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Review Article

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ADVANCEMENTS IN FORMULATIONS AND TECHNOLOGIES FOR COLON-TARGETED DRUG DELIVERY

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ABSTRACT
Background: Colonic administration of drugs may enhance drug absorption, reduce adverse reactions, and facilitate delivery to specific therapeutic targets. **Objective:** Delivering pharmaceuticals to the colon poses challenges that require innovative formulation strategies. **Methodology**: Various formulation approaches have been explored for colon-targeted drug delivery systems. These approaches target the colon using formulation components that interact with GI physiology parameters such as pH, colonic flora, and enzymes. **Result and Discussion:** The article discussed the various research studies conducted for colon targeting involving novel strategies such as pH-dependent, enzyme-dependent, Ligand-Receptor-based, new technologies, Phloral, and magnetically derived approaches. It also explored the translational technologies, such as in vivo, in vitro, and in silico, which expedite the transition from fundamental research to clinical application and enhance therapeutic outcomes. **Conclusion:** In conclusion, the most relevant preclinical studies, encompassing in vitro, in vivo, and in silico research, are delineated to facilitate the strategic advancement of novel colon-targeted therapeutics.

INTRODUCTION

The predominant and most accessible method for patients to get medications is the oral route. However, it poses numerous obstacles. The current colon drug delivery systems encounter multiple constraints, such as inadequate bioavailability resulting from gastric acids and enzymes degradation, erratic drug release due to fluctuating gastrointestinal transit durations, and degradation by intestinal microorganisms. These drugs often face difficulties in attaining targeted release in the colon, as they may be absorbed prematurely in the gastrointestinal tract. Moreover, restricted formulation alternatives and insufficient control over the release of drugs lead to suboptimal treatments. Adverse effects, including irritation and discomfort, might diminish patient adherence, while conventional distribution techniques may be burdensome for users. These problems emphasize the need for advanced drug delivery technology.

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CDDS involves modified release pathways to ensure the drug reaches the colon before being released from an oral dosage form. This approach is advantageous for addressing multiple ailments, including inflammatory bowel diseases, colon carcinoma, and various localized gastrointestinal disorders [1,2]. Designing efficient CDDS requires a thorough understanding of the colon's physiology, microbiology, and structural characteristics, as these elements elucidate the colon's distinctive environment, surface area, microbes, pH levels, and prolonged transit time [3,4].

The 1.5-meter-long colon is a multifaceted organ comprising both the proximal colon (cecum, ascending colon, transverse colon) and the distal colon (descending colon, sigmoid colon, rectum, and anus). The large surface area may cause interactions between drugs and absorption challenges [5]. The fermentation of undigested dietary components by diverse microbiota in the colon results in the synthesis of short-chain fatty acids, such as butyrate, propionate, and acetate. Microorganisms can be used to develop microbial-induced drug delivery systems, in which drugs are released upon interaction with specific bacterial enzymes [6]. The pH level within the colon is typically alkaline, generally exceeding that of the GI system, which ordinarily ranges from 5.5 to 7.5. The pH may fluctuate due to diet, microbial activity, short-chain fatty acid production, and alterations in the function of bicarbonate transporters in the colonic epithelium. Targeted strategies can exploit the colon's elevated pH environment by developing pH-sensitive medication delivery systems. The colon has been recognized for its prolonged transit period, as monolithic tablets may extend to 20 to 30 hours [7,8]. The gradual movement enhances drug absorption and interactions with the intestinal mucosa, permitting the pharmaceuticals to remain in the colon for an extended duration. This characteristic is advantageous for extended-release formulations as it prolongs the therapeutic effect at the target site. Polymers are the essential element for attaining an effective colon-targeted system. CDDS strategies integrate traditional and new procedures, significantly contributing to the field. Colon-targeted medication delivery tactics combine conventional and innovative methods, thus considerably advancing the area [5].

This review article focuses on the various novel strategies for CDDS. An elaborate discussion of the environment that encompasses the colon facilitates developing strategies. The multi-stimuli targeted technology-based methods focus on the mechanisms that commonly operate in vivo. A comprehensive summary of relevant preclinical studies for new colon-targeted drugs will conclude the review, with emphasis on the best in vivo, in vitro, and in silico models for the translation of innovative treatment. Figure 1 represents the overview of the various formulation strategies discussed in this article.

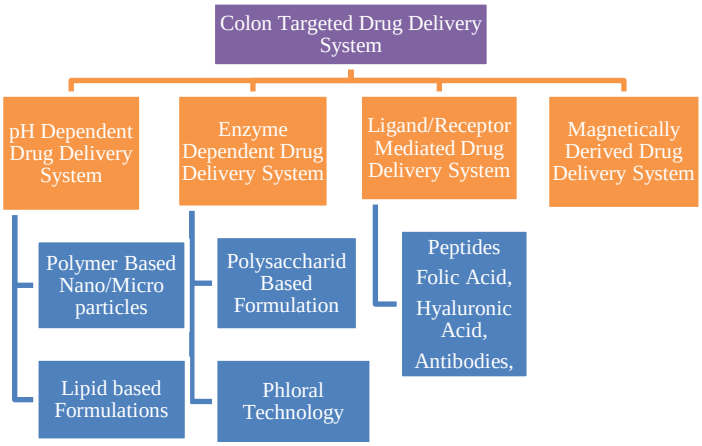

Figure 1: Represents the various formulation approaches for colon-targeted Drug delivery

