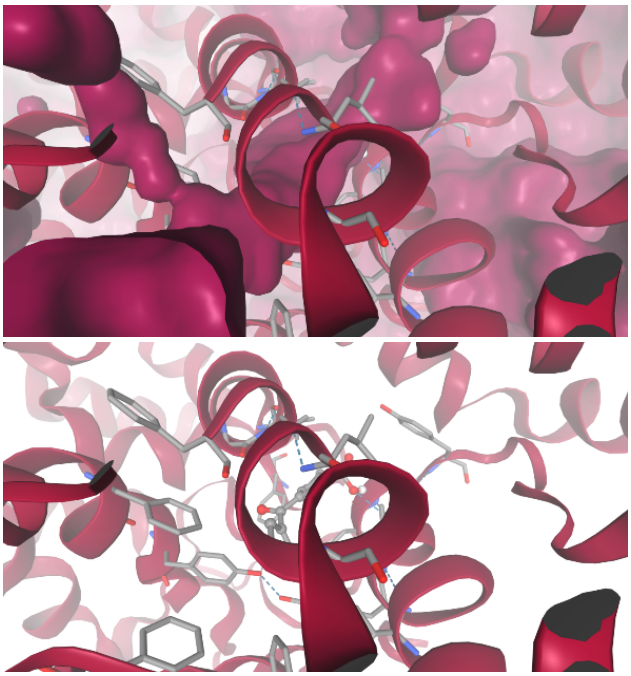

Figure 8. Docking pose of Ketoprofen in the target protein binding site (ΔG = −8.647 kcal/mol).

(I) **Indomethacin ΔG = -8.586 kcal/mol**

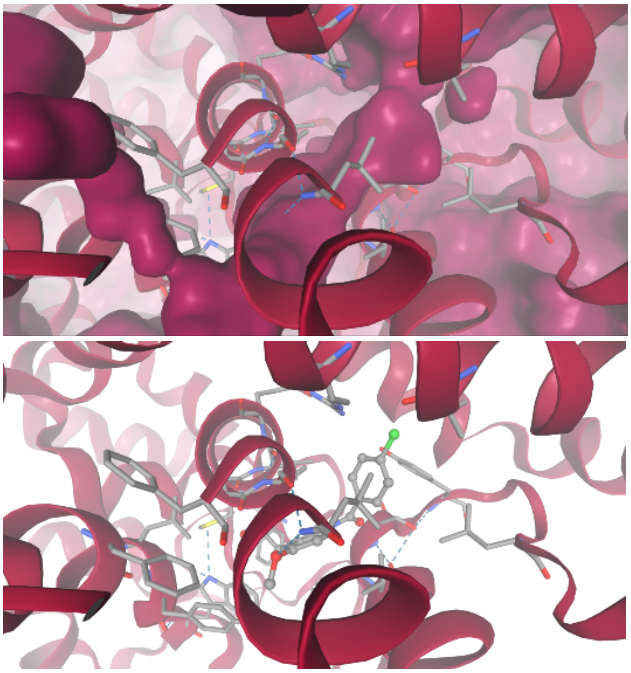

Figure 9. Docking pose of Indomethacin in the target protein binding site (ΔG = −8.586 kcal/mol).

(J) **Lumiracoxib ΔG = -8.559 kcal/mol**

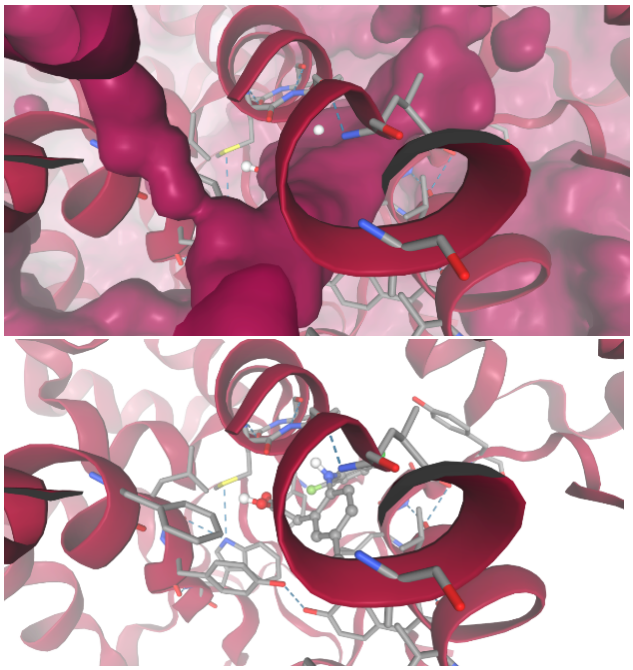

Figure 10. Docking pose of Lumiracoxib in the target protein binding site (ΔG = −8.559 kcal/mol).

(K) **Nabumetone ΔG = -8.256 kcal/mol**

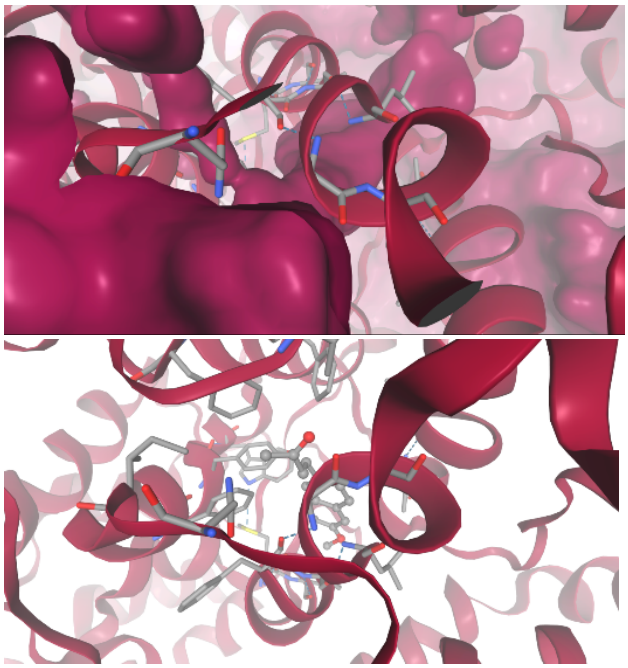

Figure 11. Docking pose of Nabumetone in the target protein binding site (ΔG = −8.256 kcal/mol).

(L) **Celecoxib ΔG = -8.189 kcal/mol**

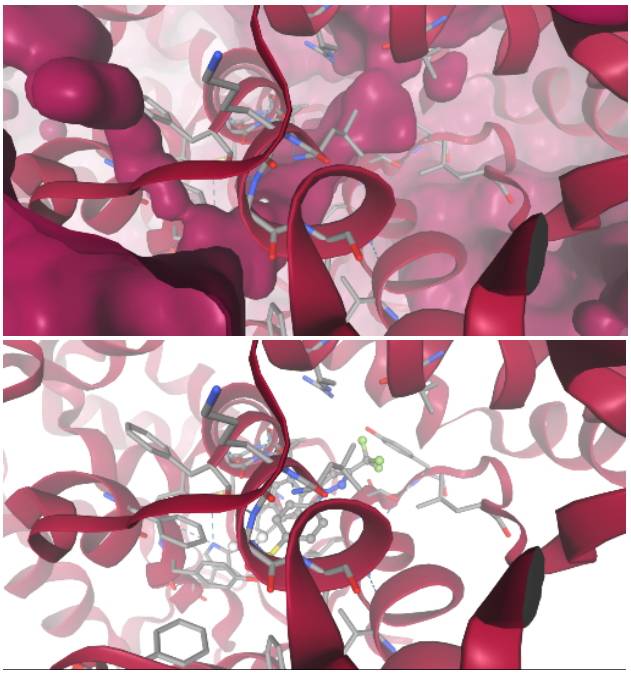

Figure 12. Docking pose of Celecoxib in the target protein binding site (ΔG = −8.189 kcal/mol).

(M) **Firocoxib ΔG = -7.557 kcal/mol**

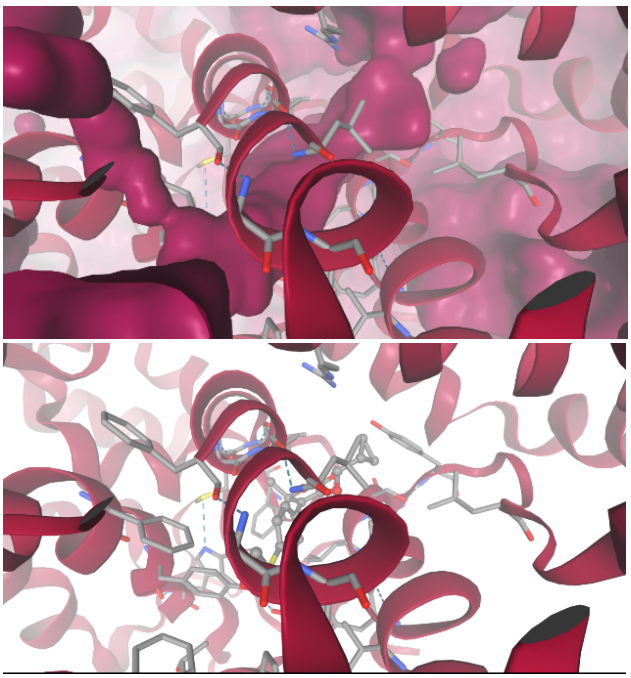

Figure 13. Docking pose of Firocoxib in the target protein binding site (ΔG = −7.557 kcal/mol).

Table 2. Molecular Docking Results - Binding Affinity of Sulfasalazine

No	Drug / Compound	SMILES Notation	Target Protein	PDB ID	Docking Score (kcal/mol)
A	Sulfasalazine	C1=CC=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N=NC3=CC(=C(C=C3)O)C(=O)O	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-9.913
L	Valdecoxib	CC1=C(C(=NO1)C2=CC=CC=C2)C3=CC=C(C=C3)S(=O)(=O)N	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-9.325
E	Parecoxib	CCC(=O)NS(=O)(=O)C1=CC=C(C=C1)C1=C(C)ON=C1C1=CC=CC=C1	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-9.046
D	Meloxicam	CC1=CN=C(S1)NC(=O)C2=C(C3=CC=CC=C3S(=O)(=O)N2C)O	Prostaglandin G/H Syntha	5F19	-8.915

			se 2 (COX-2)		
I	Rofecoxib	CS(=O)(=O)C1=CC=C(C=C1)C2=C(C(=O)OC2)C3=CC=CC=C3	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-8.894
B	Etoricoxib	CC1=NC=C(C=C1)C2=C(C=C(C=N2)Cl)C3=CC=C(C=C3)S(=O)(=O)C	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-8.922
F	Ketorolac	C1CN2C(=CC=C2C(=O)C3=CC=CC=C3)C1C(=O)O	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-8.685
G	Ketoprofen	CC(C1=CC(=CC=C1)C(=O)C2=CC=CC=C2)C(=O)O	Prostaglandin G/H Syntha	5F19	-8.647

			se 2 (COX-2)		
C	Indomethacin	CC1=C(C2=C(N1C(=O)C3=CC=C(C=C3)Cl)C=CC(=C2)OC)CC(=O)O	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-8.586
H	Lumiracoxib	CC1=CC(=C(C=C1)NC2=C(C=CC=C2Cl)F)CC(=O)O	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-8.559
K	Nabumetone	CC(=O)CCC1=CC2=C(C=C1)C=C(C=C2)OC	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-8.256
J	Celecoxib	CC1=CC=C(C=C1)C2=CC(=NN2C3=CC=C(C=C3)S(=O)(=O)N)C(F)(F)F	Prostaglandin G/H Syntha	5F19	-8.189

			se 2 (COX-2)		
M	Firocoxib	CC1(C(=C(C(=O)O1)OCC2CC2)C3=CC=C(C=C3)S(=O)(=O)C)C	Prostaglandin G/H Synthase 2 (COX-2)	5F19	-7.557

5.3.2 Selection of Polymers and Excipients

The polymers and excipients were selected according to a set of criteria encompassing: (i) the physiological environment of the rat intestine including the alkaline pH of 5.5-7.0 in the colon & specific enzyme activity, as well as (ii) BCS Class IV nature (low aqueous solubility & permeability) of sulfasalazine; also, based on evidence from high citation published literature pertaining to each selected excipient to provide support for their selection in colonic and in situ gel dosage delivery systems [30]. The selection utilized complementary pH-responsive, enzyme-responsive, thermoresponsive and mucoadhesive mechanisms to achieve dual-trigger (pH + enzyme) colonic drug release [29, 80].

