EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 1
Year: 2025

COLON TARGETED DRUG DELIVERY: AN EXTENSIVE OVERVIEW

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Article Received on 15/11/2024 **Article Revised on 04/12/2024** **Article Accepted on 25/12/2024**

ABSTRACT
In addition to providing access to specific therapeutic targets, colon drug administration may improve drug absorption while lowering off-target effects. Formulation development is necessary for drug delivery to the colon since the unique physiological surroundings of the colon might provide difficulties for both oral and rectal dose forms. The international pharmaceutical market for biologics has expanded recently, and the growing need for a better drug delivery system emphasizes the significance of colonic drug delivery as a non-invasive method of delivering macromolecules. Better patient compliance and reduced expenses are two therapeutic advantages that this method may offer. Consequently, a number of strategies, including pH-dependent, enzyme-triggered, receptor-mediated, and magnetically driven systems, have been investigated in order to accomplish efficient local and systemic colon drug delivery. Recent developments in a variety of methods for creating colon-targeted drug delivery systems and their pharmaceutical uses are discussed in this study. The best in-vitro, in-vivo, and in-silico preclinical research are then conducted.'

KEYWORDS: Drug delivery system, Bioavailability, Biologics, Colon targeting, preclinical investigation.

INTRODUCTION

The importance of colon drug delivery is growing as a result of several pharmacological advantages and prospects that have been identified recently.[1] By targeting the colon, drug can be more effectively treated for local conditions, provide support to local therapeutic aims, lower systemic exposure of drug and related toxicity, as well as even increase drug bioavailability.[2,3,4] For a number of bowel diseases, following ulcerative colitis, amebiosis, colonic cancer, Chron's disease, and other colonic pathologies, the localization of colon medicine administration offers substantial benefits and minimizes adverse effects.[5] Furthermore, it makes it possible for protein and peptide drug to be distributed efficiently throughout the body, which would otherwise be negatively impacted by significant pH variations across the stomach and also the small intestine.[6] Either the oral or rectal approach is used to target the colon. Since it is difficult to target particular places in the colonic area and causes discomfort for patients, the rectal route is not commonly utilized and is typically saved as a backup method for emergencies and relives. Because of its greater patient acceptance rate, non-invasiveness, ease of administration, and efficacy—all of which increase the likelihood of treatment adherence—the oral route is recommended. Additionally, It is a safe administration technique that doesn't require sterile production conditions, which facilitates the design of such systems and offers industrial flexibility and lowers production costs.[7,8] Because the colon is the furthest region of the digestive system, it is hard to administer the highest dose of drug that does not breakdown or absorb in the small intestine or stomach. The stomach's very acidic pH may hasten the digestion of pH-sensitive drug and proteins, but proteolytic enzymes in the gastrointestinal tract and small intestine can also denature them. Furthermore, a medicine with low solubility may dissolve poorly in the colon due to its low water content. The microenvironment around a disease location in the colon differs significantly from normal and healthy tissues. Patients having colonic diseases also have mucosal damage, an imbalance of critical antioxidants, and an elevated level of reactive oxygen species (ROS) with inflammatory cytokines. To optimize colonic drug delivery, a variety of formulation options have been examined, including pH-sensitive systems, enzyme-triggered systems, along with magnetically driven systems. Because of the fact that during formulation creation, It is crucial to consider the modifications that take place in the microenvironment surrounding disease sites. Studies have also been conducted on receptor-

mediated systems that selectively interact with certain receptors that are overexpressed at the site or sites of illness in order to improve the specificity at disease sites. This paper discusses preclinical research, smart polymers, variables influencing colonic drug delivery systems, and new developments in different formulation processes for creating drug delivery systems that target the colon.[8,9]

DRUG CRITERIA FOR A COLON-SPECIFIC DELIVERY

The following physico-chemical/therapeutic requirements should be met by drug that are formulated into a colon-targeted delivery system.

- In order to treat intestinal diseases, these drugs need first show local effects in the colon. Examples of substances with these effects include non-peptide drug like oxyprenolol and peptide drug like amylin.
- Secondly, the upper gastrointestinal system may not absorb these drugs as well as it should. Antianginal drugs like isosorbide dinitrate fall within this category. Drugs like 5-flurouracil and capecitabine, which are used to treat colon or rectal cancers, work well with CDDS.
- The third need is that the drugs must either have a higher risk of first-pass metabolism (corticosteroids, for instance) or be very susceptible to undergoing digestion in the digestive system by the presence of acid or enzymes (peptide drugs, such as insulin and gonadorelin, for instance).

LIMITATIONS OF COLONIC DRUG DELIVERY

There are particular restrictions and difficulties involved in the formulation as well as creation of drug forms tailored to the colon. The colon is a problem since it is situated within the distal portion of the gastrointestinal tract (GIT). To arrive to the intended location, a dose form taken orally must pass through the whole alimentary canal. There is a large variety of pH levels, fluid quantities, and transit periods in the complicated physiology of the GIT. Furthermore, the physiological complexity is increased by the existence of food as well as metabolic enzymes. The effective and dependable transport of drugs to the colon is hampered by these variables.

The solubility of the drug is the second part. The solubilization of the drug may be a rate-limiting issue for colonic absorption because of the neutral pH, greater viscosity, and reduced volume of colonic luminal fluid.

Lastly, it may be difficult to keep the drug stable in the stomach. The stability of the drug may be adversely affected by non-specific interactions between the drug and the colonic contents, including food scraps, digestive fluids, mucus, and even feces.[10] Moreover, the drug may be broken down by the gut bacteria's enzymes, making it useless.

FACTORS AFFECTING COLON-TARGETED DRUG DELIVERY AND BIOAVAILABILITY

- Physiologic and Anatomical Aspects
- Transit Time from the Intestine to the Colon
- Colonic Fluid Volume
- Viscosity of Colonic Luminal Contents
- Colonic pH
- Metabolic Enzymes in the Colon
- Aspects of Formulation

CLASSICAL METHODS TO ACHIEVING COLONIC DELIVERY

Precursor/Prodrugs

After being digested by enzymes like those in the colon, Inactive drug molecules known as prodrugs release the active component. The degree of this hydrolysis should be substantially greater in the colon and much less inside the upper gastrointestinal system in order to maximize the delivery of drug tailored to the colon. One of the most studied classes of chemicals in this area are azo conjugates.[40,41] However, because it depends on the drug molecule's functional groups, this approach is not highly adaptable. Kim et al. developed a metronidazole prodrug. that, when added to the cecal contents of rats, was converted to the active drug.[11] The absorption of prodrug in systemic circulation was shown to be significantly low as compared to that of oral metronidazole, and it did not undergo the same small intestine metabolism as metronidazole. Kim et al. developed a metronidazole prodrug containing a sulphate group in a separate study. When rat cecal contents were present, they showed that this formulation broke down and released active metronidazole, but it remained intact in the upper intestine. As with the first prodrug, upon oral administration, a significantly less amount of the conjugated prodrug was absorbed and broken down in the small intestine than the active drug. Consequently, very little entered the systemic circulation. By conjugating metronidazole with pectin, Vaidya et al. used the prodrug method. The quantity of drug release from this formulation was then contrasted with that obtained using pectin microspheres that physically retained the drugs.[12] In the upper gastrointestinal system, the pectin-metronidazole (PT-ME) prodrug released drug far less than pectin microspheres containing metronidazole. However, both *in vitro* as well as in *vivo* studies demonstrated that combining the drug with pectin is effective in directing its administration towards the colon because no drug release by the PT-ME prodrug occurred at low pH, while nearly all of the metronidazole physically bound in pectin microspheres was released in the same environment. With the PT-ME prodrug, a far greater proportion of the drugs is broken down in the colon.