VARIOUS APPROACHES FOR COLON-TARGETED DRUG DELIVERY

pH-dependent drug delivery system

The pH level of the gastrointestinal tract varies in most places; the lower ileum and colon are basic, while other parts are acidic. A pH-dependent system is a controlled drug release mechanism developed to release pharmaceuticals at varying pH values into the organism. The process of pH degradation is a simple approach to modifying drug delivery within the colon, which involves combining dosage forms with pH-sensitive biocompatible polymers to adjust pH levels. This is particularly useful for site-specific antibiotic administration. Eudragit® is a methacrylic acid copolymer commonly used for oral coatings and changing pH. Drug delivery systems that can be temporally controlled are proposed to have pH oscillators and membrane diffusion properties. The temporal control drug delivery systems are designed to release medication at precise intervals or in response to specific conditions, enhancing efficacy and minimizing adverse effects. Two proposed methodologies for such systems include pH oscillators and membrane diffusion characteristics. pH oscillators exploit the varying pH levels throughout different regions of the GI tract, responding to these fluctuations to release drugs at targeted sites. Membrane-based

drug delivery systems mainly depend on controlled diffusion through a membrane surrounding the drugs. These membranes permit the drug to diffuse at a regulated rate, which can be affected by environmental variables, such as pH, temperature, or the presence of particular enzymes. Together, pH oscillators and membrane diffusion properties allow for the precise, time-controlled release of drugs, optimizing treatment efficacy while minimizing side effects.

Drug release and failure in vivo were significantly influenced by the pH-dependent system, which had significant fluctuations in critical parameters, including pH, fluid volume, transit time of GI, and drug release variability. Lizbeth et al., [9] formulated nanogels bio-conjugated with shark antibodies (VNAR) for colon cancer that have 15% reduced cell viability and release kinetics of 5-FU per second at pH 5 as compared to pH 7.4 [9].

In another study, Abdulsalam et al., [10] prepared silicon nanoparticles which are specific to the pH level and allow for the expulsion of catechin through the supercase II transport mechanism at column pH less than 7, which could be an effective method for targeting its delivery to individuals within the colon [10].

As discussed above, various new approaches have been developed for CDDS to overcome the limitations of a conventional system. Figure 2 represents the various strategies, which include the enzyme-based system, receptor-based drug delivery system, magnetic-based drug delivery system, and Phloral technology. The pH-dependent drug delivery system can be achieved using polymer-based nano/micro particles and lipid-based formulation, which is discussed in detail below, and various examples are discussed in Table 1.

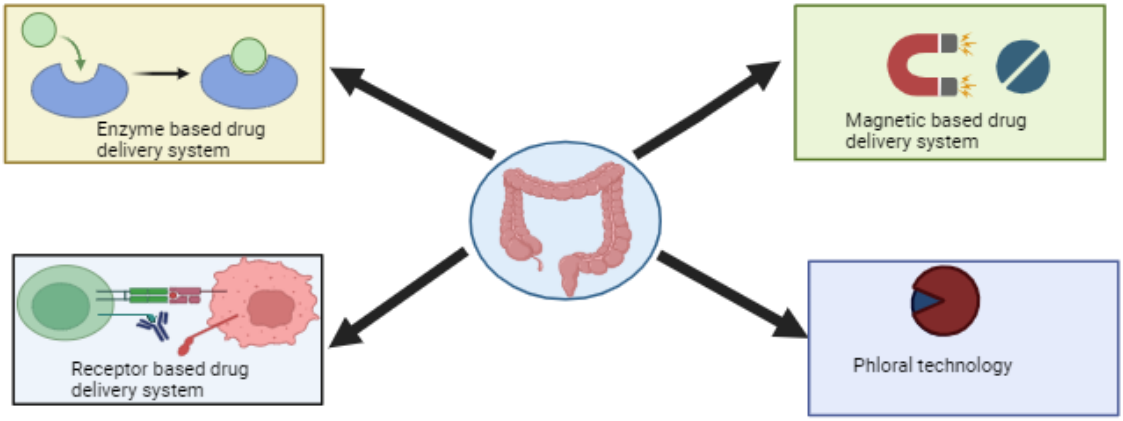

Figure 2: Representation of different strategies for colon-targeted drug delivery

Lipid-based formulation

In lipid-based drug delivery, liposomes were the most significant component. Liposomes are small, manufactured spherical vesicles potentially composed of cholesterol and natural phospholipids. Owing to their dimensions and hydrophobic-hydrophilic characteristics, liposomes serve as efficient vehicles optimized for drug delivery [11]. Because of its higher biocompatibility behaviour and biodegradability, the liposome carrier system became the first nano platform recognized for analytic use declared by the US FDA [12]. Makeen et al., [13] developed Gefitinib encapsulated nanostructured lipid carriers (NLCs), and the results demonstrated that optimised NANOGEF was 4.5 times more cytotoxic to HCT-116 cells than Gefitinib alone [13]. Alaaeldin et al., [14] formulated liposomes of Thymoquinone and Propolis for ulcerative colitis, results revealed that both thymoquinone and propolis liposomes combination were efficient in treating ulcerative colitis [14].

Polymer-Based Nano-/Micro-Particles Formulations

Polymers are regarded as crucial compounds in the drug delivery system. Because of polymers, numerous innovative medication delivery systems have advanced. Generally, biodegradable polymers exhibit hydrophilic behaviour and minimal swelling at acidic pH. Various colonic bacteria secrete numerous enzymes that can lead to the hydrolysis of glycolytic bonds, which is why biodegradable polymers are useful.

These polymers maintain their characteristics for a specified duration before gradually disintegrating into soluble molecules later eliminated from the body. Sivakumar R et al., [15] designed colon-specific delivery using nanoparticles loaded with sulfasalazine for inflammatory bowel disease, showing that these nanoparticles have a greater release capacity and are hence more effective as a treatment for inflammatory bowel disease [15].

Enzyme-based drug delivery system

Colon targets can be reached by enzyme response in polymeric systems made with degradable polymers responsive to colon enzymes. This tactic can also be applied to prodrugs, which activate in response to enzymes [16]. Since intestinal enzymes have a high substrate specificity, they are unique stimuli utilized increasingly as triggers in controlled release systems. For colon cancer targeted therapy, veratridine-loaded mesoporous silica nanoparticles (MSNs) were sealed with an enzymatically cleavable protein. Only colorectal tumor sites will receive drugs from the particles. MMP-7 protease is predominantly secreted by colorectal cancer cells; therefore, its release will boost tumor cell veratridine concentration, improving the novel therapy [17].