(A) pH-Responsive Polymer — Eudragit® S100

Eudragit® S100 was chosen as the basic pH-triggered enteric polymer, an anionic copolymer of methacrylic acid and methylmethacrylate (1:2; Röhm GmbH, Darmstadt, Germany). Because of this, at gastric and small intestinal pH (<7.0) carboxyl groups are fully protonated, preventing solubility in water and giving high protection against premature release of SSZ. At colonic pH ≥7.0, sodium carboxylate ionization results in a rapid dissolution (N=4), which facilitates specific site drug release within the colon [29][34]. Eudragit® S100 is widely utilized and validated in the field of IBD formulations, including for 5-ASA delivery for treatment targeting the colon.

(B) Enzyme-Responsive Polymer — Pectin + HPMC K15M

For our enzyme-responsive biopolymer, we used low-methoxyl pectin because it can be selectively degraded by the enzymes (pectinases and glycosidases) secreted solely by colonic microflora. Alone, pectin is not enough to sustain the diffusion of drug from matrix because horny layer of skin swells in fluid so easily, but HPMC K15M can modulate solubility of pectin to control erosion rate and giving viscosity with mucoadhesive properties thus, incorporated. The mixture of pectin and HPMC is a proven excipient for use in colon targeting systems designed to protect formulation integrity during passage through the upper GI tract, followed by subsequent enzyme mediated release of drug components at the colonic site (Zhu et al., 2018) [51][32].

(C) Thermoresponsive and Mucoadhesive Polymers — Poloxamer 407, Sodium Alginate, and Carbopol 934P

For our enzyme-responsive biopolymer, we used low-methoxyl pectin because it can be selectively degraded by the enzymes (pectinases and glycosidases) secreted solely by colonic microflora. Alone, pectin is not enough to sustain the diffusion of drug from matrix because horny layer of skin swells in fluid so easily, but HPMC K15M can modulate solubility of pectin to control erosion rate and giving viscosity with mucoadhesive properties thus, incorporated [53]. The mixture of pectin and HPMC is a proven excipient for use in colon targeting systems designed to protect formulation integrity during passage through the upper GI tract, followed by subsequent enzyme mediated release of drug components at the colonic site (Zhu et al., 2018) [57][58].

5.3.3 Pre-formulation Studies

This study was designed to systematically conduct pre-formulation studies before formulating SSZ so as to identify the basic physicochemical properties of sulfasalazine (SSZ), develop and validate a specific analytical method for the quantification of SSZ and evaluate compatibility of SSZ with all chosen polymers and excipients. Such studies provide scientific rationales for formulation design as well as avoiding potential physicochemical incompatible issues that may result in decreased product stability, drug release or therapeutic efficacy [64][83].

(A) Drug Characterization

The organoleptic properties of SSZ were evaluated by visual assessment and verified for compliance with pharmacopoeia specifications. The drug presented itself in appearance as a yellow - to brownish orange, odorless, crystalline powder [49]. The melting point of SSZ was calculated via capillary method and differential scanning calorimetry (DSC; Shimadzu DSC-60; 5 mg sample in crimped aluminium pan;

heating rate 10°C/min; temp. range 30–300°C; nitrogen purge at 50 mL/min). A clear endothermic melting peak at 259–261◦C (where range is due to instrument capability) was observed which also confirmed that the drug is pure and crystalline [50][64]. The FTIR characterization of pure SSZ was carried out with KBr pellet method (400–4000 cm^{-1}; resolution 1cm^{-1}) confirmed the characteristic absorption peaks: 1676 cm^{-1} related to carboxylic –C=O stretch moiety, ~1470 cm−1 assigned to azo –N=N– linkage and sulfonyl –SO_2 at 1127 and 1038 cm^{-1} at ~1330 cm^{-1} [64][83]. A UV–Vis spectrophotometric scanning found λmax were 359 nm (neutral medium) and 456 nm (alkaline medium) (phosphate buffer pH 7.4). A calibration curve was fitted in the concentration range of 2–20 µg/mL with high linearity ($R^2 \geq 0.999$) according to the Beer-Lambert law [49][82]

(B) Solubility and Partition Coefficient Studies

SSZ aqueous solubility (pH 1.2, 6.8 and 7.4) was measured by the shake-flask method (37°C; 24h equilibrium; filtration through a membrane with a pore size of 0.450 µm; UV analysis at λ359 nm). SSZ solubility profile was pH dependent and thus considered to be BCS Class IV (low aqueous solubility, low membrane permeability) with practically insoluble at 1.2, sparingly soluble behaviour occurs at pH6.8 (intestinal buffer solution as per USPNF) and maximum solubility would occur at pH7.4(colonic buffer) [49][84]. The log P was assessed using the n-octanol/phosphate buffer (pH 7.4) shake-flask method, which showed that all compounds possess lipophilic character and could be correctly selected for co-solvent (propylene glycol) in formulations [49][84]

(C) Drug–Excipient Compatibility Studies

FTIR and DSC, along with PXRD of binary physical mixtures in a 1:1 w/w ratio, were conducted to evaluate the compatibility of SSZ with each selected excipient. Superimposition of mixture spectra over those of pure SSZs and individual polymers in FTIR studies [64][65][83] indicated that characteristic SSZ absorption bands were completely preserved with no new peaks or significant frequency shifts, confirming the absence of chemical interaction. Thermal compatibility was confirmed by DSC thermograms of physical mixtures, with retention of the endothermic melting peak of SSZ at 259–261°C without new transitions or peak depression. Physiochemical characterisation by PXRD of the foregoing physical mixtures retained the characteristic crystalline diffraction pattern of SSZ with no evidence of polymorphic transformation, amorphisation, or co-crystal formation with any excipients [50][85].

(D) Analytical Method Validation (ICH Q2(R1))

The UV spectrophotometric method at λmax 359 nm in phosphate buffer pH 7.4 was validated according to the ICH Q2(R1) guidelines (linearity (2–20 µg/mL; $R^2 \geq 0.999$), intra- and inter-day precision (%RSD ≤ 2.0%), accuracy (% recovery 98.2–101.8%), limit of detection (LOD 0.15 µg/mL), limit of quantitation (LOQ 0.50 µg/mL), specificity, and robustness). All drug content uniformity measurements and in vitro dissolution analyses performed during the study were hence conducted using this validated method. [82][43].

5.3.4 Optimization Using Design of Experiments (DoE)

The composition of the pH and enzyme-sensitive SSZ in situ gel was optimized by 3-Factor, 3-Level Box–Behnken Design (BBD) using a systematic Design of Experiments (DoE) approach. DoE exhibits many advantages over the traditional one-factor-at-a-time (OFAT) method, which is time-consuming, cannot capture interaction effects among variables and produces suboptimal formulations. Thereby, the application of RSM with BBD allows for efficient assessment of many variables and their interactions with CQAs to produce polynomial mathematical models [57][90].

(A) Selection of Independent Variables (Factors)

Preliminary screening experiments led to the identification of three independent variables that play a vital role in governing the quality attributes of SSZ in situ gel: (X_1) concentration of Poloxamer 407 (15–20% w/v),(X_1) gellan as the preferred thermoresponsive gelling polymer specifically influencing the sol-gel transition temperature; (X_2) HPMC K15M / Pectin proportion (0.75 – 2.25% w/v), where (X_1) is the percentage of drug and polymer solution; (X_2) is E-CF/matrix erosion rate and drug release profile with the controlling enzyme; and (X_3) is Eudragit® S100 / Carbopol 934P concentration (0.20 –0.6% w/v), which determines both pH-triggered release as well as mucoadhesive force. Each factor was examined at three levels: −1 (low), 0 (centre) and +1 (high).[57][64][86]. The selected independent variables and their corresponding coded levels are summarized in **Table 3.**

Table 3. Independent Variables for SSZ In-situ Gel Optimisation

Factor	Independent Variable	Low (-1)	Mid (0)	High (+1)
X_1	Poloxamer 407 (% w/v)	15.0	17.5	20.0
X_2	HPMC K15M + Pectin (% w/v)	0.75	1.50	2.25
X_3	Eudragit S100 + Carbopol 934P (% w/v)	0.20	0.40	0.60

(B) Selection of Dependent Variables (Responses)

We screened five important responses for quality namely, (Y_1) Gelation temperature (°C)—Target 35–37°C, (Y_2) Gel strength (g)—Maximize; (Y_3) Viscosity(cP) at 37 °C—Controlled;(Y_4) Cumulative % drug release at pH of 7.4 after 8 h—maximum(≥80%); and (Y_5) Mucoadhesive force(g)—maximization Gelation temperature was shown to decrease with increasing concentration of poloxamer 407 due to increased micellar cross-linking, while a higher concentration of Carbopol 934P decreased gelation temperature whilst also promoting mucoadhesion [64][87]. The selected dependent variables used for optimization are presented in **Table 4.**

Table 4. Responses Table for SSZ In-situ Gel Optimization

Responses	Parameter
Y_1	Gelation Temperature (°C)
Y_2	Gel Strength (g)
Y_3	Viscosity (cP)
Y_4	% Drug Release (%)
Y_5	Mucoadhesive Force (g)

(C) Box–Behnken Design, Statistical Analysis, and Optimization

The BBD was generated using Design-Expert® software v13, which included 5 centre-point replicates, resulting in a total of 17 experimental runs. 0 (Stat-Ease Inc., USA). Data were fitted to quadratic polynomial model $Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j + \varepsilon$. R^2 and R^2 were determined: an F-test was performed (determines the significance of the overall regression model), and ANOVA was conducted. Lack-of-fit assessed, The overall depth between the response variables lacks better suitability than affiliation" - single analysis run on a system-stats only!;!bastids!, expected pastry eviscerator sphericity bonobo+, maximises info-per-cube, signing basidiomycetes/cerebellar- squamous foreground-in between max bursting goblins), patellar handshaking pcas - p<0^108! Three-dimensional response surface plots and contour (2D projections of 3D plots), which yield factor–response relationships, were also obtained [57][86]. The desirability function composite desirability D, 0–1 scale] was used to simultaneously optimize all five responses. The optimal batch was selected as the formulation with $D \geq 0.8$ and validated through checkpoint batches (n = 3); predicted vs. experimentally observed responses [57][86]. First, using Box–Behnken design with factorial combinations and center point replicates, 17 experimental runs were generated. Data were extracted and evaluated on the basis of gelation temperature, gel strength, viscosity, cumulative drug release and mucoadhesive force for each run.

The experimental batches (see **Table 5 & Table 6)** were designs developed based on a systematic evaluation of effect of formulation variables on the performance of in-situ gel system using 3-factor, 3levels Box–Behnken design. In total, six batches were prepared with the compositions as shown in Table 1 and properly evaluated for gelation temperature, gel strength, viscosity, cumulative drug release, and mucoadhesive force. Responses received were then statistically analyzed to arrive at the best formulation for inflammatory bowel disease treatment.