The drug can also be covalently attached to a carrier to maintain its formulation while it passes through the small and big intestines. Drugs can stick a variety of carrier molecules, including as amino acids, cyclodextrin,

glucuronide, and dextran. An azo link can be used to attach it to a carrier. Enzymes and bacteria in the colon degrade all of these links. Modasiya et al. evaluated the use of sodium alginate and hydroxypropyl methylcellulose (HPMC) for the most effective colon administration of compressed, matrix as well as enteric-coated curcumin tablets.[13] *In vitro* experiments demonstrated that the drug was promptly released from the framework and enteric-coated tablets in circumstances comparable to those seen in the stomach and small intestine. Additionally, it was shown that raising HPMC levels facilitated curcumin's straight transit to the colon and dramatically decreased its release in the upper GIT.

Colon-Specific Biodegradable Delivery Systems

Numerous anaerobic bacterial species that thrive in the colon get their energy from breaking down substrates such as indigestible polymers. These species, which are exclusive to the colon, include bacteria, eubacteria, enterococci, clostridium, and enterobacteria. They create a variety of enzymes, including xylosidase, glucuronidase, nitroreductase as well as azoreductase, to ferment these polymers.[14] Since these enzymes are exclusively present in the colon, this appears to be a more practical approach for colon-specific delivery.[50] The degree of enzymatic breakdown can be changed by chemical alterations made to polymers utilized in the creation of CDDS. To create a hydrogel of bovine serum albumin (BSA), for instance, Roos et al. produced the acetyl derivative of guar gum (AcGGM). They found that the degree of substitution (DS) of modified AcGGM determined how quickly it was hydrolyzed by β-mannase. The hydrolysis rate actually dropped as DS rose, suggesting that the side chain impeded the enzyme. However, the amount of BSA released increases dramatically when β-mannase was introduced.; With this enzyme present, 95% of the BSA came out after 8 hours, compared to just 60% without it. Azo-aromatic polymers, which are degradable by azoreductases, are some of the most extensively studied classes of prodrugs. As a result, they can be used to coat molecules of drugs, like peptides, to stop them from being broken down by peptidases in the small intestine as well as the stomach while still permitting the release of drugs in the colon. In one investigation, Pursuant to the *in vitro* and *in vivo* experiments, the colon-specific microorganisms broke down these polymers and liberated the metronidazole only in the colon.[14]

Matrix-Based Systems

Using polymer matrices to gather and release drugs inside the colon is another method of colon-targeted drug delivery. These matrices may be biodegradable or pH-sensitive. Ahmad et al. used Assam Bora rice starch, a naturally occurring polymer, to create matrix tablets that contained metronidazole. The produced tablets were put through *in vitro* drug release tests with goat cecal content, 0.1 Normal Hydrochloric Acid and phosphate buffer having a pH of 7.4. In the slightly acidic environment, the results demonstrated a continual release of the medicine from the tablets, which was thought to be caused by the polymer's slow erosion and disintegration. However, the drug's release was seen all over the GIT.

Timed-Release Systems

The idea behind timed-released formulations is that the drug will enter the colon following a predetermined period of time. This method depends on the small intestine's transit period, which is estimated to be three to four hours.[15] Each person has a different stomach emptying time, which is also affected by the food they eat. Additionally, transit time throughout the colon may be impacted by colon-related disorders such ulcerative colitis along with irritable bowel syndrome. To accomplish colon-specific distribution, Gazzaniga et al. combined a timed-release strategy with pH-sensitive polymers.[16] A formulation was created with a drug-containing core encased in three polymeric layers (two pH-sensitive layers and a hydrophilic layer). Because of the hydrogel development and pH protection, the *in vitro* assessment findings showed a prolonged drug release.

Bioadhesive Systems

Bioadhesive systems facilitate the absorption of poor absorbable drugs by enabling a formulation to remain touch with an organ—here, the colon—for long time. Ahmad et al. created a bioadhesive microsphere (BAM) that will deliver metronidazole to the colon with specificity using Assam Bora rice starch.[17] It was discovered that these BAMs increased the drug's Intake in the colon and had a longer retention period there. *In vitro* drug release tests indicate that less than 25% of the metronidazole was released in a small intestine simulation and just 10–12.5% in stomach-like environments. Nonetheless, the cecal content quickly discharged more than 90% of the drug. The drug had only been administered when the BAM reached the colon, according to additional *in vivo* testing, and it was pharmacologically as effective as commercial ones.

Multiparticulate Systems

Compared to single-unit systems, multiparticulate systems feature smaller particles and, according to research, may travel via GI tract more readily, permitting them to enter the colon sooner.[59] One type of multiparticulate structure that can be loaded with drugs for colonic distribution is a microsphere. Macrophages have the ability to absorb microspheres made of biodegradable materials. Only when Eudragit® polymers dissolved in the small intestine's alkaline pH was metronidazole liberated. It was believed that chitosan was vulnerable to colonic enzyme breakdown since there was an increase in drug release when rat cecal contents were present. Eudragit® S 100, a pH-sensitive polymer, has been applied to microspheres containing the polysaccharide pectin in a multiparticulate system developed by Vaidya et al. According to the *in vitro* drug release experiments, the stomach's acidic pH did not cause the release of metronidazole. However,

metronidazole was constantly released after the system was in the colon's more alkaline environment. It was demonstrated that metronidazole release was much greater when rat cecal contents were present, suggesting that this mechanism was not only pH-sensitive but also biodegradable. Additionally, *in vivo* tests that showed the concentration of metronidazole in different sections of the GIT also showed that this approach could selectively target the antibiotic to the colon. In contrast to unmodified pectin microparticles, based on a (Pec-ATP) compound, Perera et al. combined and assessed microparticles and discovered that they were noticeably more stable *in vivo*.[62] Based on this investigation, these particles seemed to improve choice for colon-targeted administration. Liu et al. recently created guar gum base microspheres to transport budesonide to the colon.[63] The pharmacokinetic studies and *in vivo* assessment of the colon targeting indicated that the generated budesonide microspheres effectively delivered budesonide to the colon in significant amounts.