In another study, mesoporous silica nanoparticle (MSN)-based enzyme-responsive materials for colon-specific drug delivery were developed using guar gum as a capping layer to encapsulate 5-fluorouracil. Enzymatic breakdown of guar gum by colonic enzymes in the simulated colonic microenvironment notably released 5FU from GG-MSN. The drug showed anticancer effects in colon cancer cell lines in vitro, as flow cytometry and biochemical analysis showed. In varied simulated GI settings, the drug-loaded GG-MSN system showed near-flawless 'zero release' features without enzymes [18].

The incidence of colonic microflora is 1011-1012 CFU/ml and comprises anaerobic bacteria. Biodegradable polymers are preferred for the delivery of drugs into the colon, as biodegradable enzymes are found only in this organ. The drug is protected against the stomach and small intestine's environment, allowing it to enter the large bowel. Microorganisms, enzymes, or the polymer itself break them down once they enter the large intestine, lowering their molecular weight and, as a result, their mechanical strength [16]. The enzyme-dependent drug delivery system can be achieved using a polysaccharide-based formulation and a new technology, namely Phenol technology.

Polysaccharide based formulations

Polysaccharide-based formulations are relatively natural formulation concepts as they are not chemically modified. In addition, polysaccharides are inexpensive and easily available in various structures with many characteristics. The polysaccharide-based drug formulation is much more effective for CTDD. However, these systems possess limitations, including a broad molecular weight range and alterable chemistry of the polysaccharide. Variability in molecular weight can influence the release profile, potentially resulting in uneven release of drugs in the colon. High molecular weights can inhibit release, whereas lower molecular weights might induce early release in the upper GI tract. The capacity to alter the chemical structure of polysaccharides (e.g., via crosslinking or esterification) provides modification. Still, it may lead to unpredictable behavior, including modified stability or drug degradation, which impacts the system's efficacy and dependability in targeting the colon. Zhongqun Yue et al., [19] designed to increase anti-cancer efficacy, utilizing *Bletilla striata* to encapsulate andrographolide nano micelles, then incorporating AG into BSP-VES micelles (AG@BSP–VESS). The findings indicate that the formulation exhibits excellent stability, biosafety, sustained release of the drug at the targeted location, and possible anticancer efficacy [19]. Lee et al., [20] developed emulsion gels for postoperative adhesions of 5-fluorouracil, the *in-vitro* release study results revealed that sustained release of drug for 12 hours, and *in-vivo* indicated significant increases in Tmax (up to 4.03 times) and AUC (up to 2.62 times) [20].

Phloral® Technology

It is the first double-action colonic drug delivery. It contains resistant starches, including amylose and amylopectin, along with Eudragit®S. It is a single-layer coating system with separate release mechanisms [21]. It will keep the integrity of the tablet during its passage from the stomach to the small intestine. Besides, it has the characteristics of a starch structuring agent and modifies the swelling capacity. The colonic microbiota uses resistant starch as a substrate, offering an alternate way to initiate medication release if Eudragit® S's essential pH threshold is unmet. This technology has effectively proven promising results in IBD treatment, irrespective of patients' nutritional status [22]. OPTICORE™, an optimized colonic release combination system, has been developed to rapidly release drugs from the colon region. It is the first-in-class validated coating technique for colon alignment [23]. The internal layer of OPTICORE™ is based on the advantages of Duocoat® technology to accelerate the release of the drug from the enteric-coated dosage form. The outer layer of OPTICORE™ consists of a double release of Phloral® technology. Table 1. Summarizes the advanced lipid, polymer, and polysaccharide-based formulation developed for treating and diagnosing colon diseases.

Table 1: Advanced formulation approaches for the treatment and diagnosis of colon diseases

Formulation		API	Lipids/Polymers	Diseases	Key finding	Ref
Lipid based formulations	NLCs	5-Fluorouracil (5-FU)	Compritol and Oleic acid.	Colon cancer	Showed effectiveness in a cytotoxicity assay against cancer cells and shows greater oral bioavailability and sustained release in the colon.	[24]
	NLCs	Gefitinib	Stearic acid, Sesame oil.	Colon cancer	In HCT-116 cells, the MTT test revealed a 4.5-collapse increase in cell death when compared to gefitinib alone.	[13]
	NLC (BBR-NLCs)	Berberine	Glyceryl behenate and olive oil.	Ulcerative Colitis	Berberine-loaded NLCs decrease the symptoms of ulcerative colitis.	[25]
	NLC (8-QO-Pt-loaded NLC/RFV)	8-Oxyquinolinate-Platinum (II)	Capric triglyceride lipids	Colorectal cancer	It improved colon delivery and demonstrated increased cytotoxicity against the colonrectal cancer cell line HT-29.	[26]
	LIPOSOMES (5FU-chitosome)	5-fluorouracil (5FU)	Cholesterol	Colon Cancer	PEL treatment reduces HT-29 cell viability and also enhances 5-FU permeability and inhibits colon cancer cell growth.	[27]
	Camptothecin loaded liposomes	Camptothecin	Lipoid S 100 PEG and polydopamine	Colorectal cancer	Both (PEG) and (PDA) coatings increased epithelial permeability and promoted anticancer activity.	[28]
	LIPOSOMES (Lipo-CAZ-UA-Chi)	Ceftazidime and Usnic Acid	Cholesterol	Colorectal Cancer-Inducing E. coli	Showed antimicrobial properties activity and potential for oral delivery against germs that cause colon cancer.	[29]
	LIPOSOMES (Zanthoxylum alkyl amides liposomes)	Zanthoxylum alkylamides	Cholesterol	Caco-2 cell in colon	Caco-2 cells absorbed a passively adapted Zanthoxylum alkyl amide liposome formulation with superior absorption than submerged ones.	[30]
	Liposomes (LIP-TQ and LIP-PP)	Thymoquinone and Propolis	Cholesterol, Phospholipid 90 H® (PL90H) and Soy Phospholipid	Ulcerative Colitis	Combined treatment with these formulations was more effective in reducing UC inflammation.	[14]
	Solid lipid nanoparticles (QuR-SLN)	Quercetin	Stearic acid (SA) and tripalmitin (TpN)	Colorectal cancer	Resulted in cell death due to apoptosis. Potential cytotoxicity effects and ideal carrier in treatment therapy.	[31]
	SLNs (FEX-CS-SLNs)	Fexofenadine	Cetyl Palmitate	ulcerative colitis	Showed reverse Ulcerative colitis symptoms, and restored intestinal mucosal integrity.	[32]
	SLNS (DZN-FA SLNs)	Daidzein	Stearic acid, pluronic F-127	Colon cancer	Showed excellent inhibitory properties on Caco-2 cells.	[33]