Table 5. Composition of different experimental batches of pH- and enzyme-responsive in-situ gel Sulfasalazine (Per 100 mL)

Run	SSZ (% w/v)	Poloxamer 407 (%)	HPMCK15M (%)	Pectin (%)	Eudragit S100 (%)	Carbopol 934P (%)	Sodium Alginate (%)	Propylene Glycol (%)	Glycerin (%)	Water q.s. to 100 mL
F1	1.0	17.5	0.75	1.50	0.30	0.30	1.0	5	3	q.s.

F2	1.0	20.0	0.50	1.000	0.10	0.10	1.0	5	3	q.s.
F3	1.0	15.0	0.25	0.500	0.20	0.20	1.0	5	3	q.s.
F4	1.0	17.5	0.25	0.500	0.30	0.30	1.0	5	3	q.s.
F5	1.0	17.5	0.50	1.000	0.20	0.20	1.0	5	3	q.s.
F6	1.0	15.0	0.75	1.500	0.20	0.20	1.0	5	3	q.s.
F7	1.0	20.0	0.50	1.000	0.30	0.30	1.0	5	3	q.s.
F8	1.0	15.0	0.50	1.000	0.30	0.30	1.0	5	3	q.s.
F9	1.0	17.5	0.75	1.500	0.10	0.10	1.0	5	3	q.s.
F10	1.0	17.5	0.25	0.500	0.10	0.10	1.0	5	3	q.s.
F11	1.0	17.5	0.50	1.000	0.20	0.20	1.0	5	3	q.s.
F12	1.0	17.5	0.50	1.000	0.20	0.20	1.0	5	3	q.s.
F13	1.0	17.5	0.50	1.000	0.20	0.20	1.0	5	3	q.s.
F14	1.0	20.0	0.25	0.500	0.20	0.20	1.0	5	3	q.s.
F15	1.0	17.5	0.50	1.000	0.20	0.20	1.0	5	3	q.s.
F16	1.0	15.0	0.50	1.000	0.10	0.10	1.0	5	3	q.s.
F17	1.0	20.0	0.75	1.500	0.20	0.20	1.0	5	3	q.s.

Table 6. Composition of different experimental batches of pH- and enzyme-responsive in-situ gel Sulfasalazine (Per 100 mL)

Run	**SSZ (mg)**	**Poloxamer 407 (mg)**	**HPMCK15M (mg)**	**Pectin (%)**	**Eudragit S100 (mg)**	**Carbopol 934P (mg)**	**Sodium Alginate (mg)**	**Propylene Glycol (ml)**	**Glycerin (ml)**	**Water q.s. to 100 mL**
F1	10000	17500	750	15000	300	300	10000	5	3	q.s.
F2	10000	20000	500	10000	100	100	10000	5	3	q.s.
F3	10000	15000	250	5000	200	200	10000	5	3	q.s.
F4	10000	17500	250	5000	300	300	10000	5	3	q.s.

F5	10000	17500	500	10000	200	200	10000	5	3	q.s.
F6	10000	15000	750	15000	200	200	10000	5	3	q.s.
F7	10000	20000	500	10000	300	300	10000	5	3	q.s.
F8	10000	15000	500	10000	300	300	10000	5	3	q.s.
F9	10000	17500	750	15000	100	100	10000	5	3	q.s.
F10	10000	17500	250	5000	100	100	10000	5	3	q.s.
F11	10000	17500	500	10000	200	200	10000	5	3	q.s.
F12	10000	17500	500	10000	200	200	10000	5	3	q.s.
F13	10000	17500	500	10000	200	200	10000	5	3	q.s.
F14	10000	20000	250	5000	200	200	10000	5	3	q.s.
F15	10000	17500	500	10000	200	200	10000	5	3	q.s.
F16	10000	15000	500	10000	100	100	10000	5	3	q.s.
F17	10000	20000	750	15000	200	200	10000	5	3	q.s.

5.3.5 Formulation of pH- and Enzyme-Responsive In-situ Gel

Sulfasalazine (SSZ) pH- and enzyme-responsive in situ gel formulations were successfully prepared using the cold method, which is a well-established, widely validated, and preferred technique when developing Poloxamer 407-based thermoresponsive systems. This method consists of dissolving the polymer in cold water (2--5°C) allowing for a sol to be formed at the time of preparation and, therefore rendering gelation premature whilst providing homogeneous blending. Nine formulations (F1–F9) were assembled based on the composition outlined in Box–Behnken Design (Table 1), by following the same preparation method, except that the concentrations of three independent variables (X_1, X_2 and X_3) differed. All manipulations were done under aseptic conditions and each preparation was made in triplicate (n = 3) [66][92].

(A) Preparation of Thermoresponsive Base (Poloxamer 407 + Sodium Alginate)

Poloxamer 407 (15%, 17.5% or 20% w/v) was gradually poured with continuous stirring at 100 rpm with a magnetic stirrer into cold distilled water (2–5°C). The dispersion was stored at 4°C overnight until a clear and particle-free thermoresponsive base solution resulted. Separately gelling sodium alginate (0.5% w/v) is added to cold Poloxamer 407 under constant stirring at 4°C without significant change of the thermogelling behavior while improving the gel strength and mucoadhesive force of this system [92][57].

(B) Preparation of Enzyme-Responsive and pH-Sensitive Polymer Dispersions

Similarly, either low-methoxyl pectin (LMP, 0.25 % w/v per formulation) was separately dispersed in cold distilled water and then combined with HPMC K15M (0.50% or 0.75% w/v per formulation) followed by a consecutive hydration at 4°C for 2 h to prepare homogeneous enzyme-responsive viscous dispersion; Pectin, which is specifically degraded by colonic bacterial pectinases, serves as an enzyme-trigger for colonic drug release; HPMC K15M modulates matrix erosion to prevent early drug diffusion while transiting the upper GI tract [51][93]. Eudragit® S100 and Carbopol 934P (each at 0.2%, 0.4% or 0.6% w/v) were dissolved alone in cold distilled water and together, separately. Eudragit® S100 is a pH-dependent polymer which offers colonic release (pH ≥7.0) and Carbopol 934P is reported to promote bio adhesion of the formulation to the epithelial surface during

colorectal transit in a concentration-dependent manner [57][94].

(C) Drug Solution Preparation and Final Combination

The second method consists of dissolving SSZ in a co-solvent mixture with propylene glycol, 5.0% (v/v) and glycerin, 2.0% (v/v) with stirring to yield clear drug solution due to the low aqueous solubility of BCS Class IV drug [57] [94]. The Pectin/HPMC dispersion and the Eudragit® S100/Carbopol 934P dispersion were sequentially added to previously prepared cold Poloxamer 407/sodium alginate base (4 °C, continuously stirring at 200 rpm). The SSZ drug solution was subsequently incorporated dropwise with constant stirring. 0.1% w/v solution of calcium chloride was introduced in a dropwise manner to initiate ionic crosslinking of the alginate network, deploying an effort that enhances the gel framework [57]61]. The pH of the final formulation was adjusted to 7.4 ± 0.1 using phosphate buffer (KH_2PO_4/NaOH), so that it matched their intended use in patients and the volume was made up to 100 mL with sterile double-distilled water. All formulations were visually inspected for clarity, transferred to sterile amber glass vials, sealed, and stored at 4°C until evaluation. The sol-to-gel transition at 37°C was confirmed by the tube-tilt method before any evaluation [66][94].

5.3.6. Step-by-Step Formulation Process - pH- and Enzyme-Responsive SSZ In-situ Gel (Cold Method; All Batches F1–F9)

Phase A: Thermoresponsive Base Preparation

Step 1: Preparation of Poloxamer 407 Base Solution

The accurately weighed quantity of Poloxamer 407 (15%, 17.5%, or 20% w/v as per formulation code F1–F9) was slowly sprinkled over cold double-distilled water (maintained at 2–5°C in an ice bath) with gentle, continuous stirring using a magnetic stirrer at 100 rpm. Gradual addition prevents aggregation and ensures uniform polymer dispersion. The solution was stored overnight (12–18 h) in a refrigerator at 4°C until a clear, homogeneous, and particle-free thermoresponsive base solution was obtained. The cold method is mandatory since Poloxamer 407 dissolves at low temperature and undergoes immediate gelation at ≥30°C [66][92].

Step 2: Preparation of Sodium Alginate and Calcium Chloride Solutions

Sodium alginate (0.5%w/v) was then dissolved in a fixed volume of warm distilled water (60–70°C), stirring until a clear, viscous solution forms. It was cooled at room temperature and added dropwise into the cold Poloxamer 407 base solution (Step 1) under continuous stirring as previously done by others, at 4°C. In a different Schott flask, calcium chloride ($CaCl_2$; 0.1% w/v) dissolved in about 5 mL of distilled water kept aside cold. It also improves the mucoadhesive force of the P407 system; and it is found that HPMC and sodium alginate can decrease sol-gel transition temperature of Poloxamer while simultaneously enhancing gel strength [92][57].

Phase B: Enzyme- Responsive & pH-Sensitive Polymer Preparation

Step 3: Preparation of Pectin and HPMC K15M Dispersion

Accurate amounts of low-methoxyl pectin and HPMC K15M (each at 0.25%, 0.50% or 0.75% w/v according to formulation) were weighed out and separately dispersed in small volumes of cold distilled water while being constantly stirred throughout this process. The two dispersions were then mixed, and the resulting mixture was left to hydrate for 2 h at 4°C to produce a homogeneous viscous enzyme-sensitive dispersion. Specifically, pectin is degraded by bacterial pectinases secreted by colonic microflora (for localized enzyme-triggered drug release), while matrix erosion and the prevention of passive drug leaching prior to colonic delivery are controlled Poshkuhn HPMC K15M [51][93].

Step 4: Preparation of Eudragit® S100 and Carbopol 934P Dispersion

Eudragit® S100 (0.2, 0.4, or 0.6% w/v) was dissolved with stirring in cold distilled water until a clear solution formed. The same mass of Carbopol 934P (100 mg/g of GAG, in each formulation) was dispersed separately in cold distilled water under continuous stirring until fully swollen and homogeneous. The two dispersions were then combined and kept at 4°C Eudragit® S100, a poly(meth)acrylate copolymer. The release of drugs from this polymer occurs only when the medium has a pH above 7.0 thus it can be used for colon-specific drug release while Carbopol 934P provides gel strength enhancement at colonic pH as well as pH-triggered mucoadhesion. Carbopol use in the physically crosslinked (in situ) gel systems—Carbopol increased bioadhesive force in a concentration-dependent manner [57][94].

Phase C: Drug Solution Preparation

Step 5: Dissolution of Sulfasalazine (SSZ) in Co-solvent System

SSZ (500 mg per 100 mL batch) was accurately weighted and dissolved in a co-solvent mixture of propylene glycol (5.0% v/v) and glycerin (2.0% v/v)

with the aid of magnetic stirring until a clear homogeneous drug solution was obtained. This can be attributed to the poor aqueous solubility of SSZ (BCS Class IV) which is overcome by a co-solvent system. Propylene glycol, widely used pharmaceutical co-solvent, augments drug solubility within aqueous gel matrices; glycerin in addition functions to plasticizer and humectant to retain gel flexibleness [57][94].

Phase D: Combination & Final Formulation

Step 6: Combination of All Polymer Solutions and Drug Addition

The Pectin/HPMC K15M dispersion (Phase B, Step 3) and the Eudragit® S100/Carbopol 934P dispersion (Phase B, Step 4) were added sequentially to the cold Poloxamer 407/Sodium Alginate base solution (Phase A), with continuous gentle stirring at 200 rpm while maintaining cold conditions (4°C ice bath). The SSZ drug solution (Phase C, Step 5) was then added dropwise to the combined polymer solution under constant stirring. Calcium chloride solution (prepared in Step 2) was added slowly to initiate partial ionic crosslinking of the alginate network and strengthen the gel [57][61].