Two different types of pellets made up the drug in the centre of the formulation in the multiparticulate system that Beckert et al. manufactured and assessed.[16] Pellet A, the first kind, contained an external polymer that only decomposed at pH levels greater than 5.5 and an interior polymer coating that allowed the drug to be delivered continuously. After six hours at pH 6.8, the polymer covering decreased drug release from pellet B by less than 20%. At pH 7.2, however, approximately half of the drug was released in the same length of time. It has been demonstrated that a mix of both of these pellet forms and their polymer coverings offers the possibility of targeted drug delivery to the colon. In a recent research, Agarwal et al. developed and reported on calcium alginate-carboxymethyl cellulose (CA-CMC) beads for the oral administration of colonic drugs.[65] The researchers investigated the combination properties of CA-CMC, such as pH sensitivity, breakdown via colonic microorganisms, and preferred colonic mucoadhesivity, in order to provide colon-specific delivery of the anticancer drug 5-flurouracil. Additionally, it was demonstrated that the CA-CMC beads exhibited a notably high mucoadhesive at colonic pH and deteriorated when there are colonic bacteria.

Prior research has also documented the utilization of nanoparticles as transporters for drugs taken orally intended for the colon.[17,18] Because macrophages can absorb nanoparticles at inflammatory parts of the colon, the system can stay at the target location for an extended time. The effectiveness of this approach depends on protecting the nanoparticulate systems from being absorbed either broken down by enzymes or Payer's patches prior to entering the colon. The findings demonstrated that these more recent nanoparticle formulations enabled long lasting effects in the colon. Additionally, it was discovered that the limited drug release in the simulated stomach juice negates the need for an enteric coating.

Polysaccharide-Based Delivery Systems

Due to their many benefits, polysaccharide-based delivery methods are increasingly being used for colon-specific drug administration. Utilizing polysaccharides has several benefits, including as accessibility, ease of modification, safety, stability, and biodegradability. Mundargi et al. evaluated the effectiveness of a number of polysaccharides in colon-targeted metronidazole administration.[19] The findings demonstrated that the kind and concentration of the polysaccharide utilized in the formulation do affect the metronidazole release rate. Gauri et al. formulate matrix tablets of metronidazole using variables quantities of guar gum and xanthan gum.[70] The amount of drug was determined by evaluating tablets *in vitro* in 0.1 Normal Hydrochloric acid, pH 7.4 buffer with phosphate, and pH 6.8 phosphate buffer solution containing 4% w/v rat cecal content out from the matrix tablets throughout the first five hours, which reflected time spent in the small and large intestines, ranged from 12 to 33%. Additionally, it was noted that the matrix tablets' growing xanthan gum content made them more vulnerable to colonic enzymes and postponed the release of the drug.

In CDDS, it has been discovered that using a mixture of polysaccharides rather than just one is more efficient in ensuring colon-specific delivery. Because oral cellulose does not undergo systemic absorption, cellulose derivatives are commonly combined to create these delivery methods. Cellulose esters fall into two categories and can be utilized in drug compositions. The solubility of non-enteric cellulose esters, like cellulose acetate, is pH-independent as well as they are insoluble in water. These can be applied on permeable and insoluble coatings. Enteric cellulose esters, including hydroxypropyl methylcellulose phthalate (HPMCP) as well as cellulose acetate phthalate (CAP), exhibit pH-dependent solubilities. They dissolve after the pH hits a specific range, although they remain insoluble in extremely acidic environments. The degree of esterification affects the pH where the polymer dissolves. Examples of carbohydrate blends that has investigated include pectin-HPMC, chitosan-HPMC, guar gum-chitosan, chitosan-pectin, and dextran-chitosan.[20]

Because they are innocuous to the organisms and can be broken down by the intestinal enzymes, galactomannan, polysaccharides including pectin, chondroitin sulphate, chitosan, and amylose are perfect for colon-specific administration, as was discussed in the preceding sections. It is thought that using such polysaccharides in thin-layer coatings could enable more rapid and efficient drug delivery to the target areas than other formulations that use them as compressive coatings or inside matrix systems. Water loving polysaccharide like pectin that gels and can change the way drugs are delivered. Ethyl cellulose (EC), is commonly added to the coating layer along with the pectin to shield the drug's core and lessen water permeability. Wakerly et al. used pectin and an aqueous EC (Surelease®) dispersion to paracetamol

tablets. The findings of the coated tablets' *in vitro* test show that the more pectin there was in the film, the faster the drug was released. The pectin/EC ratios of these film coatings varied. The drugs spread via the EC and the holes that developed in the film coating subsequent to the pectin was broken down by pectinolytic enzymes.[21]

Colon Targeting by Coatings

By shielding the active component from the stomach's and the proximal small intestine's acidic pH, the drug's incorporation into pH-sensitive polymers enables delayed release. Targeted drugs distribution to the colon is made possible by these polymers' breakdown in the terminal ileum's higher basic pH. There are several drawbacks to this strategy, even though these polymers become more soluble as pH rises. The lag time at the ileo-cecal junction or ascending colon may be too long, and the formulation may dissolve before it enters the small intestine as a result of pH fluctuations in the GIT. Methacrylic acid-based polymers, often known as Eudragit®, are pH-sensitive polymers commonly used in the development of colon-targeted drug delivery systems.

Enteric-soluble polymers may break down at the greater pH levels of the intestines yet are insoluble in the stomach's acidic environment. Much research has focused on using these polymers as coatings in formulations intended to selectively deliver active pharmacological ingredients to the colon (Fig. 1). These two polymers were combined in various proportions to provide a covering with the optimal rate of dissolution. Moreover, coatings that contain These polymers' purpose is to be quite thick in order to postpone their breakdown and provide a regulated or extended release of the drug.[22] Obitte et al.[77] investigated the ability of the hydrophobic polymers Eudragit® L-100 as well as Landolphia owariensis latex (LOL) to control metronidazole release with a colon-targeting effect. The *in vitro* dissolving studies showed that when the pH increased, so did the drug release.

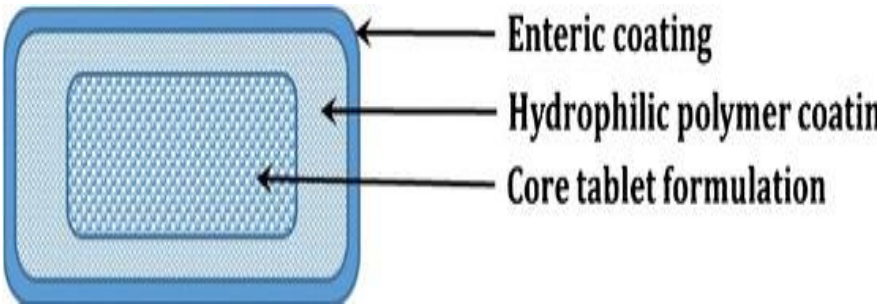

Figure 1: Diagrammatic illustration of the enteric-coated colon-targeted drug delivery system's cross-section.