Formulation		API	Lipids/Polymers	Diseases	Key finding	Ref
	SLNs (OXA-SLNs)	Oxaliplatin	Stearic acid, Tripalmitin	Colorectal Cancer	Showed higher cytotoxicity and showed increased apoptosis in colon cancer cells.	[34]
Polymer-Based Nano-and Micro-Particles Formulations	Sulfasalazine loaded nanoparticles	Sulfasalazine	Eudragit S100, Polyvinyl Alcohol (PVA) and Starch	Inflammatory bowel syndrome	Showed better release, improved the therapeutic effect of inflammatory bowel syndrome.	[15]
	(5-ASA-HbNPs) Nanoparticles	5-aminosalicylic acid	Dextran Sulfate Sodium (DSS)	Ulcerative Colitis	In this study, 5-ASA-HbNPs reduced disease activity and treated UC model mice.	[35]
	(CTB-PS-CS) Nanoparticles	Capecitabine	Potatoes Starch (PS) and (Poly(D-glucosamine))	Colon cancer	Carbohydrate-based nanoparticles showed improved colon histology, indicating antitumor activity in the colon.	[36]
	Tegafur-chitosan-coated polycaprolactone Nanoparticles	Tegafur	Chitosan and Polycaprolactone	Colon cancer	Anticancer activity was increased by roughly four times, while the half-maximal inhibitory doses in HT-29 and T-84 cells were decreased by around 3.5 times.	[37]
	Cur–Art Nio-NPs Nanoparticles	Curcumin and Artemisinin	NA	Colon cancer cells	High SW480 cell cytotoxicity. Downregulated BCl_2, Rb, and Cyclin D1 and upregulated Bax, Fas, and p53.	[38]
	Mesalamine Loaded Microspheres	Mesalamine	Eudragit S-100, PLGA' Sod. Alginate	Colon-specific drug delivery	Two-fold increase in drug release and strong caecal biodegradability in comparison to previous methods.	[39]
	Microparticles (MP-LP-V)	Lactobacillus plantarum	Sodium Caseinate and Chitosan	Immunomodulatory	Showed immunomodulating effect in mice and shown superior protection against lacto inactivation.	[40]
	Resveratrol-loaded microparticles	Resveratrol	Carboxymethyl Cellulose, Tragacanth Gum and Fish Gelatin (FG)	Colon targeted delivery	Delivered resveratrol into the colon without premature release in simulated gastric circumstances.	[41]
	Quercetin loaded microparticles	Quercetin	Chitosan and Hydroxypropyl Methylcellulose (HPMC)	Inflammatory bowel diseases	*In-vitro* results showed significant release of quercetin. Potential for use in clinical settings to treat acute IBD.	[42]
Polysaccharide based Nano formulations	Chitosan hydrogel loaded nanoparticles	Puerarin	*Bletilla striata* polysaccharide	Ulcerative colitis	Excellent mucosal penetration, colon drug retention, and drug release control.	[43]
	Polysaccharide-based emulsion gels	5-fluorouracil	Pectin and Xantham gum	Postoperative tissue adhesions	*In-vivo* anti-adhesion efficacy results showed significant anti-adhesion capacity ($P < 0.01$). Showed no cytotoxicity to epithelial cell lines or fibroblasts.	[20]
	Xanthan gum-based multi particulate	Metronidazole	Xanthan gum and Xanthan gum	Colon targeting	Efficiently manage metronidazole delivery. Does not alter in the colon or stomach.	[44]

Formulation	API	Lipids/Polymers	Diseases	Key finding	Ref
Polyguluronic acid polysaccharides formulation	Doxorubicin	Alginate	Colon cancer	Showed sensitive drug release in response to GSH depletion, which improves therapeutic effectiveness and drug accumulation at tumour locations.	[45]
Pectin-based nanocarriers	Pectin	Pectin	Colon Targeted	Exhibited excellent stability, safety, and gelling ability with low toxicity, immunogenicity, and biodegradability.	[46]
Mesalazine-loaded macroparticles compressed tablet	Mesalazine	Opuntia ficus Indica polysaccharide	Ulcerative colitis	In-vivo result confirmed probiotic mass and medicine are more effective at preventing illness.	[47]
Fucoidan-based irinotecan-loaded nanoparticles	Irinotecan	Fucoidan (FDC), Gamma-polyglutamic acid	Colorectal carcinoma	Showed more cellular absorption, mainly in p-selectin-positive colorectal cancer cells HCT116.	[48]
Budesonide pellets	Budesonide	Tamarind gum and Microcrystalline cellulose (MCC)	Ulcerative colitis	It is effective in targeting the colon, and its 2.5-3 g ratio with 6% enteric coating provides an optimized formulation.	[49]
CCM-loaded S-SNEDDS	Curcumin	Pectin	Colon tumour	Showed an initial 5-hour drug release of less than 10% and a 5- to 10-hour burst release in rats.	[50]

Ligand/receptor-based drug delivery system

Another technique for colon-targeted therapy is a ligand/receptor-based drug delivery system. This system aims to attain effective localized treatment of colon diseases with minimized toxic side effects and improve specificity towards targets at disease sites. Various ligands, including peptides, folic acid, hyaluronic acid, and antibodies, can facilitate this drug delivery process.