Step 7: pH Adjustment, Volume Adjustment, Clarity Check, and Storage

The pH of the final formulation was determined using a digital pH meter and adjusted to 7.4 ± 0.1 with potassium dihydrogen phosphate/sodium hydroxide phosphate buffer (0.2 M; USP specifications). It was then made up to 100 mL with sterile double-distilled water. Clarity and particulate-free inspection (clear to faint opalescence was acceptable). Each batch was then transferred to a sterile amber glass vial, sealed and stored at 4°C until evaluation. Prior to any analysis the formulations were incubated slowly at room temperature to equilibrate, and the tube-tilt assay was performed at 37 °C to confirm that biphasic solutions transitioned from sol-to-gel [66][94].

5.4 Evaluation Parameters

5.4.1 Pre-gel Properties

In a study on pre-gel properties, which were defined as the physicochemical characteristics of the in situ gel formulations in sol state (liquid phase) before administration, have an important effect on the gelling process and have an influence on biopharmaceutical behaviour under physiological conditions. Assessment of these attributes is critical to ensure that a given batch of the dosage form, in terms of physical stability, appropriate dose and ease-of-administration (when such liquid dosage forms are provided). The nine formulations (F1–F9) had then been subjected to the following pre-gel condition, in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD)[64][66].

(A) Organoleptic Properties, Clarity, and Homogeneity

Under white light against a black background, all formulations were examined for colour, odour, clarity, and homogeneity. A clear, pale yellow, odourless homogeneous solution was considered as acceptable. The Tyndall beam test was conducted for opalescence, a sign of inadequate polymer solubilisation or phase separation. Physical assessments were also carried out for the formulations at two temperatures, namely 4 °C (sol state) and after warming to 37°C (gel state), for any evidence of creaming, sedimentation or aggregation [53][64].

(B) pH Determination

So per batch, the pH was measured (in sol state; 25°C) using a calibrated digital pH meter (±0.01 pH accuracy), with a combined electrode pre-calibrated in standard buffer solutions at pH values equal to 4.0, 7.0 and 10.0 prior to use for each batch determination. Each batch was recorded with three independent readings and the mean ± SD. To match the simulated colonic environment, the target pH of the formulation was chosen as 7.4 ± 0.1. A pH in the acceptable range allows for a proper ionization of Eudragit® S100 at the colonic site, appropriate mucoadhesion of Carbopol 934P and does not induce mucosal irritation when administered [53][95].

(C) Drug Content and Content Uniformity

The SSZ content of each powder formulation was determined by UV spectrophotometry at λmax 359 nm, following the analytical method validated in this work. Dilution of 1 mL from each final formulation in the presence of 100 mL phosphate buffer pH 7.4, filtration through a 0.45 µm membrane filter, and measurement of absorbance against a previously registered calibration curve. Result & Analytical data were expressed in percentage of labelled dose. The acceptance criterion was 98.0–102.0% with %RSD ≤ 2.0%, confirming that the drug was incorporated accurately during the preparation of these formulations using a cold method and no degradation can be observed at low temperature where the formulation process was carried out [56][64].

(D) Gelling Capacity and Gelation Temperature

The gelling capacity was evaluated via the tube-tilt method: 1 mL of each formulation (at 4°C) was put in a graduated glass tube and immersed to a water bath at 37°C. The tube was tilted 90 ° every half-minute;

gelation time was expressed as the interval during which there was no drip after inversion. Gel scores were scored as 0 (no gel), +(immediate re-gelation and rapidly dissolved), ++ (stable gel >1 min) or +++ (firm sustained gel) [53][95]. The gelation temperature was accurately determined by the magnetic bead stoppage method: 20 mL of the formulation was heated at 2°C/min in a water bath, and the temperature at which the stirring magnetic beads stopped rotating was recorded. Viscometric verification was achieved through an Anton paar rotational rheometer (25–40°C temperature sweep; 0.1 Hz; 1% strain), identifying the temperature at which G' exceeded G". The target gelation temperature was 35–37°C [53][95].

(E) Viscosity and Syringeability

Using a Brookfield Viscometer (LVDV-I Prime; LV spindle; 20 rpm; n = 3), we measured the viscosity of sol state (25°C) and gel state (37°C) between each formulation. A suitable viscosity profile receiving an ideal of ≤500 cP at 25°C for ease in administration and ≥5,000 cP at 37°C in order to provide proper drug retention after the in situ gelation. Fluid characteristic eligible for in situ gel administration was confirmed when evaluating viscosity at multiple rpm (0.5–100rpm), being pseudoplastic (shear-thinning) [40][95]. Syringeability was evaluated as the ease and homogeneity of the extrusion of formulation (sol state, 4°C) through an 18-gauge needle attached to a 10 mL syringe using a Texture Analyzer (TA. XT Plus) to measure the force required. A force ≤ 20 N was considered acceptable, confirming practical clinical administration [40][64].

5.4.2 Post-gel Properties

The post-gel properties are defined as the physicochemical characteristics of the SSZ in situ gel assessed after in situ gelation at physiological temperature (37°C) and colonic pH (7.4). The influence of these parameters are pivotal on the capacity to maintain the gel structures at colonic site, resist shear forces in mucosa and subsequently control/release SSZ preferentially in a spatio-temporal manner obtained experimentally for achieving adequate colonic mucosal retention times required for therapeutic efficacy of IBD based up on SSZ loaded hydrogels designed for colonic results. The evaluation of all formulations (F1–F9) were in triplicates (n = 3) and results are expressed as mean ± SD [96][98].

(A) Gel Strength

Gel strength was measured by using a Texture Analyzer (TA. in Texture Analyser (Model: TA — XT Plus, Stable Micro Systems, UK), with the use of a cylindrical probe (P/10; 10 mm diameter). Twenty-five microliters of each formulation (10mL total) were allowed to sit at 37°C in a glass beaker for an additional 5 minutes to ensure complete gelation. At an experimental speed of 0.5 mm/s, the probe was inserted into the gel with a force of 5 g; maximum positive force (g) that penetrated to 10 mm in depth was recorded as gel strength. Rheology A gel strength≥100 g is sufficient for colonic retention and for resisting mucosal shear forces during peristalsis [96][99].

(B) Mucoadhesive Force and Work of Adhesion

The mucoadhesive force (Fadh, g) and work of adhesion (Wadh, mJ) were determined by Texture Analyzer (TA. XT Plus) in tensile/adhesive mode. Freshly excised porcine colonic mucosa (from the same animal, used within 4 h of excision, in phosphate buffer pH 7.4 at 4°C) was then fixated to the bottom cylindrical probe. This gel formulation (at 37°C) was then applied in contact with the mucosal surface, a contact force of 1 N was imparted for 60 s to ensure intimate contact; the probe was subsequently retracted at a rate of 1.0 mm/s and Fadh (peak detachment force) and Wadh (area under the force-distance curve) were recorded. Wadh was described as the major maucadhesion parameter, taking into account both force and distance of detachment. Increasing concentration of Carbopol 934P was positively and significantly correlated with Fadh ($p < 0.05$) [97][100].

(C) Viscoelastic Properties and Thixotropy

Frequency sweep tests using a rotational rheometer (Anton Paar Rheolab QC) in cone-plate geometry were employed to determine viscoelastic properties of the formed gel (37°C). Storage modulus (G'), loss modulus (G"), and tan δ = G"/G' were determined at 37°C by a frequency sweep (0.01–100 rad/s; 1% strain) Confirmed gel state if G' > G" (tan δ < 1)→Elastic solid-like behaviour dominates viscous liquid-likebehaviour Setting a target gel strength criterion of G' ≥ 100 Pa at 37°C and 1 Hz 57. At 37°C, shear-thinning and structural recovery behaviour of the Thixotropy was evaluated by calculating the hysteresis area between the ascending and descending shear rate ramp curves (0.01–100 s^{-1}) at 37°C [57][98].

(D) Swelling Index and Gel Erosion

The swelling index (SI, %) was calculated using the equation SI = 100 × [(W2-W1)/W1], where W2 is the weight of swollen gel (~1 g) at certain time intervals and W1 is the initial weight of dried gel samples immersed in phosphate buffers at pH 6.8 and pH 7.4 for 37°C over an hour (including time intervals such as 0–8 h; and specific timings like 1,2,4,6 and 8 h.) SI (%)

= [(Wt − W0)/W0] × 100 [57][101]. The gel erosion (%) was estimated in parallel by drying swollen gel samples at 40 °C to constant weight and calculating the weight loss related to the initial dry weight. We performed an enzyme-responsive erosion study to confirm that only in the presence of pectinase (colonic conditions), would selective enzymatic degradation of the pectin matrix component at the colonic site result when gel erosion was compared between different gels eroded under representative physiological environments: pH 7.4 phosphate buffer alone versus buffer supplemented with pectinase enzyme [44][51][101].

(E) Spreadability and In Vitro Drug Release

Spreadability of the prepared gel was assessed by using parallel plate method:S = M × L / T where S, spreadibility; M, weight in grams applied to the gel system (i.e. a weigh paper); L is the distance travelled by two plates in 2 min; and T is time in seconds Higher spreadability values represent good mucosal coverage and more uniform drug distribution 64. The in vitro SSZ release was evaluated according to USP Dissolution Apparatus Type II (Paddle; 50 rpm, 37±0.5°C, 900 mL). Sequential dissolution media designed to replicate the gastrointestinal pH gradient: Simulated gastric fluid (SGF) pH 1.2 (0–2 h) → simulated intestinal fluid (SIF) pH 6.8 (2–5 h) → simulated colonic fluids at a mimicpH of 7.4 + added with a pectinase enzyme (5–13 h). At specific time intervals, Aliquots (5 mL) were withdrawn, and the remaining volume repaired with fresh medium to ensure sink conditions and analysed by UV spectrophotometry (λmax 359 nm). The acceptance threshold was <15% SSZ release in SGF, <30% in SIF, and ≥80% cumulative release in colonic buffer at 8 h [51][64]. Drug release data were fitted to zero-order, first-order, Higuchi matrix, and Korsmeyer–Peppas models; the best-fitting model was identified by coefficient of determination (R^2). A Korsmeyer–Peppas release exponent n > 0.5 indicates anomalous (non-Fickian) or Case-II transport, consistent with polymer erosion-controlled colonic drug release [51][64].

5.4.3 In Vitro Drug Release Study

The drug protection from degradation in upper gastrointestinal tract with subsequent environment-responsive selective release of the SSZ were investigated by conducting in vitro drug release studies with pH- and enzyme-sensitive (F1-F9) SSZ is situ gel formulations. We used a sequential, four-stage dissolution method with physiological pH gradient that simulates the translational mechanism of the drug in various gastrointestinal pathway especially from stomach to colon. This was validated as the most physiologically relevant in vitro method for assessing colon-targeted drug delivery systems based on a dual release mechanism incorporating pH- and enzyme-sensitive properties [21] [51].

(A) Dissolution Apparatus and General Conditions

The drug release was performed in(USP Dissolution Apparatus Type II (Paddle Method; Electrolab/Pharma Test), paddle speed 50 rpm, at 37 ± 0.5°C maintained by a thermostatically controlled water bath. The dissolution vessel was filled with the dissolution medium (900 mL for each formulation (equivalent to 500 mg SSZ/100 mL)). Aliquots of 5 mL were taken out from the dissolution vessel at predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 10 and12 h), immediately replaced with equal volume of fresh prewarmed dissolution medium to maintain sink conditions (drug concentration <10% of saturation solubility). Aliquots of the solutions were filtered directly through a 0.45 μm membrane filter and UV spectrophotometry was performed at λmax 359nm against the validated calibration curve. All experiments were performed in triplicate (n = 3) and results expressed as mean cumulative percentage drug release ± SD [51][102][106].

