AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

A Review on: Colon Targeted Delivery by Time- Dependent Polymeric Nanoparticles for Colon Cancer.

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ABSTRACT

Oral administration of different dosage forms is the most common form of administration due to greater patient compliance. Time-dependent drug delivery systems have been widely used for colon-targeted delivery. This study, Eudragit RS 100 was used as a Time-dependent polymer. Single pH-dependent NPs (pH-NPs), single time-dependent NPs (Time_NPs), and dual pH/time-dependent NPs (pH-NPs) were prepared using the oil-in-water emulsion method. In present study colonic drug delivery is prepared in form of nanoparticles by using oil in water or Nano precipitation method. Nanoparticles having various advantages over tablets or capsules as short gastric residence time, improved bioavailability.

Keywords: colon targeted, Time-dependent nanoparticles, Eudragit RS100, colon cancer.

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Received 18 April 2017, Accepted 25 April 2017

Please cite this article as: Kotkar AA *et al*., A Review on: Colon Targeted Delivery by Time- Dependent Polymeric Nanoparticles for Colon Cancer. American Journal of PharmTech Research 2017.

INTRODUCTION

Cancer is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases. Colorectal cancer, also called colon cancer or large bowl cancer includes cancerous growth in the colon, rectum and appendix (Wikipedia, 1994). The basic goal of novel drug delivery system is to achieve a steady state blood or tissue level that is therapeutically effective non toxic for an extended period of time.[1] Nanotechnology is the designing of practical frameworks at molecular or nano scale. Particles are considered as nanoparticles when one dimension is 100 nanometers or less in size[2]. The properties of numerous common materials change when framed from nanoparticles [3]. This is commonly in light of the fact that nanoparticles have a more prominent surface area per weight than bigger particles; this makes them be more receptive to certain molecules. It is conceivable that medications as nanoparticles may give better solubility, prompting better absorption3. Additionally, medications may be contained inside of a molecular transporter, either to shield them from stomach acids or to control the drug release to a particular focused area, diminishing the probability of side effects. Nanoparticular drug delivery systems are submicron-sized particles (3-200 nm), devices, or systems that can be made, utilizing an assortment of materials including polymers (polymeric nanoparticles, micelles, or dendrimers), lipids (liposomes), virus (viral nanoparticles), and even organometallic compound (nanotubes). Capecitabine is a prodrug that is changed over to fluorouracil in the body tissues taking after the oral route. It is broadly utilized as a part of the treatment of metastatic colorectal growth and breast malignancy, since it is promptly ingested from the gastrointestinal tract. The prescribed every day dosage is huge, i.e., 1500 mg/m2 and it has a short disposal half-life of 0.5–1h4. The unwanted impacts with capecitabine incorporate bone-marrow depression, cardiotoxicity, looseness of the bowels, sickness and retching, stomatitis, dermatitis, and so on. Subsequently, preparing capecitabine as a controlled release (CR) medication would give more noteworthy or more in vitro and in vivo antitumor movement. [3].

Anatomy and Physiology Of Colon [19]

The GI tract is divided into stomach, small intestine and large intestine. The large intestine extending from the ileocecal junction to the anus is divided in to three main parts. Theseare the colon, the rectum and anal canal. The entire colon is about 5 feet (150 cm) long, and is divided in to five major segments. Peritoneal folds called as mesentery which is supported by ascending and descending colon. The right colon consists of the cecum, ascending colon, hepatic flexure and the right half of the transverse colon. The left coloncontain the left half of the transverse colon,

descending colon, splenic flexure and sigmoid. The rectum is the last anatomic segment before the anus. The human colon were shown in Figure3.The major function of the colon is the creation of suitable environment for the growth of colonic microorganisms, storage reservoir of faecal contents ,expulsion of the contents of the colon at an appropriate time and absorption of potassium and water from the lumen. The absorptive capacity is very high, each about 2000ml of fluid enters the colon through the ileocecal valve from which more than 90% of the fluid is absorbed [4].(Figure: 1)