The antibodies and their variants, which include fragment antigen-binding and fragment crystallizable region fusion proteins, were engineered for targeted CDDS. Development of anti-transferrin receptor antibody-conjugated liposomes for localized drug delivery to the inflamed intestinal mucosa, the *ex vivo* studies showed increased accumulation of anti-transferrin immunoliposomes in the inflamed mucosa of rats compared with non-specific immunoliposomes [51,52]. Studies demonstrated that the conjugation of nanoparticles with folic acid might be used to develop targeted drugs. For example, Navya Ajitkumar et al., [53] folic acid-chitosan functionalized polymeric nanocarriers encapsulating irinotecan and quercetin for colon cancer treatment. Results revealed that formulation has a potential substitute for intravenous IRI formulations in rats [53]. Another ligand, Hyaluronic acid (HA), is a polysaccharide found in mammalian connective tissues and plays an essential physicochemical and biological role within the extracellular matrix. It suppresses the growth of some cancer cells, such as those found in the breast, prostate, bladder, melanoma, and fibrosarcoma cells, in a dose-dependent way. Because HA binds to the CD44 receptor, overexpressed in many malignancies, studies have focused on using targeted, selective delivery systems conjugated with a drug delivery system [54]. Recently, interest has been shown in the peptide as a potential ligand for CTDDS [55]. It enhances the drug delivery system's transport capacity by acting as a carrier to deliver anticancer medications to locations [56]. Andretto et al., [57] formulated a peptide-based hydrogel Nano system to encapsulate intestinal inflammation and conducted studies on their effectiveness in a murine model of intestinal swelling; The results revealed that the disease score can be enhanced with oral drug administration [57]. Table 2 summarizes the various ligand-based formulations for treating and diagnosing colon diseases.

Table 2. Advanced Ligand-based formulation approaches for treatment and diagnosis of colon diseases.

Formulations	API	Ligands	Diseases	Key Finding	Ref
Fc-fused PD-L1 nanoparticles	α-Cyclo dextrin (α-CD)	Fc-fused PD-L1 antibody	Acute and chronic colitis	Improved mouse colitis model PD-L1 effectiveness and safety. Regulated D-L1-Fc accumulation and release at colonic inflammatory sites.	[58]
Irinotecan and Quercetin loaded NPs	Irinotecan and quercetin	Folic acid-chitosan conjugates	Colon cancer	Potential replacement for intravenous IRI formulations in rats.	[53]
Lactoferrin nanoparticles modified with folic acid and coated with curcumin	Curcumin	Folic acid-modified lactoferrin	Ulcerative colitis	Decreased inflammation, enhanced the colonic mucosal barrier, reduced colitis symptoms, and brought the intestinal flora back into equilibrium.	[59]
Conjugated chitosan modified PLGA nanoparticle	alpha-terpineol	Folic acid conjugated chitosan	Colon cancer	Inhibitory effect on cancer cells as well as a pro-apoptotic effect that was demonstrated in HT-29 cells.	[60]
Hyaluronic acid/geltine hydrogels loaded microspheres	Curcumin	Hyaluronic Acid	Inflammatory bowel disease	Prevented macrophages from secreting the pro-inflammatory cytokines TNF-α and IL-6 which shown the greatest therapeutic impact in animals with colitis.	[54]
Hyaluronan enema-based suspension	Dextran sulphate sodium	Sodium hyaluronate	Ulcerative Colitis	In a mouse model of colitis, intestinal epithelial protection and reduced inflammation were observed.	[61]
Methotrexate loaded nanoparticles	Methotrexate	hyaluronic acid	inflammatory bowel disease	*In-vivo* imaging demonstrated intestinal accumulation and extended retention time of MTX in animals with colitis.	[62]
Hyaluronic acid–doxorubicin loaded NPs	Doxorubicin	Hyaluronic acid	colorectal cancer	It reduces the expression of inflammatory and intestinal apoptotic markers in mice.	[63]
Peptide-Based Hydrogel	-	Peptide	Intestinal Inflammations	Showed improvement in disease score.	[57]
Anionic lipid-based nanoformulation	-	Thuricin CD	Clostridioides difficile	When both peptides are present in the intestinal area, thuricin CD is active at low doses.	[64]
Peptides derived from turtle peptide improved Sodium sulphate dextran	Dextran sodium sulfate	Turtle peptide	Ulcerative colitis	Modified the makeup of the gut microbiota and suppressed inflammatory factors.	[65]
Quaternary chitosan nanoparticles	Curcumin	Peptides from egg whites	Ulcerative colitis	Hydrophobic nutrients and peptides reversed chronic illnesses.	[66]

Magnetically Driven Drug Delivery System

In a magnetic nanoparticle drug delivery system, magnetic particles such as iron act as carriers and are guided by a magnet. It enables drug delivery materials, including micelles, liposomes, and polymers, to have imaging and controlled release properties [67]. The mAb198.3, a monoclonal antibody specific to FAT1, enhances the targeted treatment of colorectal cancer. mAb198.3 bonded directly to both Erythro-Magneto-Hemagglutinin Virosomes (EMHVs) implanted in magnetized erythrocyte-based carriers and Super-paramagnetic Nanoparticles (spmNPs).