INTEGRATED APPROACHES FOR ACHIEVING COLONIC DELIVERY

A number of integrated strategies have been investigated recently to accomplish colon-specific administration of drugs. These methods construct the delivery systems using physical phenomena like osmotic pressure or physiological parameters like luminal pressure.

Pressure Controlled Delivery

Because the Water reabsorption makes the contents of the large intestine more viscous, peristalsis causes its luminal pressure to rise higher than that of the small intestine.[78] The colonic luminal pressure has been used in a number of researches to create colon-specific delivery of drugs devices. Takaya et al. created capsules that use luminal pressure to deliver drugs to the colon. Due to increased colonic pressure, these systems enable the introduction of drugs to the colon instead of the small intestine; nevertheless, the reabsorption of water from the colon results in very viscous material, which might be a challenge for site-specific delivery.[23]

Controlled Delivery via Osmotic

Although osmotic controlled administration of drugs has been a concept for some time, it has only been popularized in the last ten to fifteen years when it comes to designing oral dosage forms that are particular to the colon. One system that is controlled by osmotic pressure is the OROS-CT. It is made out of a hard gelatine capsule that dissolves in the small intestine's pH and lets water in. It then swells as a result, forcing the drug out. There may be five to six units in each capsule, and each unit is encased in an enteric covering that is impermeable to drugs, preventing water from penetrating the stomach's acidic environment (Fig. 2). However, as soon as the capsule reaches the small intestine's higher pH, this covering dissolve and the water enters. There is a semipermeable membrane inside the enteric coating that contains both a drug and an osmotic push compartment. The gel that is created in the push compartment as a result of the water's swelling leads it to be driven out of an opening in the membrane next to the drugs compartment. The pace at which water enters establishes how quickly the drug leaves. To stop the release of the within the small intestine, these systems are additionally created to postpone the absorption of the drugs until the enteric coating dissolves.[24]

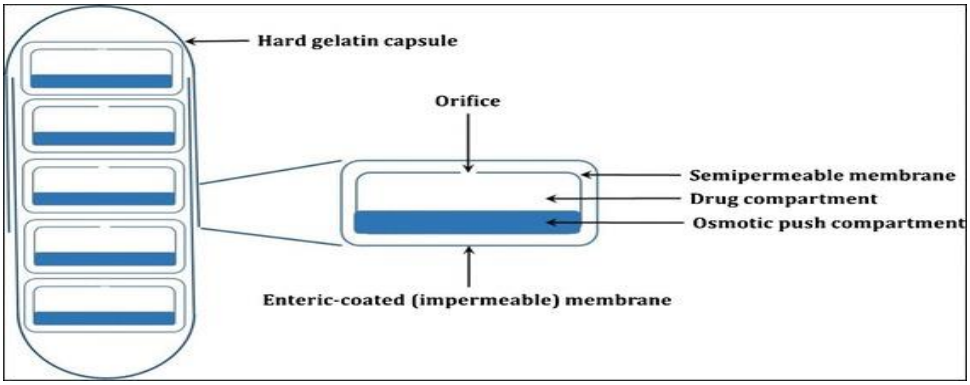

Figure 2: Diagrammatic illustration of the OROS-CT colon-targeted drug delivery system's cross-section.

Pulsincap Systems

Because the stomach empties differently and gastrointestinal transit varies due to peristalsis or conditions like IBS, time-dependent methods may not be the most efficient for delivering drugs to the colon. In order to achieve colon-targeted administration, it may be advantageous to include a timed-release system with pH-sensitive features. One formulation that makes use of both of these strategies is the pulsincap system.[25] The system is made from with a water-soluble cap covering the hydrogel plug, a water-insoluble capsule body holding the drugs, and a hydrogel plug sealing the opening end of the drug-containing capsule body (Fig. 3). To prevent the drug from escaping in the stomach, the capsule is also coated with an acid-insoluble film. Within the intestinal tract, the hydrogel plug starts to swell as its enteric coating dissolves. The length of the plug and the degree of insertion determine how much lag time is allowed by the swelling of the plug before the drug is released. A pulsincap system was created by Abraham et al., and they evaluated a number of polymers for the plug material. The formulations were tested at pH 1.2 for a two-hour period, pH 7.4 for three hours, and pH 6.8 for seven hours in order to simulate stomach fluid. Since there was no significant drug release within five hours of the trial starting, the study came to the conclusion showed the colon may be effectively targeted by this modified pulsincap system for metronidazole.

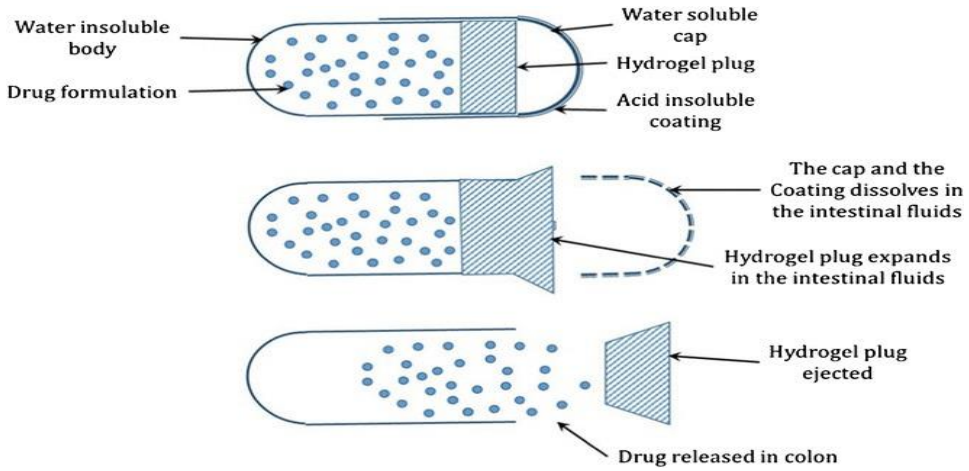

Figure 3: Diagrammatic illustration of the Pulsincap colon-targeted drug delivery system's operation.

EVALUATION AND PRECLINICAL INVESTIGATION

***In vitro* evaluation**

Disintegration, dissolution, and stability of novel products are critical evaluation characteristics of colon-targeted drug delivery systems, along with their *in-vitro, in vivo* association is complex.