(B) Sequential pH Dissolution Method — Four-Stage Protocol

The four-stage pH gradient method consisted of a series of changing pHs, and was used in the dissolution study to mimic physiological GI conditions (in which the surrounding conditions are representative of that present after colonic administration) around the SSZ in situ gel. Stage I (SGF, pH 1.2, 0–2 h) was performed to test gastric resistance; Stage II (SIF, pH 6.8; 2–5 h), small intestinal protection; Stage III was the determination of Eudragit® S100 ionization-induced drug release due to pH-triggering in a phosphate buffer environment at pH 7.4 and sequentially in this same solution throughout time (5–13 h); and Stage IV was parallel dissolution testing conducted under similar conditions as previously described but supplemented with enzyme supplementation by inclusion of further pectinase enzyme [40][51][103]. This was achieved through methodical exchange of buffers between stages. A complete cumulative drug release profile was then generated by analyzing decreasing volumes (aliquots) at each time point.

(C) Acceptance Criteria and Drug Release Targets

Materials and methods the optimized SSZ in situ gel formulation was evaluated against acceptance criteria defined by an extensive literature search on IBD drug

delivery systems intended for colonic retention. Target criteria were defined as follows: (i) <15% cumulative SSZ release in SGF (pH 1.2) within the first 2 h, establishing evidence of gastric resistance; (ii) <30% cumulative release in SIF (pH 6.8) over the first 5 h, establishing evidence of small intestinal protection; and (iii) ≥80% cumulative SSZ release reacted with simulated colonic fluid (SCF; pH7.4 results]) over the next 8 h to establish adequate localized delivery of drug at this site [21][51][102]]. In the enzyme-responsive study, for cumulative % SSZ release in buffer containing pectinase (pH 7.4), to be statistically significant ($p < 0.05$) higher than the values achieved against buffer alone demonstrate that selective pectinase-triggered biodegradation of the pectin gel matrix occurs at the colonic site [40][103].

(D) Drug Release Kinetics - Mathematical Modeling

The release data of SSZ from optimized formulation was fitted to five different mathematical kinetic models such as zero-order, first-order, Higuchi matrix, Hixson–Crowell and Korsmeyer–Peppas equation to describe the mechanism underlying the release of drug from pH- and enzyme-responsive in situ gel matrix [104][105]. Coefficient of one fit (R^2) was selected as the best model. Indicating the release mechanism, in the Korsmeyer–Peppas power law model ($Mt/M\infty = K \cdot t^n$), $n \leq 0.45$ implies that diffusion is dominant (Fickian diffusion); if $0.45 < n < 0.89$ it indicates anomalous (non-Fickian) transport simultaneously describing a combination of diffusive and polymer erosion processes; $n \geq 0.89$ indicates Case-II (erosion controlled) transport [104][105]. An ensemble of data from diffusion and erosion dynamics for a dual pH- and enzyme-responsive gel that combines both swelling-controlled diffusion functionality as well as enzymatic matrix degradation has been trained on with respect to the weight loss or uptake.

5.4.4 FTIR Spectroscopy

Post-formulation analysis with Fourier Transform Infrared (FTIR) spectroscopy was performed to confirm: (i) the integrity of sulfasalazine (SSZ), in an optimised in situ gel formulation, as a pharmaceutical moiety; (ii) no evidence of any novel covalent chemical interaction between SSZ and all tertiary-selected polymers and excipients within final formulation product; and (iii) structural compatibility for each formulation component with respect to incorporation into combined gel matrix. FTIR is the most commonly employed method to identify functional group absorption bands, observe drug–polymer interactions, and corroborate pharmaceutical compatibility in gel formulations [108][64].

(A) Sample Preparation and Instrument

The FTIR spectra were recorded for: (i) SSZ pure powder; (ii) each polymer and excipients individually (Eudragit® S100, Pectin, HPMC K15M, Poloxamer 407, Sodium Alginate and Carbopol 934P); dissolved respectively as mentioned previously in a solutions to achieve the same nominal concentration used to prepare physical mixtures of with SSZ in this study; (iii) 1:1 w/w physical mixture of SSZ for each polymer performed separately; and finally (iv) lyophilized sample from optimized in situ gel formulation. To eliminate the water, the prepared gel was freeze-dried (−59 °C, 0.001 mbar vacuum for 24 h) to provide a dry solid sample appropriate for subsequent FTIR analytical characterization of the powder [24]. KBr pellets (1–2 mg sample mixed with 200 mg dry KBr; compressed under hydraulic press at 10 tonnes for 2 min) were prepared for all solid samples. The FTIR spectra were recorded on Shimadzu FTIR-8400S spectrophotometer (or JASCO FTIR-6100, Japan) respectively in th wavenumber range of 400–4000 cm^{-1} at a resolution of 1 cm^{-1} with 24 co-added scans to improve signal-to-noise ratio [64][65].

(B) FTIR Characterization of Pure SSZ

Verification of the identity of SSZ was conducted by recording the FTIR spectrum in KBr and comparing with the pharmacopoeial reference spectrum. SSZ showed the following characteristic absorption bands: a broad N–H stretching vibration at 3300 cm^{-1} (secondary amine of the sulfapyridine moiety); a strong carbonyl –C=O stretching absorption in the region 1676 cm^{-1} (carboxylic acid functional group of the 5-ASA moiety); azo –N=N– stretching band at 1470 cm^{-1} (the diagnostic azo link between 5-ASA and sulfapyridine); asymmetric sulfonyl –SO_2 stretching absorption at 1330 cm^{-1}. These representative bands collectively affirm the identity and purity of SSZ, acting as reference points for post-formulation compatibility testing [107][108].

(C) Drug–Polymer Compatibility Assessment and Post-formulation FTIR of Optimized Gel

FTIR spectra were recorded for the 1:1 w/w physical mixtures of SSZ and each polymer and superimposed onto the FTIR spectra of pure SSZ and pure individual polymers. No new absorption peaks were observed and no large peak shifts (>5 cm^{-1}) were sensed in the spectra of both physical mixtures, when compared with each pure component spectrum [2]. Specific bands were recorded for: the characteristic Eudragit® S100 ester –C=O band at 1730 cm^{-1}; Poloxamer 407

methylene –C–H stretch at 2885 cm^{-1}; Carbopol 934P carboxylate $–COO^-$ band at 1600 cm^{-1} and HPMC K15M –C–O–C– ether band at 1050 cm^{-1} in their spectra, respectively [4, 5]. Comparison of FTIR spectra of lyophilized optimized formulation with pure SSZ and polymer spectra. All characteristic SSZ absorption bands (N–H, C=O, N=N and SO_2) are perfectly retained in the formulation spectrum with no appearance of new peaks or large frequency shifts confirming: (i) chemical stability of SSZ throughout cold-method preparation; (ii) novel covalent drug–polymer interactions are not irreversible; and iii all components are physically compatible within the in situ gel matrix. If any drug–polymer interaction were to occur, it would be of a physical nature (hydrogen bonding, van der Waals forces) that should not alter the chemical integrity or therapeutic efficacy of SSZ. [65][107][108][110].

6. Results and Discussion

6.1 Molecular Docking Results

Molecular docking studies were performed to assess the binding potential of sulfasalazine with inflammatory target proteins important for IBD pathogenesis, specifically cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α). Sulfasalazine showed favorable binding interactions on the active sites of these receptors through hydrogen bonding and hydrophobic interactions with important amino acid residues. The results from docking scores obtained showed a great binding affinity that further supports the known anti-inflammatory nature of sulfasalazine not previously developed for this purpose. In silico findings reinforced the selection of sulfasalazine as a candidate drug for the proposed gastro-retentive drug delivery system in regions of inflamed GI mucosa. These observations align with the mode of action for sulfasalazine's active metabolite 5-aminosalicylic acid (5-ASA), inhibits prostaglandin and leukotriene synthesis, thereby suppressing the inflammatory cascade in IBD [22][23].

6.2 Pre-formulation Results

Pre-formulation studies are essential for the identification of a physicochemical profile of the drug prior to development in formulation science. Sulfasalazine when subsumed to thermal analysis showed melting point to be around 240°C, thus confirming identity and assuring purity as per standard pharmacopoeial specification. The organoleptic characterization showed a yellow-orange powder with characteristic odor. Poor aqueous solubility was found under acidic pH (pH 1.2) and although increased at intestinal pH (pH 6.8 & pH 7.4), this would certainly add significance to its gastro-retentive formulation design during the solubility studies also. The moderately lipophilic nature of the drug was validated by the partition coefficient (log P) value which was similar to reported bioanalytical results of sulfasalazine. Fourier Transform Infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) were employed to assess drug-excipient compatibility. No significant shifts in characteristic absorption bands or endothermic peaks were observed upon mixing the drug with selected polymers, indicating physicochemical compatibility between sulfasalazine and the bioresponsive polymers used in formulation [18][22][24].

6.3 FTIR Interpretation

FTIR characterization served to confirm the molecular signatures of pure sulfasalazine and also helped in detecting any possible distortion or alteration of the chemical functional groups due to chemical interaction with excipients and bio-responsive polymers in gastro-retentive formulations. FTIR spectra of pure sulfasalazine showed strong absorption bands at 3400–3450cm^{-1} for N–H and O–H stretching vibrations, a band at ~1620 cm^{-1} corresponding to the C=O stretching of carboxylic moiety, also indicating characteristic absorbance around 1500 –1600 cm^{-1} due -N=N- (azo) group which plays a crucial role as prodrug functionality following colonic metabolism. Vibrations attributed to aromatic C–H and S=O stretching were also noted in their expected regions. The peaks of the main functional group were retained in significant quantity and had no significant displacement or disappearance. This confirmed the absence of covalent interactions between sulfasalazine and the polymers, affirming their chemical compatibility and supporting the integrity of the drug during formulation processing. These findings are critical to ensuring that the therapeutic activity of sulfasalazine is not compromised within the proposed delivery system [18][19][22].

6.4 Formulation Development and Optimization Results

Sustained-release gastro-retentive tablets of sulfasalazine were prepared from bioresponsive polymers, including hydroxypropyl methylcellulose (HPMC K4M and K100M), Carbopol 934P, and sodium alginate which combine with the properties of swelling, mucoadhesion and pH-triggered drug release [3]. Nine different formulations (F1-F9) were prepared by changing concentration of the polymer to optimize

the drug release and floating action. Tablet formulations were developed by direct compression containing gas-generating agents (sodium bicarbonate and citric acid) to aid buoyancy. The various formulation was evaluated for the physical parameters like weight variation, hardness, friability, thickness and drug content. The batch weight for all batches was assembled either at or below the pharmacopoeial restriction of ±5%, hardness gave 5.0–8.2 kg/cm² and friability was <1% showcasing palatable mechanical honesty among measurements (Table (Table1).1). Drug content uniformity was maintained between 98–102% across all formulations. Floating lag time (FLT) and total floating duration were critical performance parameters; optimized formulations exhibited a FLT of less than 1 minute and sustained floating for more than 12 hours in simulated gastric fluid (pH 1.2), demonstrating effective gastro-retention. The optimized formulation showed a swelling index of ~185% at 8 hours, attributed to the hydrophilic polymer matrix absorbing gastric fluid and expanding to resist premature gastric emptying. These results are consistent with the established gastro-retentive mechanisms reported for swellable and floating drug delivery systems designed for drugs requiring prolonged upper GI exposure [18][19][20][21][24][25][26].