The investigation found that mAb198.3 formulations efficiently targeted the colon cancer cells, requiring roughly 200 times lower antibody concentrations to achieve the therapeutic index. Other investigations included the development of smart silica

gate magnetic mesoporous particles for targeted colon drug delivery. Hydrocortisone (S2) and safranin O (S1) were utilized to treat inflammatory intestinal disorders. A mouse model was used for the experiments. *In-vitro* tests revealed that S1 and S2 microparticles had a regulated release in the colon's reducing environment. In contrast, in vivo pharmacokinetic results revealed that S1 in the colon released safranin O in a concentrated manner. To treat colitis caused by 2,4,6-trinitrobenzenesulfonic acid, S2 microparticles were tried [67–69].

Technology translating for actual patient benefits

***In-vitro* Evaluation**

Despite the challenge of reconciling *in vitro* analysis results with estimated *in vivo* outcomes, evaluating disintegration time, dissolution rate, and stability of CDDS is a crucial step in developing methodologies that account for the potential physiological characteristics of the colon [70,71]. One may identify the management of formulations at various pH by carrying out parallel dissolving trials in various buffers. Intentional dissolving investigations of a colon-specific formulation were carried out in multiple conditions replicating pH ranges and timeframes that are likely to be encountered at various places in the gastrointestinal tract.

The formulation intended for colon delivery, which includes sophisticated dissolution approaches combined with drug stability formats - like bicarbonate buffers, simulated intestinal fluids, animal fluids/tissues, human faecal slurries, and relevant hydrodynamics - combined with specially designed permeability studies, has been subjected to attack for several decades [72].

Dissolution for oral dosage forms

Bicarbonate buffers

USP-standard buffers, including phosphate, citrate, acetate, and hydrochloric acid, are critical to determining the drug release in pharmaceuticals with a wide range of pH and with definite ionic substance and buffer capacity. Bicarbonate buffers for the biorelevant dissolution experiment of CTDDS were envisaged in 2005. This represents the intestinal fluid's buffer volume more than phosphate buffers. The constant evaporation of CO_2, which raises the pH of the buffer solution, is the bicarbonate medium's drawback. Both automatic and manual techniques are developed to maintain the bicarbonate buffer solution's pH during the test [73].

Bile salts and enzymes

Simple aqueous buffer systems are not the same as colonic fluid. Enzymes and bile salts are crucial for solubilizing and releasing drugs from particular formulations. Colonic enzymes are essential for evaluating the release of drugs from formulations using the enzymatic degradation of coatings [74] . Microflora from digestive fluids or human or animal excrement can create enzymes [75]. Microbial enzymes that operate on carbohydrates (such as glycoside hydrolase, polysaccharide lyase, and carbohydrate esterase) and enzymes that metabolize pharmaceuticals (such as phosphorylases, reductases, and decarboxylases) are the two main essential enzymes in colonic fluid [76,77].

Faecal Slurries

Faecal slurries are primarily employed in predicting colon drug release, solubility, and stability. These consist of fresh feces and media simulating the physiology of the colon that stimulates microbial growth. Fresh human feces have a mean pH of 6.64 with the following inorganic contents: potassium, phosphate, sodium, calcium, and magnesium; proteins (2-25%), lipids (2-15%), and undigested polysaccharides (~25%); and microbiota (25-54%) [78,79].

Its formulation can be tailored to coincide with physiological fluctuations, including pH, bile acid levels, and buffering capacity. Yet, the inherent pH of the paste and available carbon sources have an immense influence on microbiota structure and activity. Bacteria excrete organic acids that can accumulate, reduce the pH of the media, and result in bacterial stasis/death. In static batch culture, raw medicines and formulations were incubated in feces slurry for ≤ 24 hours, with periodical sample withdrawals for dissolution and stability analysis. For prolonged incubations, continuous culture systems replenish spent culture with a fresh growth medium [80]. Advanced techniques mimic the colon regions and adjust acceptable pH and transit time. The Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME®) is a model representing the anatomy of the colon. Up to four weeks of treatment are possible with this model. Additionally, microcosms in the colonic regions of the M-SHIME® enable the cultivation of luminal and mucosal bacteria. Rodent feces may be employed; although the mouse/rat microbiome is distinct from the human colon in terms of composition, the proportion of bacteroides and bifidobacterial in rat feces is similar [81].

In-vitro colonic permeability

The biological differences in the colon from the small bowel require using unique measurements to predict colonic drug permeability. Immortalized Caco-2 cell lines are generally used for predicting permeability of through epithelia; however, they are not free from culture-dependent variability and fail to express essential enzymes, like CYP3A4, and a mucus layer [82]. The transporters expressed by Caco-2 cells include those found in the small intestine, though these are cells generated from the colonic epithelium. The investigation study, including 18 medicines of small molecular weight, was used to validate the model under specific settings, and the results showed a high correlation (R2 =0.74) between Caco-2 permeability and human intestinal absorption. Since cell lines include biological sex, which is frequently neglected, a single cell line alone cannot discriminate gender variability for a drug permeability study.

HT-29 and LS 174 T are female colonic epithelial cells, while Caco-2 and C2BBel are male. Approaches to estimating colonic drug permeability using cell lines should be conducted in both male and female cells. Organoid systems, colons-on-a-chip, and human colonic tissue chambers may be able to test the colonic permeability of drugs. They may potentially make available, for drug discovery applications, more biologically relevant epithelial landscapes than those created through standard Caco-2 permeability tests [72].

In-vivo Evaluation

When designing new formulations for the colon, *in vivo* animal models that most nearly mimic the physiological functioning of the human gastrointestinal tract are essential. The cost, opportunity, and convenience of administration commonly determine the choice of animal models, leading to more variability than physiological similarities. Animal gastrointestinal anatomy and physiology differ much more between species than does medication absorption and bioavailability. Researchers employed various animal models, like mice, rabbits, rats, and guinea pigs, and larger animals like dogs, pigs, and nonhuman primates, which are popular choices. The features of the medication or other consumable form, the expected testing limits, and the treatment indication will probably determine which animal model is best. Additionally, the equal resemblance of male and female mammals in pre-clinical research is crucial for formulations used to treat patients of both genders [83].