As seen by the presence of biowaiver programs, the industry generally accepts the United States Pharmacopeia (USP) dissolving methodologies for examining the behaviour of formulations with rapid release, particularly those that include drugs that dissolve easily. The multi-vessel USP 3 apparatus and other conventional USP apparatuses are not entirely appropriate about the biorelevant characterization of dosage forms that target the colon. Some of the causes include the presumption that disintegration is the rate-limiting element of absorption, the incompatibility of disintegrating dosage forms, and the use of excessive fluid volumes and mixing speeds. Since penetration could represent a rate-limiting stage for many colon-targeted drugs, absorption through the epithelium of the colon is often shorter than that via the small intestine. Because of these limitations, colon-specific techniques have been developed that may mix customized permeability studies with more intricate drug stability and dissolution setups (such as bicarbonate buffers,

simulated GIF, animal fluids/tissues, human fecal slurries).[26]

Dissolution for oral dosage forms

Bicarbonate buffers

Since they have established ionic strength and buffer capacity and span a broad pH range, When evaluating drug release from pharmaceutical formulations, USP-standardized buffers including phosphate, citrate, acetate, and hydrochloric acid have remained essential.[85] Nevertheless, these buffers often have no biological significance for the human gut, even if they are still in use. When it was established in 2005 that bicarbonate-based buffers closely matched the buffer capacity of intestinal fluid than commonly used phosphate buffers, the use of such buffers for biorelevant dissolution experiments of colon-targeted drugs was first proposed.[86] Fadda et al. investigated whether bicarbonate medium might predict the *in vivo* behavior of sustained and delayed release mesalazine preparations.[27] The disintegration and PK characteristics of the products in Krebs bicarbonate buffer were more akin to those observed in people than they were in regular phosphate buffer. Indeed, a research employing Lialda® (Mezavant® XL, 5-ASA) has further proven this whereby human data obtained through gamma-scintigraphy demonstrated a high association with *in vitro* drug release.[89] Prednisolone tablets produced with four distinct Eudragit® coatings have shown similar results. Sadly, the primary drawback of employing bicarbonate media is the constant evaporation of CO2, which raises the pH of the buffer. To address this issue, both automatic and human methods have been devised to effectively stabilize the pH of the bicarbonate buffer during dissolution tests.[28] Asacol® 400 mg tablets and generic colon-targeted 5-ASA tablets were dissolved in bicarbonate buffer utilizing a unique flow-through cell technique, and the results showed a strong correlation in the *in vivo* the plasma PK measurements in 48 male healthy volunteers.

Adding enzymes and bile salts

Simple water buffer systems are very different from colonic fluid because bile salts and enzymes play a significant role in mediating drug release and solubilization from specific formulations.[29] By adding biorelevant quantities of proteins, phospholipids, and bile salts, as well as controlling buffer capacity, osmolality, and surface tension, Vertzoni et al. created simulated colonic fluids (FaSSCoF and FeSSCoF) that replicate the fasting and fed states.[93] The researchers showed that compared to normal buffers, FaSSCoF and FeSSCoF were better able to forecast the solubility of three poorly soluble drug in GIF. Bile salts, for instance, improve the solubility of lipid loving drugs in a way that is appropriate for the human colon when added to buffers. Colonic enzymes must be added in order to assess drug release from targeted formulations that employ enzymatic degradation of polysaccharide-based coatings. Microbiota derived from intestinal fluids or human or animal feces can actively manufacture enzymes inside media.[30] Although adding biorelevant enzyme concentrations to buffers might replicate *in vivo* circumstances if cell-free media are selected, it would be preferable to incorporate a variety of enzyme classes to fully understand the colon's wide range of metabolic functions. The microbiota's carbohydrate-active enzymes (such as glycoside hydrolases, polysaccharide lyases, and carbohydrate esterases) and drug-metabolizing enzymes (such as phosphorylases, reductases, and decarboxylases) are important components of colonic fluid.[95-98] The capacity of colonic microbiota to modify drug bioavailability (typically in a patient-specific way) is a significant PK factor that must be searched for during the characterization of novel active compounds, even if it is not frequently taken into account during pharmaceutical development.[31]

Faecal slurries

Faecal slurries are mostly use to anticipate drug release, solubility, and stability in the colon. They are made comprised of raw fecal material and conditions that are intended to replicate colon physiology and promote microbial growth.[1] Microbiota (25–54% of solid fecal material), undigested polysaccharides (about 25%), proteins (2–25%), lipids (2–15%)along withinorganic elements (such as potassium, phosphate, sodium, calcium, and magnesium) are all present in healthy human feces, which have an average pH of 6.64. Although concentrations of 10–25% are typical because they enable precise volume measurement and efficient homogenization of fecal material with support media, the percentage weight of fecal material per volume of support media can vary greatly (ranging from 1 to 60% as described in the literature).[102] Electrolytes (such as sodium, phosphate, magnesium, as well as bicarbonate), bile salts, yeast extract, L-cysteine as well as peptone water—the latter three of which are added to support medium to promote microbial growth—are frequently included in its composition. It is possible to alter a fecal slurry's precise composition to replicate changes in physiology, including pH, bile acid content, and buffer capacity. It has to be acknowledged, although, that the initial pH of the slurry and the carbon substrates that are available had a considerable influence on the material and functionality of the microbiota; bacteria can create organic acids that build up, lower the pH of the medium, and cause bacterial stasis or death.[32]

A static batch culture is a setting where raw drugs and/or formulations are often cultured in fecal slurry for brief periods of time (≤ 24 hours) with frequent sample withdrawals to assess drug stability and dissolution. Fresh growth media is used to replenish exhausted culture in continuous culture systems, which necessitate longer incubations. More sophisticated systems may include several compartments that replicate various colonic areas and enable precise control of variables like pH or transit time across segments (Fig. 4). The Mucosal Simulator of the Human Gastrointestinal Microbial

Ecosystem (M-SHIME®) is a particular model that allows for up to four weeks of therapy and contains vasculature similar to the stomach, small intestine, and ascending, transverse as well as descending colon. In addition to luminal bacteria, mucin-covered microcosms found in the colonic sections of the M-SHIME® aid in the cultivation of mucosal microbiota. In order to imitate human colonic circumstances, investigations should be carried out in anaerobic surroundings kept at 37 °C, regardless of how complicated the fecal slurry incubations are.

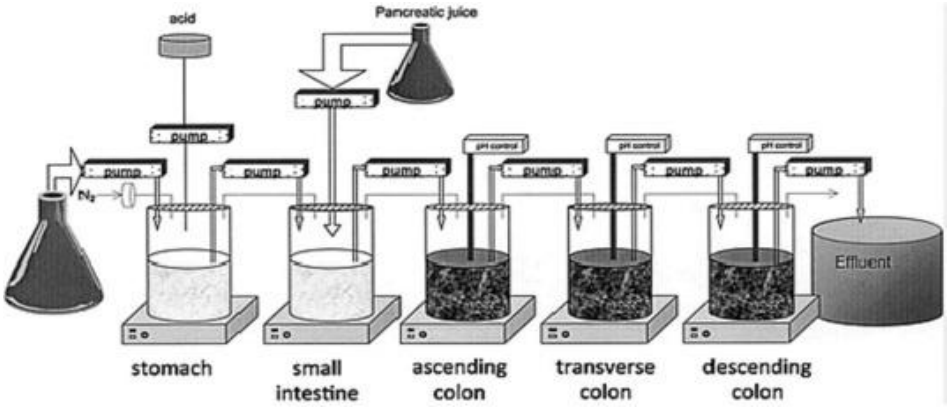

Figure 4: Diagram for the Human Intestinal Microbial Ecosystem Simulator (SHIME®).