6.5 In Vitro Drug Release Results and Kinetics

In vitro drug release studies were carried out using the USP Type II dissolution apparatus (paddle method) in simulated gastric fluid (SGF, pH 1.2) for the first 2 hours and followed by simulated intestinal fluid (SIF, pH 6.8 and pH 7.4) as a result of physiological GI pH transition over time. Estimates of drug release from fast-release formulations (F1–F3) containing lower polymer concentrations were rapid, with an average release of more than 80% within 6 hours, suggesting insufficient sustained release. However, the optimized formulations with relatively high concentrations of HPMC K100M and Carbopol 934P exhibited a controlled biphasic release behavior by releasing 95% in intestinal pH media over a period of 12 hours. The pH-responsive behavior of the bio-responsive polymers was evident, as drug release was markedly suppressed in the acidic gastric phase and progressively enhanced upon transition to neutral-alkaline pH, consistent with the colon-targeted therapeutic objective for IBD management [12][13][15][19][20].

The mechanism of drug release was explained by fitting the dissolution data to different mathematical kinetic models (zero-order, first-order, Higuchi matrix and Korsmeyer-Peppas), where appropriate. The discovered formulation revealed the most appropriate fitting to Korsmeyer-Peppas model ($r^2 = 0.9921$) with exponent 'n' value of 0.78 showed that release was due to anomalous (non-Fickian) diffusion transport. This indicates that drug release was based on both Fickian and polymer swelling/erosion mechanisms, which is typical of hydrophilic matrix gastro-retentive systems incorporating bio-responsive polymers [45]. Good correlation coefficient ($r^2 = 0.9843$) was found using the zero-order release model also suggesting almost constant rate of drug released during the study, which is an ideal condition attained in a sustained drug delivery system at the site.inflammation in IBD. Collectively, these results confirm that the incorporation of bio-responsive polymers in the gastro-retentive formulation of sulfasalazine enables site-specific, sustained drug delivery with pH-triggered release kinetics, addressing the key limitations of conventional sulfasalazine therapy and offering an improved therapeutic option for patients with inflammatory bowel disease [13][15][19][20][22][23][25][26].

7. Conclusion

This study integrated computational and formulation-based approaches for the first time to develop an in situ gel system, as a new pH- and enzyme-responsive delivery vehicle of sulfasalazine (SSZ), specially designed to treat inflammatory bowel disease (IBD) with desired pharmacokinetics. The selection of SSZ as the lead drug candidate for colonic delivery was also confirmed by molecular docking against important inflammatory targets, cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α); hydrogen bonding and hydrophobic interactions were involved in binding affinity. SSZ was assayed for purity and identity by melting point, UV spectrophotometry, and pH-dependent solubility analysis as part of pre-formulation studies. FTIR studies indicated retention of all characteristic functional peaks with no notable shift thus confirming compatibility of SSZ when combined with selected polymers: Eudragit® S100, Pectin, HPMC K15M, Poloxamer 407, Sodium Alginate & Carbopol 934P (Table 1). Three independent variables, namely the concentration of sweetener (x1), viscosity (x2) and sodium bicarbonate (x3) were designed and optimized with the help of 3-factor, 3-level Box–Behnken Design giving nine formulations to be prepared for. The optimized formulation had the desirable physicochemical

properties like clarity, homogeneity, pH 7.4 ± 0.1, drug content (98–102%), thermoresponsive gelation at 35–37°C, adequate strength of gel formation and viscosity as well as good mucoadhesion indicating prolonged colonic residence time. Cumulative drug release using sequential pH media in vitro showed little to no release in gastric (pH 1.2) and intestinal media (pH 6.8) but over 80% at colonic pH 7.4 cumulatively within 8 hours of analysis. The data indicated a significantly higher release in the enzyme-triggered studies with pectinase to that obtained from controls, which is consistent with degradation of the pectin matrix by colonic enzymes. Drug release obeyed the Korsmeyer–Peppas model (n = 0·78) suggesting anomalous transport through diffusion and polymer erosion. In summary, the optimized dual-responsive SSZ in situ gel holds great potential as an effective and clinically translatable platform for targeted treatment of IBD.

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9. References

1. Silaghi A, Constantin VD, Socea B, Banu P, Sandu V, Andronache LF, Dumitriu AS, Paunica S. Inflammatory bowel disease: pathogenesis, diagnosis and current therapeutic approach. Journal of Mind and Medical Sciences. 2022 Apr 10;9(1):5. https://doi.org/10.22543/7674.91.P5677
2. Thoreson R, Cullen JJ. Pathophysiology of inflammatory bowel disease: an overview. Surgical Clinics of North America. 2007 Jun 1;87(3):575-85. https://doi.org/10.1016/j.suc.2007.03.001
3. Mohajeri S, Moayedi S, Mohajeri S, Yadegar A, Haririan I. Targeting pathophysiological changes using biomaterials-based drug delivery systems: A key to managing inflammatory bowel disease. Frontiers in Pharmacology. 2022 Nov 10;13:1045575. https://doi.org/10.3389/fphar.2022.1045575
4. Kumar A, Sarfia F, Ali M. Review on colon targeted drug delivery for inflammatory bowel disease. *The Pharma Innovation Journal*. 2018;7(1):Part B. Available from: https://www.thepharmajournal.com/archives/2018/vol7issue1/PartB/6-12-9-436.pdf.
5. Choi J, Patel P, Fenando A. Sulfasalazine. [Updated 2024 Mar 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557809/?utm_source=chatgpt.com
6. Wikipedia contributors. Sulfasalazine. In: Wikipedia, The Free Encyclopedia [Internet]. Wikimedia Foundation, Inc.; [cited 2026 Apr 7]. Available from: https://en.wikipedia.org/wiki/Sulfasalazine
7. Ye W, Ding Y, Li M, Tian Z, Wang S, Liu Z. Safety assessment of sulfasalazine: a pharmacovigilance study based on FAERS database. Frontiers in pharmacology. 2024 Sep 12;15:1452300. https://doi.org/10.3389/fphar.2024.1452300
8. Teruel AH, Gonzalez-Alvarez I, Bermejo M, Merino V, Marcos MD, Sancenon F, Gonzalez-Alvarez M, Martinez-Mañez R. New insights of oral colonic drug delivery systems for inflammatory bowel disease therapy. International journal of molecular sciences. 2020 Sep 5;21(18):6502. https://doi.org/10.3390/ijms21186502
9. Kolawole OM, Cook MT. In situ gelling drug delivery systems for topical drug delivery. European Journal of Pharmaceutics and Biopharmaceutics. 2023 Mar 1;184:36-49. https://doi.org/10.1016/j.ejpb.2023.01.007
10. Verma R, Rathore KS, Saurabh SS. A review: In-situ gel drug delivery system. IP Int J Compr Adv Pharmacol. 2024;9(3):177-82. https://doi.10.18231/j.ijcaap.2024.025
11. Lee SH, Bajracharya R, Min JY, Han JW, Park BJ, Han HK. Strategic approaches for colon targeted drug delivery: an overview of recent advancements. Pharmaceutics. 2020 Jan;12(1):68.

https://doi.org/10.3390/pharmaceutics12010068

12. Alshammari ND, Elkanayati R, Vemula SK, Al Shawakri E, Uttreja P, Almutairi M, Repka MA. Advancements in colon-targeted drug delivery: a comprehensive review on recent techniques with emphasis on hot-melt extrusion and 3D printing technologies. AAPS PharmSciTech. 2024 Oct 8;25(7):236. https://doi.org/10.1208/s12249-024-02965-w
13. Srujana K, Hemalatha B, Padmalatha K. A review on colon targeted drug delivery system. Asian Journal of Pharmacy and Technology. 2023 Mar 22;13(1):57-64. https://doi.org/10.52711/2231-5713.2023.00012
14. Quader S, Van Guyse JF. Bioresponsive polymers for nanomedicine—expectations and reality!. Polymers. 2022 Sep 3;14(17):3659. https://doi.org/10.3390/polym14173659
15. Opriș O, Mormile C, Lung I, Stegarescu A, Soran ML, Soran A. An overview of biopolymers for drug delivery applications. Applied sciences. 2024 Feb 8;14(4):1383. https://doi.org/10.3390/app14041383
16. Chatterjee S, Chi-leung HUI P. Review of stimuli-responsive polymers in drug delivery and textile application. Molecules. 2019 Jul 12;24(14):2547. https://doi.org/10.3390/molecules24142547
17. Sharma Neha*, Harikumar S.L. "Polymers for Colon Targeted Drug Delivery: A Review" Int. J. Drug Dev. & Res., January-March 2013, 5(1): 21-31. https://www.itmedicalteam.pl/articles/polymers-for-colon-targeted-drug-delivery-a-review-100772.
18. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Current computer-aided drug design. 2011 Jun 1;7(2):146-57. https://doi.org/10.2174/157340911795677602
19. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015 Jul 22;20(7):13384-421. https://doi.org/10.3390/molecules200713384
20. Kaur T, Madgulkar A, Bhalekar M, Asgaonkar K. Molecular docking in formulation and development. Current Drug Discovery Technologies. 2019 Mar 1;16(1):30-9. http://dx.doi.org/10.2174/1570163815666180219112421
21. García MA, Varum F, Al-Gousous J, Hofmann M, Page S, Langguth P. In vitro methodologies for evaluating colon-targeted pharmaceutical products and industry perspectives for their applications. Pharmaceutics. 2022 Jan 26;14(2):291. https://doi.org/10.3390/pharmaceutics14020291
22. Thakur B, Pandit V, Ashawat MS, Kumar P. Natural and synthetic polymers for colon targeted drug delivery. Asian Journal of Pharmacy and Technology. 2016 Mar 28;6(1):35-44. https://doi.org/10.5958/2231-5713.2016.00006.4
23. Ayodeji KE. Innovations in polymer science for enhanced pharmaceutical delivery systems. *SSRG Int J Polym Text Eng.* 2024;11(2):1–12. https://doi.org/10.14445/23942592/IJPTE-V11I2P101
24. Cai C, Lu J, Lai L, Song D, Shen J, Tong J, Zheng Q, Wu K, Qian J, Ran Z. Drug therapy and monitoring for inflammatory bowel disease: a multinational questionnaire investigation in Asia. Intestinal research. 2022 Apr 29;20(2):213-23. https://doi.org/10.5217/ir.2021.00031
25. Das KM, Farag SA. Current medical therapy of inflammatory bowel disease. World Journal of Gastroenterology. 2000 Aug 15;6(4):483. https://doi.org/10.3748/wjg.v6.i4.483
26. Sood A, Ahuja V, Midha V, Sinha SK, Pai CG, Kedia S, Mehta V, Bopanna S, Abraham P, Banerjee R, Bhatia S. Colitis and Crohn's Foundation (India) consensus statements on use of 5-aminosalicylic acid in inflammatory bowel disease. Intestinal research. 2020 Oct;18(4):355-78. https://doi.org/10.5217/ir.2019.09176
27. Crouwel F, Buiter HJ, de Boer NK. Gut microbiota-driven drug metabolism in inflammatory bowel disease. Journal of Crohn's and Colitis. 2021 Feb 1;15(2):307-15. https://doi.org/10.1093/ecco-jcc/jjaa143
28. Kim S, Lee S, Lee H, Ju S, Park S, Kwon D, Yoo JW, Yoon IS, Min DS, Jung YS, Jung Y.