Colonic anatomy of animals

The anatomy of the colon differs significantly among species because of evolution driven by particular nutritional absorption and processing requirements. Mice and rats are valuable models for studying colorectal cancer because they have intestinal anatomy and functions similar to humans. Nevertheless, they lack submucosal adipose tissue and have a non-sacculated colon [84]. Additionally, dogs probably have larger intraluminal pressures compared to humans. Consequently, dogs' gastrointestinal tracts may break down formulations faster and be prone to mechanical stress. Pigs' gastrointestinal tracts are physically similar to those of humans, especially in terms of the length of intestine segments and surface area. Due to their cecotrophic lifestyle, rabbits have a well-defined caecum; they expel faeces that are contaminated with microbes that must be reingested to absorb nutrients fully [84].

Colonic fluids of the animal

The animal's gastrointestinal fluid volume and pH are essential for accurately predicting the breakdown, dissolution, and absorption of drugs in humans. It has been found that the intestinal pH of a healthy pig, rabbit, and guinea pig was comparable to that of a human. The colonic pH values of a beagle breed dog ranged from pH 5 to 8, with high pH between the small intestine and colon. The use of rodents to examine pH-stimulated colonic delivery formulations must, therefore, be done with some caution if the researcher intends to extrapolate the release of a formulation to humans. However, although undigested food may limit drug solubility, rats, fed or not, appear to be the animal model with the most equivalent free fluid volume to that of humans. As shown in humans, age can cause major physiological changes in animals employed in drug delivery experiments. The young rats of 4 weeks had a smaller colon, lower fluid volumes, and a larger buffer capacity than the elderly rats of 38 weeks. Human, guinea pig, rabbit, and pig colonic fluid viscosities are similar. However, the increased water content in these animals' guts may make medication-dissolving predictions inaccurate[85].

Colonic transit time of the Animal

The motility of the GI tract is one of the significant determinants of oral bioavailability of medications. Intestinal transit times among animals are highly variable. Rats have the shortest transit time of 6.2 ± 21.2 mins, which is prolonged for prokinetic drugs, while humans exhibit 21 hours of colonic transit time [86]. The

gastric emptying times of pigs significantly exceed those of humans, ranging between 68 and 233 hours for Landrace pigs, 24 to 672 hours for Yorkshire pigs, over 54 hours for Yucatan pigs, and beyond 48 hours for Gotten minipigs. Thus, pigs may not be the best model for transit-time-affected colon-targeted formulations. In contrast, the dog may be more similar to a human's colonic transit [87]. A study involving 31 healthy adult dogs of diverse breeds, including 14 females, revealed that the total gastrointestinal transit time varied from 21.57 to 57.38 hours, while the colonic transit time ranged from 7.12 to 42.88 hours. Research indicates that the beagle, a widely utilized model in pharmaceutical research, exhibits colonic and overall gut transit times comparable to humans, ranging from 25.4 ± 3.3 hours in a fasted state to 28.2 ± 4.7 hours while fed. The researchers expected that beagle colonic transit would be enhanced, as capsules may traverse the proximal and distal colons more swiftly and subsequently reside in the rectum for an extended duration [87].

Although the animal models provide insightful information, but species-specific physiology, metabolism, and immunological responses limit their applicability to humans. Humans have more genetic diversity than animals, which can cause different reactions. Controlled laboratory environments and limited animal study durations may not replicate human complexity or long-term impacts. Due to ethical and practical limits, animals cannot fully replicate human diseases, and behavioral or cognitive differences may alter therapy processing. Thus, while animal data is crucial, caution is needed when applying these findings to humans, and more clinical research is required to validate these findings.

In-silico Evaluation

The medicinal product development process is characterized by significant risk and considerable expense. Hence, a strong demand exists for forecasting in vivo behavior and expediting formulation development while safeguarding valuable assets. Various predictive technologies help in the development of new CTDDS, such as design of experiments (DoE), molecular dynamics (MD), mechanistic modeling (MM), machine learning (ML), and other, less popular methods like computational fluid dynamics and finite element analysis, as illustrated in Figure 3 [88].

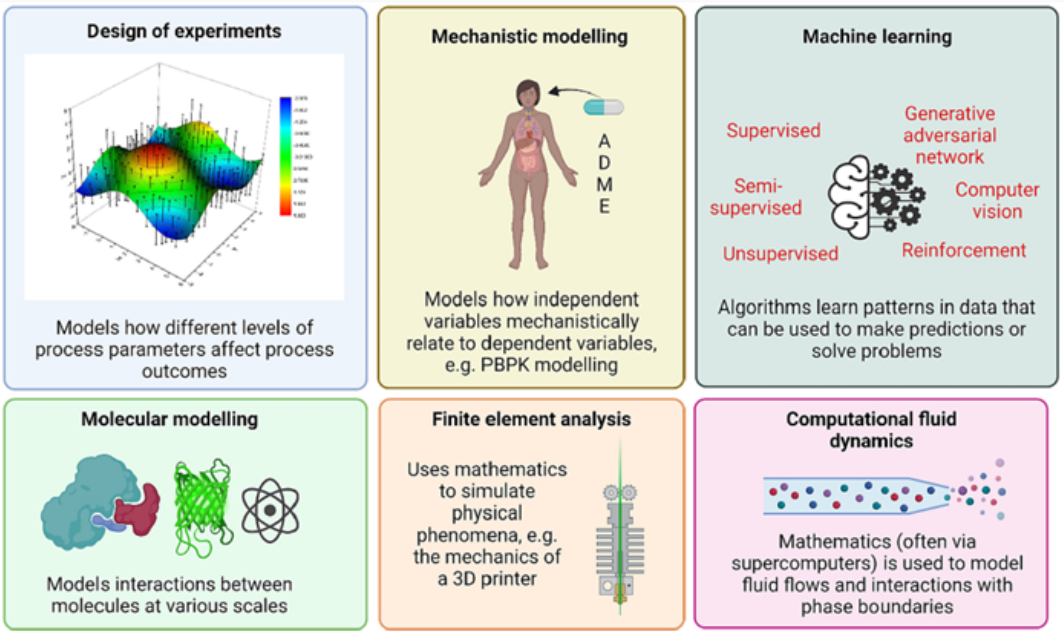

Figure 3: In-silico investigation in developing colon-targeted therapeutics. Copyright and reposted from Elsevier [89].