The various gastrointestinal system sections are shown as insulated glass jars that are joined by peristaltic pumps. It is possible to vary the pump flow rates, vessel volume, and fluid pH to represent different patient groups. To replicate the microbiota, fecal samples are implanted into colonic arteries. drug stability in the gastrointestinal system and drug effects on colonic microbiota may both be predicted using the SHIME® model. The reference image was used with permission.

Human fecal material is readily available and, with proper and consistent processing, is able to forecast formulation behaviour *in vivo*, even if it is not a true representation of the composition of the colonic microbiota. The ability to collect patient samples for research of formulation behavior in a specific disease state is another benefit.[33] Although the microbiome of mice and rats is not the same as that of the human colon, rodent feces may also be employed since they contain similar amounts of Bacteroides and Bifidobacteria. The standardization of the feed and living conditions may help reduce animal variability.

Dissolution methods for rectal dosage forms

Suppositories, rectal capsules, semisolids, and liquid preparations are common rectal dosage forms with rapid release. The lipophilicity, solubility, and particle size of the active component, as well as the physiological rectal environment, all have an impact on *in vivo* drug release from rectal dosage forms.[113] A well-designed *in vitro* dissolving system is necessary for the *in vivo* prediction of drug behaviour in the rectal environment. Although several requirements are included in the FDA-recommended Dissolution Methods Database, there are regrettably presently no officially approved *in vitro* drug release characterization techniques for rectal drug published in pharmacopoeia.[114] The database documents the usage of the paddle equipment in large amounts of aqueous solutions at different pH levels for solid rectal formulations. The lack of information on the makeup of human rectal fluid is the primary obstacle to designing suitable dissolving techniques. This hinders the creation of permeability models in addition to making it more difficult to create pertinent dissolving media.[34]

Suppositories, which come in hydrophilic or lipophilic forms based on their bases, are the most often used rectal dose forms. Hydrophilic forms are composed of polyethylene glycols (PEGs) and other non-ionic surface-active compounds that are chemically bound to PEGs. These substances can be used with a USP apparatus 1, 2, or 4 at specific pH values that are comparable to those in the human rectum since they dissolve in aqueous dissolving media. Since suppositories could float on the medium's surface, a sinker device is typically needed: Palmieri created a unique basket[116] Wider vertical slits in the so-called Palmieri basket provide a more intensive contact between the medium and the rectal formulation. Aspirin release from hydrophilic suppositories in aqueous buffer was accurately anticipated by this configuration.

Composed of solid fats, hydrophobic suppositories dissolve at body temperature, releasing the drug into the rectal fluid from the fatty basis. Based on the drug's characteristics and the base's melting pattern, the test circumstances should be assessed individually when figuring out how well the drug dissolves in different forms. Schoonen et al. created one technique in 1976,[117] who separated the medium into two compartments by fixing a lipid loving formulation beneath a circular glass plate. The plate was then submerged in 900 millilitres of physiological pH phosphate buffer. For a total of five hours, the upper compartment was continuously shaken, and samples were taken from it at predetermined intervals. Then, Klein et al. added a paddle mechanism to

this configuration. The release of paracetamol from four distinct lipophilic base suppositories was tested using an unvalidated dissolving equipment that was presented by the British Pharmacopoeia and the European Pharmacopoeia III in the early 2000s.[118] A suppository is positioned inside a thermostatically regulated flow-through cell that is filled with phosphate buffer (pH 7.4). However, an exact *in vitro-in vivo* correlation (IVIVC) cannot be assessed from this configuration due to various issues with the dissolving system's approach. In conclusion, a precise bio-predictive dissolving technique has not yet been developed, particularly for lipophilic suppositories.

Investigating *in vitro* colonic permeability

Because the colon and small intestine have different physiological characteristics, different assays should be used to estimate colonic drug permeability. Though immortalized cell lines, such Caco-2, are frequently used to forecast epithelial drug permeability, they lack a mucus layer and important enzymes, including CYP3A4, and are subject to culture-derived variability.[35] It's interesting to note that Caco-2 cells express transporters more like those in the small intestine while being generated from the colonic epithelium. However, a study of 18 small molecular weight drugs revealed a strong association (R2 = 0.74) between Caco-2 permeability and human intestinal absorption, confirming the model's applicability under certain circumstances. Furthermore, the biological sex of cell lines is frequently overlooked, which suggests that drug permeability investigations across cell lines are insufficient to detect patient diversity between males and females.

Other models for evaluating drug permeability in the colon include colons-on-a-chip, organoid systems, and Using chambers that use human colonic tissue. Lemmens et al. have thoroughly examined these models' exact parameters as well as their advantages and disadvantages. In short, by enabling more physiologically realistic epithelial landscapes that may be created to replicate a specific disease state, these technologies may provide benefits over conventional Caco-2 permeability trials.[36] Building distinct, precisely calibrated colonic tissue models that transcend conventional cell culture is another opportunity presented by the development of bioprinting Furthermore, by coculturing epithelial tissue models with colonic microbiota, new methods also enable the integration of drug permeability and microbiome stability research. These intestines-on-a-chip could sustain the long-term coculture of human and microbial cells at tunable oxygen gradients and relevant microbial diversities.

Using animal models to study colonic drug delivery

It is essential to select *in vivo* animal models that most closely mimic the physiology of the human gastrointestinal tract when creating innovative colon-targeted drugs. In actuality, cost, accessibility, and handling comfort frequently determine the choice of animal model. Often to a larger degree than physiological resemblance. Animals' gastrointestinal structure and physiology differ significantly between species, which results in a wide range of drug uptake and bioavailability.[37] Regretfully, no one animal can accurately mimic the gastrointestinal tract of humans. Because of this diversity, scientists studying colonic administration of drugs frequently employ a variety of animal models. Small rodents like mice, rats, rabbits, and guinea pigs, as well as bigger animals like dogs, pigs, and non-human primates, are popular alternatives. To maximize the effectiveness of pre-clinical development, it is crucial to acknowledge the suitability of using animal models to evaluate colon-targeted drugs in humans. The best animal model will probably vary depending on the parameters to be investigated, the indicator that has to be addressed, and the features of the dose form. Furthermore, for formulations meant to treat humans of both genders, it is crucial that male and female animals be equally represented throughout pre-clinical research. From now on, the benefits and drawbacks of using animals in pharmacological research will be examined in relation to colonic morphology, fluids, transit, and microbiota.