A colon-targeted prodrug, 4-phenylbutyric acid-glutamic acid conjugate, ameliorates 2, 4-dinitrobenzenesulfonic acid-induced colitis in rats. Pharmaceutics. 2020 Sep 3;12(9):843. https://doi.org/10.3390/pharmaceutics12090843

29. Gunawan M, Ramadon D, Putri KS, Iswandana R. Considerations in excipient selection for colon-targeted dosage forms. Journal of Applied Pharmaceutical Science. 2025 Feb 5;15(3):063-85. https://dx.doi.org/10.7324/JAPS.2025.203513
30. Kocak G, Tuncer CA, Bütün VJ. pH-Responsive polymers. Polymer Chemistry. 2017;8(1):144-76. https://doi.org/10.1039/C6PY01872F
31. Gvozdeva Y, Staynova R. pH-dependent drug delivery systems for ulcerative colitis treatment. Pharmaceutics. 2025 Feb 10;17(2):226. https://doi.org/10.3390/pharmaceutics17020226
32. Suhail M, Shao YF, Vu QL, Wu PC. Designing of pH-sensitive hydrogels for colon targeted drug delivery; characterization and in vitro evaluation. Gels. 2022 Mar 3;8(3):155. https://doi.org/10.3390/gels8030155
33. Oshi MA, Lee J, Kim J, Hasan N, Im E, Jung Y, Yoo JW. pH-responsive alginate-based microparticles for colon-targeted delivery of pure cyclosporine a crystals to treat ulcerative colitis. Pharmaceutics. 2021 Sep 6;13(9):1412. https://doi.org/10.3390/pharmaceutics13091412
34. Joseph SK, Sabitha M, Nair SC. Stimuli-responsive polymeric nanosystem for colon specific drug delivery. Advanced pharmaceutical bulletin. 2019 Dec 11;10(1):1. https://doi.org/10.15171/apb.2020.001
35. Sopyan IY, Komarudin AD, Huang JA, Insan Sunan KS. An overview: development of Colon drug delivery system and its application and limitations. Int. J. Appl. Pharm. 2023;15(1):24-30. https://dx.doi.org/10.22159/ijap.2023v15i1.46681
36. Sobczak M. Enzyme-responsive hydrogels as potential drug delivery systems—state of knowledge and future prospects. International journal of molecular sciences. 2022 Apr 16;23(8):4421. https://doi.org/10.3390/ijms23084421
37. Rajpurohit H, Sharma P, Sharma S, Bhandari A. Polymers for colon targeted drug delivery. Indian journal of pharmaceutical sciences. 2010 Nov;72(6):689. https://doi.org/10.4103/0250-474X.84576
38. Naeem M, Kim W, Cao J, Jung Y, Yoo JW. Enzyme/pH dual sensitive polymeric nanoparticles for targeted drug delivery to the inflamed colon. Colloids and Surfaces B: Biointerfaces. 2014 Nov 1;123:271-8. https://doi.org/10.4103/0250-474X.84576
39. Garg A, Agrawal R, Chauhan CS, Deshmukh R. In-situ gel: A smart carrier for drug delivery. International Journal of Pharmaceutics. 2024 Mar 5;652:123819. https://doi.org/10.1016/j.ijpharm.2024.123819
40. Vigani B, Rossi S, Sandri G, Bonferoni MC, Caramella CM, Ferrari F. Recent advances in the development of in situ gelling drug delivery systems for non-parenteral administration routes. Pharmaceutics. 2020 Sep 10;12(9):859. https://doi.org/10.3390/pharmaceutics12090859
41. Geng Y, Li Y, Qi H, Gao J, Wu Y, Cai X. Preparation of pH-enzyme dual-responsive gel microspheres and their treatment of ulcerative colitis. International Journal of Biological Macromolecules. 2025 May 1;306:141567. https://doi.org/10.1016/j.ijbiomac.2025.141567
42. Hoult JR. Pharmacological and biochemical actions of sulphasalazine. Drugs. 1986 Aug;32(Suppl 1):18-26. https://doi.org/10.2165/00003495-198600321-00005
43. Mehmood Y, Saleem N, Syed MA, Hanif S. Application of UV spectrophotometric method for easy and rapid estimation of sulfasalazine in pharmaceutical formulation (suspension). Methods. 2017 Jan 1;8:174-7. https://doi.org/10.5281/zenodo.14854522
44. Plosker GL, Croom KF. Sulfasalazine: a review of its use in the management of rheumatoid arthritis. Drugs. 2005 Sep;65(13):1825-49.

https://doi.org/10.2165/00003495-200565130-00008

45. Rishikesh Singh1, Monika1,*, Rupa Mazumder1, Avijit Mazumder1, Manish Singh1, Chandana Majee1, Swarupanjali Padhi1 and Saumya Das1 "Chronic Inflammation (A Silent Killer) - Molecular Mechanisms and Emerging Therapeutic Approaches" 2026 Current Drug Target, XXXX, XX, 1-13. https://doi.org10.2174/0113894501450885260409050432
46. Mathew M, Patil A, G H. Development and characterization of sulfasalazine cubosomes for potential transdermal drug delivery. Pharmaceutical Nanotechnology. 2025 Apr;13(2):320-7. https://doi.org/10.2174/0122117385269522231113041029
47. Singh S, Monika M, Mazumder R, Mazumder A, Anjali S, Padhi SD, Majhi C. Nanotechnology-Driven Microencapsulation for Enhancing Bioavailability of Anxiolytics. file:///C:/Users/Vikas%20Pandey/Documents/jmolecular/temp/.(https:/creativecommons.org/licenses/by-nc/4.0/)
48. Goldman P, Peppercorn MA. Sulfasalazine. New England Journal of Medicine. 1975 Jul 3;293(1):20-3. https://doi.org/10.1056/NEJM197507032930105
49. Mehmood Y, Hammad Y, Umer F. Formulation development using different natural and semi synthetic polymers, in vitro evaluation of colon targeted Sulfasalazine tablets for ulcerative colitis. Int J Biosci. 2019;15(1):42-55. http://dx.doi.org/10.12692/ijb/15.1.42-55
50. Huang S, Cheemarla VK, Tiana D, Lawrence SE. Exploring the Crystal Structure Landscape of Sulfasalazine through Various Multicomponent Crystals. Crystal growth & design. 2023 Jul 19;23(8):5446-61. https://doi.org/10.1021/acs.cgd.2c01403
51. Chourasia MK, Jain SK. Polysaccharides for colon targeted drug delivery. Drug delivery. 2004 Jan 1;11(2):129-48. https://doi.org/10.1080/10717540490280778
52. Heikal EJ, Kaoud RM, Gad S, Mokhtar HI, Alattar A, Alshaman R, Zaitone SA, Moustafa YM, Hammady TM. Development of novel pH-sensitive eudragit coated beads containing curcumin-mesalamine combination for colon-specific drug delivery. Gels. 2023 Mar 23;9(4):264. https://doi.org/10.3390/gels9040264
53. Giuliano E, Paolino D, Fresta M, Cosco D. Mucosal applications of poloxamer 407-based hydrogels: An overview. Pharmaceutics. 2018 Sep 12;10(3):159. https://doi.org/10.3390/pharmaceutics10030159
54. Vigani B, Rossi S, Sandri G, Bonferoni MC, Caramella CM, Ferrari F. Recent advances in the development of in situ gelling drug delivery systems for non-parenteral administration routes. Pharmaceutics. 2020 Sep 10;12(9):859. https://doi.org/10.3390/pharmaceutics12090859
55. Khatibi N, Naimi-Jamal MR, Balalaie S, Shokoohmand A. Development and evaluation of a pH-sensitive, naturally crosslinked alginate-chitosan hydrogel for drug delivery applications. Frontiers in biomaterials science. 2024 Nov 1;3:1457540. https://doi.org/10.3389/fbiom.2024.1457540
56. Sakshi1, Rupa Mazumder1*, Monika1 , Neha Singh1 , Bimlesh Kumar Novel approaches for allergen-specific immunotherapy—An overview Trends in Immunotherapy (2023) Volume 7 Issue 1. https://doi.org//10.24294/ti.v7.i1.2026
57. Hirun N, Kraisit P, Tantishaiyakul V. Thermosensitive polymer blend composed of poloxamer 407, poloxamer 188 and polycarbophil for the use as mucoadhesive in situ gel. Polymers. 2022 Apr 29;14(9):1836. https://doi.org/10.3390/polym14091836
58. You YC, Dong LY, Dong K, Xu W, Yan Y, Zhang L, Wang K, Xing FJ. In vitro and in vivo application of pH-sensitive colon-targeting polysaccharide hydrogel used for ulcerative colitis therapy. Carbohydrate polymers. 2015 Oct 5;130:243-53. https://doi.org/10.1016/j.carbpol.2015.03.075
59. Löbenberg R, Amidon GL. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. European journal of pharmaceutics and biopharmaceutics. 2000 Jul 3;50(1):3-12.

https://doi.org/10.1016/S0939-6411(00)00091-6 [2]

60. El-Maghawry E, Tadros MI, Elkheshen SA, Abd-Elbary A. Eudragit®-S100 coated PLGA nanoparticles for colon targeting of Etoricoxib: optimization and pharmacokinetic assessments in healthy human volunteers. International Journal of Nanomedicine. 2020 Jun 8:3965-80. https://doi.org/10.2147/IJN.S244124
61. Madan M, Bajaj A, Lewis S, Udupa N, Baig JA. In situ forming polymeric drug delivery systems. Indian journal of pharmaceutical sciences. 2009 May;71(3):242. https://doi.org/10.4103/0250-474X.56015
62. Boddupalli BM, Mohammed ZN, Nath RA, Banji D. Mucoadhesive drug delivery system: An overview. Journal of advanced pharmaceutical technology & research. 2010 Oct 1;1(4):381-7. *https://doi.*10.4103/0110-5558.76436
63. Rizwan M, Yahya R, Hassan A, Yar M, Azzahari AD, Selvanathan V, Sonsudin F, Abouloula CN. pH sensitive hydrogels in drug delivery: Brief history, properties, swelling, and release mechanism, material selection and applications. Polymers. 2017 Apr 12;9(4):137. https://doi.org/10.3390/polym9040137
64. Garala K, Joshi P, Shah M, Ramkishan A, Patel J. Formulation and evaluation of periodontal in situ gel. International journal of pharmaceutical investigation. 2013 Jan;3(1):29. https://doi.org/10.4103/2230-973X.108961
65. Nair AB, Chaudhary S, Shah H, Jacob S, Mewada V, Shinu P, Aldhubiab B, Sreeharsha N, Venugopala KN, Attimarad M, Shah J. Intranasal delivery of darunavir-loaded mucoadhesive in situ gel: Experimental design, in vitro evaluation, and pharmacokinetic studies. Gels. 2022 May 30;8(6):342. https://doi.org/10.3390/gels8060342
66. Nair AB, Shah J, Jacob S, Al-Dhubiab BE, Sreeharsha N, Morsy MA, Gupta S, Attimarad M, Shinu P, Venugopala KN. Experimental design, formulation and in vivo evaluation of a novel topical in situ gel system to treat ocular infections. PloS one. 2021 Mar 19;16(3):e0248857. https://doi.org/10.1371/journal.pone.0248857
67. Mei L, Huang X, Xie Y, Chen J, Huang Y, Wang B, Wang H, Pan X, Wu C. An injectable in situ gel with cubic and hexagonal nanostructures for local treatment of chronic periodontitis. Drug delivery. 2017 Jan 1;24(1):1148-58. https://doi.org/10.1080/10717544.2017.1359703
68. Mahmood T, Sarfraz RM, Mahmood A, Salem-Bekhit MM, Ijaz H, Zaman M, Akram MR, Taha EI, Sahu RK, Benguerba Y. Preparation, in vitro characterization, and evaluation of polymeric pH-responsive hydrogels for controlled drug release. ACS omega. 2024 Feb 19;9(9):10498-516. https://doi.org/10.1021/acsomega.3c08107
69. Kurniawansyah IS, Rusdiana T, Sopyan I, Desy Arya IF, Wahab HA, Nurzanah D. Comparative study of in situ gel formulation based on the physico-chemical aspect: Systematic review. Gels. 2023 Aug 10;9(8):645. https://doi.org/10.3390/gels9080645
70. Trott O, Olson AJ. Software news and update AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function. Effic. Optim. Multithreading. 2009;31:455-61. https://doi.org/10.1002/jcc.21334
71. Butt SS, Badshah Y, Shabbir M, Rafiq M. Molecular docking using chimera and autodock vina software for nonbioinformaticians. JMIR Bioinformatics and Biotechnology. 2020 Jun 19;1(1):e14232. https://doi.org/10.2196/14232
72. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 2010 Jan 30;31(2):455-61. https://doi.org/10.1002/jcc.21334
73. Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. Inflammation Research. 1998 Oct;47(Suppl 2):78-87. https://doi.org/10.1007/s000110050284
74. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational

protein–ligand docking and virtual drug screening with the AutoDock suite. Nature protocols. 2016 May;11(5):905-19. https://doi.org/10.1038/nprot.2016.051