Design of Experiment

DoE is a widely used method in pharmaceutical research to forecast the effects of certain process attributes on process results. Numerous research studies employ DoE to optimize the composition of enteric polymer-based colon-targeted coatings for successful in vivo administration. In addition to optimizing coating composition, DoE can be used for modeling and predicting the effects of other formulation parameters, such as

the size or shape of dosage forms or the production process on colonic drug release reliability. For large datasets where the number of independent variables is more than 10, or for complex, nonlinear relationships between the independent and dependent variables, DoE is less beneficial. Alternative techniques for prediction may be more suitable in these instances [90,91].

Molecular modeling and mechanistic modeling

Molecular modeling is a computational method for simulating molecule interactions using classical and quantum physics equations. Depending on the experiment's needs and resources, numerous techniques depict molecular systems, including coarse-grained and full atomistic models. MD has proven itself as a reliable in silico method for drug discovery because of its capacity to precisely predict dynamic interactions at the atomic level, such as by making it possible to analyse drug-target binding.

In drug development, there are many applications of MD in formulation design and mapping the mechanisms of drug loading and release in stimuli-responsive formulations for targeted dose formulations that are highly explicable in vivo responses [90,92]. MM includes mathematical formulas that show how independent and dependent variables interact. The formulas derive from predictions based on current experimental data and serve as simplified visualizations. PBPK models estimate how ADME influences drug concentrations in plasma and tissues and are thus used in drug development to design drugs. GI-Sim and GastroPlus, two popular PBPK programs, can estimate the intestinal absorption of 14 drugs with sufficient accuracy to replace *in vivo* localized absorption in dogs during preclinical drug development. MM is capable of handling both small and enormous datasets, determining the mechanical interactions between variables inside a model, and understanding the mathematical foundation upon which predictions are based [93].

Machine Learning

Machine learning (ML) is potentially used to enhance drug development across all stages, from discovery to clinical trials, while reducing associated costs. ML is primarily categorized into two types: supervised and unsupervised. Supervised learning uses labeled datasets, such as a big drug dataset with colonic fluid solubilities [93]. The algorithm in supervised machine learning learns how to sample properties, such as medication solubility in intestinal fluid, to impact label prediction. Unsupervised learning employs unlabelled datasets, and one might use methods such as dimension reduction and clustering to identify data patterns. Semi-supervised learning uses unsupervised techniques to identify unlabelled data and then applies them to supervised machine learning problems. New developments in machine learning, including generative models, multi-task learning, active learning, and reinforcement learning, offer more ways to make use of existing experimental data and increase the effectiveness of upcoming experiments [94–96]. Furthermore, based on excipient composition, an ANN performed better than conventional design-of-experiment (DoE) models in forecasting nanoparticle size and drug loading efficiency. It also forecasts intricate drug-microbiome interactions, demonstrating its wide range of applications in improving drug development procedures [94,97]. Reker et al. demonstrated the powerful synergy of machine learning (ML) and molecular dynamics (MD) simulations to identify 100 new solid drug nanoparticles, formed through self-assembling drug-excipient combinations. The researchers were able to characterize two of these nanoparticles, both *in vitro* and *in vivo*, which revealed the potential of these approaches. This work highlights the value of combining advanced *in-silico* technologies with big data analytics to speed up and improve the process of formulating drugs while opening new avenues for better, more targeted drug delivery systems [98]. In another study, ML predicted 5-aminosalicylic acid release from polysaccharide-based coatings in simulated human, rat, and dog colonic environments. Polysaccharides were characterized using Raman spectra for ML characteristics. Validation on 8 novel polysaccharide-coated drug release profiles showed the method's generalizability and dependability. The model study also helped explain how chemical properties affect the colonic distribution. This study's RF model simplifies colon-targeted formulation coating creation, which can improve future colonic medication delivery programs' efficiency, sustainability, and success. This technique can pre-rank polysaccharide coatings before experimental validation of top hits, minimizing experimental needs for new formulations [99].

CONCLUSION

Novel promising drugs can be developed using modern knowledge of colonic physiology, novel formulation approaches, and enhanced *in vitro, in vivo, and in silico*

evaluation technologies. However, caution must be taken when applying these findings to humans, and more clinical research is needed to validate these findings, as animals cannot fully replicate human diseases due to ethical and practical limitations.

Future colon-targeted therapy research should improve medication delivery technologies that target the colon while minimizing systemic side effects. Exploring the gut microbiome's function in drug metabolism and disease development could lead to tailored therapy. Future research should also examine noninvasive colon disease detection methods and improve animal models to replicate human colon physiology for preclinical testing better. In addition to the integrated methodologies, exploring nanotechnology appears to be a promising domain for advancing research in medication targeting within the colon.

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All the authors contributed to the manuscript. Ritik Singh Rana drafted it. Yogita Ale revised, supervised, and visualized it. Vikash Jakhmola supervised the whole work. Pankaj Pant and Neha Kukreti provided the funding acquisition and resources.

FINANCIAL ASSISTANCE
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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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