Animal colonic anatomy

The wide variation in colonic morphology among species is most likely caused by the way that different species absorb and digest nutrients, which has affected the shape and functions of the gut throughout evolution. The colon of omnivorous species and herbivore is longer than that of carnivores, which helps them absorb nutrients from diets high in fibre and low in protein. Although rats and mice are thought to be ideal models for colorectal cancer illness because they've human-like intestinal architecture and functions, they had non-sacculated colons, no adipose tissue in the submucosa, and rats in particular have considerably bigger caecum's because they consume more fiber.[38] Dogs, in contrast, have no sacculation's, a shorter colon than humans, and tiny caecums. Additionally, dogs' intraluminal pressures are probably higher than those of humans, which means that formulations that are susceptible to mechanical stress may break down sooner in the GI tract of dogs. Pigs are considered a good model for nutritional absorption because of their GI tract structure, which is more similar to that of humans in terms of the intestinal surface area and the relative length of the intestinal segments.[39] The GI tract pressure in pigs is thus similar to that in humans. Because they are cecotrophic, meaning they emit feces covered with bacteria that must be reinvested for full nutritional absorption, The caecum of rabbits is clearly delineated.

Animal colonic fluids

Understanding the pH and amount of the GI fluids in animals is necessary to make an accurate prediction about the disintegration, solubility, and absorption of drugs in humans. Enteric coatings can take use of the

intraluminal pH rise that occurs between the ileum and the colon in humans to facilitate site-specific drugs delivery. It has been discovered that the intestinal pH values of healthy guinea pigs, rabbits, and pigs are similar to those of humans. Beagles have varying colonic pH values (pH 5–8), indicating a pH rise between the small intestine and colon. The intraluminal pH of rats falls from around 7.0 in the distal small intestine to approximately 6.0 in the caecum. This is frequently lower than the pH of a person's natural caeces.130 pH-dependent coating polymers, such as Eudragit® S and FS, may function incorrectly, and drugs that dissolve at basic pH may precipitate at lower pH levels. Therefore, if researchers want to extend dosage form release to people, they should exercise caution while using rats to evaluate pH-triggered colonic administration formulations. Conversely, the rat appears to be the animal model with the most human-like relative free fluid volume, especially when fed, however undigested food may impede the breakdown of drugs.

The age of the animals used in drug delivery studies should also be considered because aging can result in significant changes in physiology, just as it does in humans. For example, juvenile rats (4 weeks) had shorter colons, lower total GI fluid amounts, and greater colonic buffering ability than older rats (38 weeks). It may be impossible to predict how well drugs will dissolve in humans due to the relatively increased water content in these species' guts, although the consistency of colonic fluid is identical in people, guinea pigs, rabbits, and pigs.[40]

Animal colonic transit

One important component in assessing the bioavailability is GI motility of drugs taken orally. Animals' intestinal transit times differ greatly from one another. The intestinal transit is substantially shorter in rodent (about 62.2 ± 21.2 min) than humans, who have a median colonic transit of 21 hours. This transit may be extended by administering prokinetic drugs.[134] However, side effect may disrupt with clinical measures and/or test drugs Catabolism and anabolism thus caution should be used when Simultaneous use of prokinetic drugs with test formulations. When male landrace pigs are fasting, the average duration of transit of a 26 x 13 mm The telemetric Smart Pill capsule as it progresses through their colons is 53.77 ± 31.68 hours, and after a high-calorie meal, it is 102.47 ± 59.54 hours. With averages of 68–233 hours for America sowherd pigs, 24–672 hours for large white pigs, >54 hours for Yucatan pigs, and >48 hours for Göttinger minipigs, pigs' stomach emptying periods are similarly significantly greater than those of humans. Pigs are therefore probably not the best model to use when examining colon-targeted formulations that are impacted by transit duration. The dog could be more like a person in contrast. A research that looked at 31 healthy adult dogs of different breeds, 14 of whom were female, discovered that the total GI transit took 21.57 to 57.38 hours, while the colonic transit duration ranged from 7.12 to 42.88 hours.[136] Particularly, it has been discovered that the beagle, a model frequently employed in pharmaceutical research, correlates exhibiting average intestinal transit times of 25.4 ± 3.3 hours when fasted and 28.2 ± 4.7 hours when fed, in good harmony with people, as well as entire gut transits of 27.3 ± 3.3 (fasted) and 33.0 ± 4.1 (fed). However, the investigators hypothesized that beagle colonic transit would appear to be increased because capsules may move more quickly between the proximal and distal colon before remaining in the rectum for a longer period of time.

In silico modelling for colonic drug targeting

Because therapeutic development is a famously costly and risky process, it is greatly desired to be able to anticipate *in vivo* behavior and speed up formulation development while conserving critical resources.

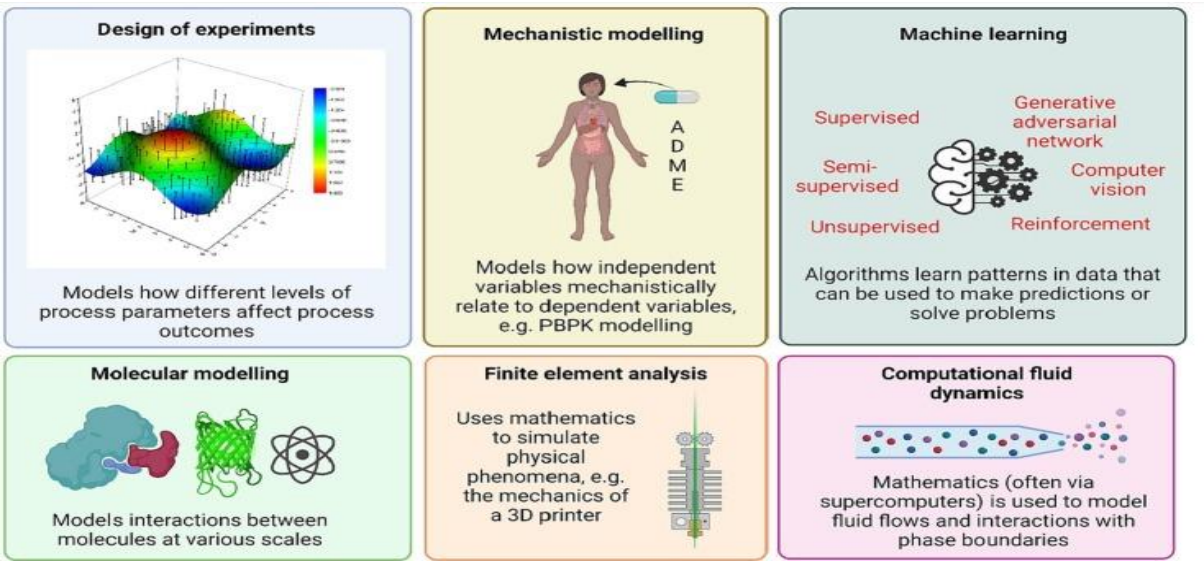

Figure 5. Potential applications of in silico methods for creating colon-targeted drugs include analyzing drug-target interactions and improving formulation manufacturing.