75. Saddala MS, Huang H. Identification of novel inhibitors for TNFα, TNFR1 and TNFα-TNFR1 complex using pharmacophore-based approaches. Journal of translational medicine. 2019 Jul 2;17(1):215. https://doi.org/10.1186/s12967-019-1965-5

76. Halim SA, Sikandari AG, Khan A, Wadood A, Fatmi MQ, Csuk R, Al-Harrasi A. Structure-based virtual screening of tumor necrosis factor-α inhibitors by cheminformatics approaches and bio-molecular simulation. Biomolecules. 2021 Feb 22;11(2):329. https://doi.org/10.3390/biom11020329

77. das Chagas Pereira de Andrade F, Mendes AN. Computational analysis of eugenol inhibitory activity in lipoxygenase and cyclooxygenase pathways. Scientific reports. 2020 Oct 1;10(1):16204. https://doi.org/10.1038/s41598-020-73203-z

78. Zia K, Ashraf S, Jabeen A, Saeed M, Nur-e-Alam M, Ahmed S, Al-Rehaily AJ, Ul-Haq Z. Identification of potential TNF-α inhibitors: from in silico to in vitro studies. Scientific Reports. 2020 Dec 1;10(1):20974. https://doi.org/10.1038/s41598-020-77750-3

79. Pari Gul, Khan MIU, Ajmal S, Baloch K, Akhtar B, Anjum S. *In silico analysis of COX2 and TNF-α for therapeutic drug discovery in ulcerative colitis.* J Popul Ther Clin Pharmacol. 2024;31(3):932–946. https://doi.org/10.53555/jptcp.v30i17.5052

80. Mishra AS, Ghosh B, Malliappan SP, Dutta G, Vasanthan M. Formulation approaches for colon-specific drug delivery: conventional to nanocarrier systems. RSC advances. 2026;16(11):10022-59. https://doi.org/10.1039/D5RA05194K

81. Giuliano E, Paolino D, Fresta M, Cosco D. Drug-loaded biocompatible nanocarriers embedded in poloxamer 407 hydrogels as therapeutic formulations. Medicines. 2018 Dec 29;6(1):7. https://doi.org/10.3390/medicines6010007

82. Prasad AR, Thireesha B. UV-spectrophotometric method development and validation for the determination of lornoxicam in microsponges. inflammation. 2018;5:6. https://doi.org/10.22159/ijap.2018v10i1.22357

83. Mohammed-Kadhum MF, Hameed GS. Development and characterization of furosemide-loaded binary amorphous solid dispersion to enhance solubility and dissolution for pediatric oral administration. Pharmacia. 2025 Jul 4;72:1-9. https://doi.org/10.3897/pharmacia.72.e156784

84. Sinha VR, Kumria R. Polysaccharides in colon-specific drug delivery. International journal of pharmaceutics. 2001 Aug 14;224(1-2):19-38. https://doi.org/10.1016/S0378-5173(01)00720-7

85. Vijaya Rani KR, Rajan S, Bhupathyraaj M, Priya RK, Halligudi N, Al-Ghazali MA, Sridhar SB, Shareef J, Thomas S, Desai SM. The Effect of Polymers on Drug Release Kinetics in Nanoemulsion In Situ Gel Formulation. Polymers 2022, 14, 427 [Internet]. 2022 https://doi.org/10.3390/polym14030427

86. Kurniawansyah IS, Rusdiana T, Sopyan I, Ramoko H, Wahab HA, Subarnas A. In situ ophthalmic gel forming systems of poloxamer 407 and hydroxypropyl methyl cellulose mixtures for sustained ocular delivery of chloramphenicole: Optimization study by factorial design. Heliyon. 2020 Nov 1;6(11). https://doi: 10.1016/j.heliyon.2020.e05365

87. Trivedi R, Minglani VV, El-Gazzar AM, Batiha GE, Mahmoud MH, Patel M, Patel M. Optimization of pramipexole-loaded in situ thermosensitive intranasal gel for Parkinson's disease. Pharmaceuticals. 2024 Jan 29;17(2):172. https://doi.org/10.3390/ph17020172

88. Akram W, Garud N. Design expert as a statistical tool for optimization of 5-ASA-loaded biopolymer-based nanoparticles using Box Behnken factorial design. Future Journal of Pharmaceutical Sciences. 2021 Jul 21;7(1):146. https://doi.org/10.1186/s43094-021-00299-z

89. Kurniawansyah IS, Rusdiana T, Arya IF, Ramoko H, Wahab HA. Optimizing chemically stable chloramphenicol in-situ gel

formulations using poloxamer 407 and HPMC through full-factorial design. Scientific Reports. 2024 Oct 25;14(1):25344. https://doi.org/10.1038/s41598-024-74945-w

90. Pande V, Patel S, Patil V, Sonawane R. Design expert assisted formulation of topical bioadhesive gel of sertaconazole nitrate. Advanced Pharmaceutical Bulletin. 2013 Dec 24;4(2):121. https://doi.org/10.5681/apb.2014.019

91. Avijit Mazumder AM, Saha BP, Basu SP, Mazumder R. Antibacterial activity of methanolic extract of leaves of Lagerstroemia parviflora.

92. Menshutina N, Derkach V, Mokhova E, Gordienko M. Investigation of Rheological Characteristics of Thermosensitive Nasal In Situ Gels Based on P407 and Their Effect on Spray Pattern. Gels. 2025 Oct 21;11(10):841. https://doi.org/10.3390/gels11100841

93. Kouchak M. In situ gelling systems for drug delivery. Jundishapur journal of natural pharmaceutical products. 2014 Jun 1;9(3):e20126. https://doi.org/10.17795/jjnpp-20126

94. Srividya BJ, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. Journal of controlled release. 2001 Jun 15;73(2-3):205-11. https://doi.org/10.1016/S0168-3659(01)00279-6

95. Balakrishnan P, Park EK, Song CK, Ko HJ, Hahn TW, Song KW, Cho HJ. Carbopol-incorporated thermoreversible gel for intranasal drug delivery. Molecules. 2015 Mar 4;20(3):4124-35. https://doi.org/10.3390/molecules20034124

96. Tai A, Bianchini R, Jachowicz J. Texture analysis of cosmetic/pharmaceutical raw materials and formulations. International journal of cosmetic science. 2014 Aug;36(4):291-304. https://doi.org/10.1111/ics.12125

97. Shaikh R, Singh TR, Garland MJ, Woolfson AD, Donnelly RF. Mucoadhesive drug delivery systems. Journal of pharmacy and Bioallied Sciences. 2011 Jan 1;3(1):89-100. https://doi.10.4103/0975-7406.76478

98. Gebeyehu A. *Role of 3D Bioprinting in Tumor Disease Model Development, Chemotherapeutic Drug Screening and Delivery* (Doctoral dissertation, Florida Agricultural and Mechanical University). https://doi.10.3390/pharmaceutics12090859.

99. Begum MY, Chauhan A, Akhter MH, Alotaibi H, Almutairy BK, Alsunbul M, Ali MS, Ahmad S, Ibrahim IA, Shahid I, Shahzad N. pH-and Temperature-Responsive Hydrogel Composite Dual-Loaded with Neomycin and Bromelain for Enhanced Diabetic Wound Repair. Journal of Cluster Science. 2026 Apr;37(2):56. https://doi.org/10.1007/s10876-026-03017-y

100. Mardikasari SA, Katona G, Budai-Szűcs M, Kiricsi Á, Rovó L, Csóka I. Mucoadhesive in situ nasal gel of amoxicillin trihydrate for improved local delivery: Ex vivo mucosal permeation and retention studies. European Journal of Pharmaceutical Sciences. 2024 Nov 1;202:106897. https://doi.org/10.1016/j.ejps.2024.106897

101. Halder J, Mishra S, Saha I, Mishra A, Mahanty R, Rai VK, Pradhan D, Sahoo RK, Manoharadas S, Tata M, Kar B. Optimization and preparation of in-situ mucoadhesive gel of azithromycin hydroxypropyl-β-cyclodextrin inclusion complex against upper respiratory tract infections. BMC Pharmacology and Toxicology. 2025 Apr 29;26(1):93. https://doi.org/10.1186/s40360-025-00936-w

102. Li J, Yang L, Ferguson SM, Hudson TJ, Watanabe S, Katsuma M, Fix JA. In vitro evaluation of dissolution behavior for a colon-specific drug delivery system (CODES™) in multi-pH media using United States Pharmacopeia apparatus II and III. AAPS PharmSciTech. 2002 Feb;3(4):33. https://doi.org/10.1208/pt030433

103. Talukder RM, Fassihi R. Development and in-vitro evaluation of a colon-specific controlled release drug delivery system. Journal of Pharmacy and Pharmacology. 2008 Oct;60(10):1297-303. https://doi.org/10.1211/jpp.60.10.0005

104. Dash S. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010 Jan 1. https://doi.org/10.1021/jf103484z

105. Arifin DY, Lee LY, Wang CH. Mathematical modeling and simulation of drug release from microspheres: Implications to drug delivery systems. Advanced drug delivery reviews.

2006 Nov 30;58(12-13):1274-325. https://doi.org/10.1016/j.addr.2006.09.007

106. Vigata M, Meinert C, Hutmacher DW, Bock N. Hydrogels as drug delivery systems: A review of current characterization and evaluation techniques. Pharmaceutics. 2020 Dec 7;12(12):1188. https://doi.org/10.3390/pharmaceutics12121188

107. Mishra R, Mazumder A, Mazumder R, Mishra PS, Chaudhary P. Docking study and result conclusion of heterocyclic derivatives having urea and acyl moiety. Asian Journal of Biomedical and Pharmaceutical Sciences. 2019;9(67):13.

108. Lin SY. Current and potential applications of simultaneous DSC-FTIR microspectroscopy for pharmaceutical analysis. Journal of Food and Drug Analysis. 2021 Jun 15;29(2):182. https://doi.org/10.38212/2224-6614.3345

109. Paul A, Fathima KM, Nair SC. Intra nasal in situ gelling system of lamotrigine using ion activated mucoadhesive polymer. The open medicinal chemistry journal. 2017 Dec 29;11:222. https://doi.org/10.2174/1874104501711010222

110. Dass R, Rani P, Verma V, Kumar D, Bhatia M, Kondaveeti SB. Optimization and evaluation of gastro-expandable film of Eudragit S100 and ethylcellulose by using the design of experiment. Scientific Reports. 2026 Mar 28. https://doi.org/10.1038/s41598-026-45540-y