DoE is a popular method in pharmaceutical research that may be used to model the effects of various process factors on particular process outcomes. Numerous research have employed DoE to optimize the enteric polymer-based colon-targeted coatings' composition for effective *in vivo* delivery.[40] It is especially helpful when researchers want to look at how certain quality qualities (like coating drug release rate) are influenced by a small quantity of process variables (like the percentage of coating polymer for enteric protection). DoE might be used to simulate and forecast the effects of additional formulation characteristics (such as dosage form size, shape, or production technique) on the dependability of drug liberation in the colon in addition to optimizing coating composition. When dealing with big datasets that have a lot of (≥ 10) independent variables or complex non-linear interactions between independent and dependent variables, DoE is less helpful. Other prediction technologies could be more suitable in certain situations.[141]

Molecular modelling applies equations from both classical and quantum physics to computationally simulate molecular interactions.[41] Full atomistic and course-grained models are two different levels of detail that may be used to depict molecular systems; the decision depends on the needs of the experiment and the resources at hand. MD has established itself as a reliable in silico method for drug discovery because of its capacity to precisely anticipate dynamic interactions at the atomic level, such as by making it possible to analyse drug-target binding. From the standpoint of drug discovery, MD might be used to find substances that have action at newly identified microbiome and other therapeutic targets within the colon. For example, MD in the near past estimated that the biflavanoid amentoflavone would block gut bacterial β-glucuronidases; these discoveries should help prevent microbiome-led drugs metabolism, There is evidence that bacterial β-glucuronidases can biotransform drugs. The influence of microbiological metabolites on metabolic pathways linked to duration has been shown elsewhere by molecular modelling, including MD; this might result in the development of new treatments to promote healthy aging through the gut. From the standpoint of drug development, MD offers a wide range of possible uses for formulation design. The loading and discharge of the drug processes of formulations that respond to stimulus might be reliably mapped using MD, allowing for the creation of tailored dose type that is extremely explicable *in vivo* behaviour. In order to optimize colon-targeted nano formulations, MD has also been utilized to comprehend the creation of polymer-coated nanoparticles generated by flow nanoprecipitation.[42]While handling a range of data types, from significantly greater sizes to the atomic level, MM and ML are clearly helpful. MM entails creating mathematical expressions that illustrate the mechanical relationships between independent and dependent variables. Since the equations are based on conjectures derived from current experimental results and cannot realistically incorporate every component that potentially influence interactions, they are simplified representations. Drug development frequently uses physiologically based pharmacokinetic (PBPK) models, which mimic how (ADME) affect pharmacological concentration in tissues and/or plasma. MM incorporates these models. PBPK models can precisely forecast how physiological variability impacts pharmacokinetics in various patient groups since important ADME parameters may be changed.[151] Studies using the popular PBPK programs GI-Sim and GastroPlus have demonstrated that both programs are capable of predicting the intestinal absorption of 14 drugs, with results sufficiently good to encourage their substitution for *in vivo* assessment of regional absorption in dogs in preclinical drug development.[152] Working with both small and big datasets, determining how variables interact mechanistically inside a model, and—most importantly—understanding the mathematical foundation of predictions are all benefits of MM. When the processes behind interactions are unclear or extremely complicated, such as when evaluating how physiological variability—which involves several biological variables—affects the effectiveness of medicinal formulations, machine learning (ML) could be a better fit.

The potential of machine learning (ML) to lower expenses and boost success across the whole drug development process— from the discovery phase to clinical trials—has drawn more attention in recent years. Machine learning (ML) includes a wide range of technologies that use different kinds of mathematical algorithms to find patterns in data. These patterns may then be used to predict experimental results for data that hasn't been tested yet. Supervised and unsupervised learning are the two main categories of machine learning.[42] A big database of drugs with known solubility in colonic fluid is one example of a dataset containing labelled samples that is used to train algorithms in supervised learning. In supervised machine learning, the algorithm would learn how the features of the sample could forecast label this would include learning how the qualities of the drug impact its solubilities in colonic fluid. The resulting machine learning model may be applied to untested samples in order to forecast their labels. (such as drugs with unknown solubilities in colonic fluid) if sample attributes are enough to predict the label. Unsupervised learning uses unlabelled datasets, and methods like dimension reduction and clustering may be used to identify patterns in the data.[156] In the case of semi-supervised learning, where unlabelled data is labelled using unsupervised techniques and then used for supervised machine learning tasks, is a combination of both learning approaches.[157] New developments in machine learning, including generative models, multi-task learning, active learning, and reinforcement learning, present further chances to take advantage of the experimental data that is

already accessible and increase the effectiveness of upcoming studies. Formulation design has started to make substantial use of machine learning. For instance, With a typical error of just 24.29 minutes, a supervised artificial neural network (ANN) trained on more than 950 formulas was able to anticipate the drug release timing of 3D printed drug. In other cases, an ANN outperformed a DoE model used for the same task in predicting the size and drug loading effectiveness of nanoparticles based on the composition of the excipient.[43] Drug-microbiome interactions have also been predicted using machine learning. Because quantum computing can handle datasets with numerous datapoints far more effectively than classical computing. Additionally, in order to take use of each *in silico* predictive technology's unique benefits, it is probable that more and more of them will be integrated into jobs. Researchers may be able to use these hybrid methodologies to get the atomic visualisation made possible by MD, the mechanistic understanding offered by MM, and the large-scale data exploration made possible by ML. To find 100 new co-clustered solid drug nanoparticles made of autonomously assembled drug-excipient combinations, for instance, Reker et al. utilized ML and MD. Two of these were satisfactorily characterized both *in vivo* and *ex vivo*. This work serves as an example of how combining cutting-edge *in silico* technology may be used to use pre-existing large data to expedite and optimize formulation development.

CONCLUSION

In recent years, the creation of drug delivery methods that target the colon has become more significant. Modern understanding of the distinct physiology of the colon, efficient formulation techniques, and sophisticated *in vitro*, *in vivo*, and in silico development tools may all be used to create new and successful drugs at this important juncture in colonic drug delivery. Targeted drug delivery to the colon systems provide patients substantial safety-oriented therapeutic benefits, effectiveness, and treatment compliance, as was covered in the study above. The physicochemical characteristics of the drug, formulations and production variables, and GI physiological considerations are some of the factors that may affect and complicate the successful creation of a colon-specific delivery system for the drug. The manufacturing techniques utilized for tackling these problems mostly focus on a single drug delivery mechanism, such as releasing the drug for absorption in the colon, avoiding the upper GIT's complex pH environment by the dosage form, and preventing the drug's release and absorption in the upper GIT.

Another strategy to focus medicine delivery precisely in the colonic area is to investigate the colon enzymes' capacity to metabolize substances. The creation of drug delivery systems in specific area like colon seems to require a mix of traditional and novel techniques in order to guarantee a proportionality between effectiveness, selective targeting, price, and patient adherence. In addition to the combined methods, future research appears to focus on the investigation of nanotechnology for drugs targeting in the colon.

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