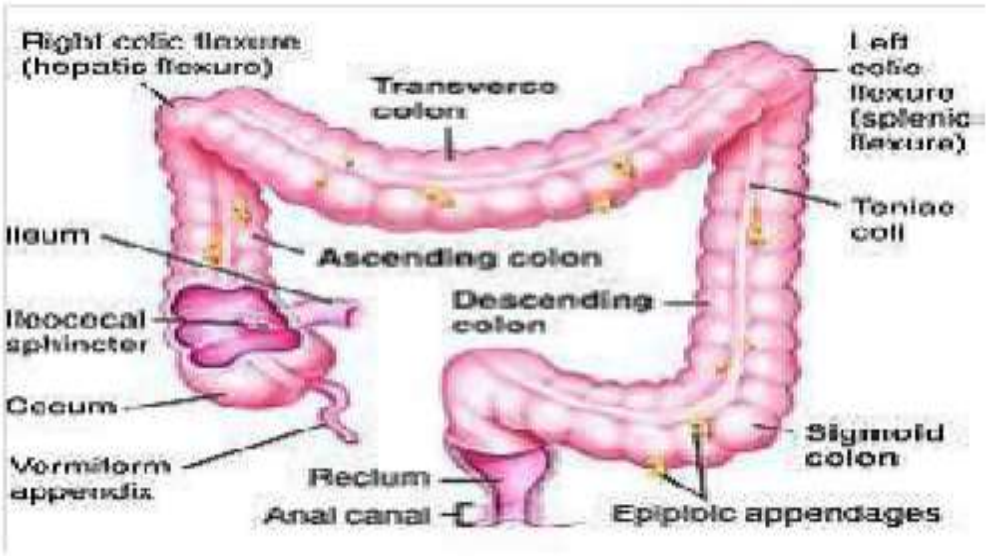

Figure 1: Anatomy of colon [12]

Advantages of colon targeting drug delivery system [19]:

1. Colon is an ideal site for the delivery of agents to cure the local diseases of the colon.
2. Local treatment has the advantage of requiring smaller drug quantities.
3. Reduces dosage frequency. Hence lower cost of expensive drugs.
4. The colon is an attractive site where poorly absorbed drug molecules may have an improved bioavailability.
5. Bypass initial first pass metabolism.
6. It has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs.
7. Possibly leading to a reduced incidence of side effects and drug interactions.
8. Improve patient compliance.
9. Targeted drug delivery system.

Limitations and Challenges In Colon Targeted Drug delivery [19]

1. The targeting of drugs to the colon is very complicated. Due to its location in the distal part of

the alimentary canal; the colon is particularly difficult to access. In addition to that the wide range of pH values and different enzymes present throughout the gastro intestinal tract, through which the dosage form has to travel before reaching the target site, further complicate the reliability and delivery efficiency.

2. In addition, the stability of the drug is also a concern and must be taken into consideration while designing the delivery system. The drug may potentially bind in nonspecific way to dietary residues, intestinal secretions, mucus or faecal matter.

3. The resident microflora could also affect colonic performance via metabolic degradation of the drug. Lower surface area and relative 'tightness' of the tight junctions in the colon can also restrict the drug transport across the mucosa and into the systemic circulation.

4. One challenge in the development of colon-specific drug delivery systems is to establish an appropriate dissolution testing method to evaluate the designed system *in-vitro*. This is due to the rationale after a colon specific drug delivery system is quite diverse.

5. Successful delivery through this site also requires the drug to be in solution form before it arrives in the colon oral tentatively, it should dissolve in the luminal fluids of the colon, but this can be a limiting factor for poorly soluble drugs as the fluid content in the colon is much lower and its more viscous than in the upper part of the GI tract.

Factors Affecting Colon Targeted Drug Delivery [15]

1. Physiological factors
2. Pharmaceutical factors

Physiological factors

Gastric emptying

Drug delivery to the colon upon oral administration depends mainly on gastric emptying and bowel transit time. Upon reaching the colon the transit time of dosage form depend on the size of the particles. Smaller particles have more transit time compared to larger particles. Diarrhea patients have shorter transit time whereas constipation patients have longer transit times.

pH of colon

The pH of GIT varies between different individuals. The food intakes, diseased state, etc. influences the pH of the GIT. This change in the pH in different parts of GIT is the basis for the development of colon targeted drug delivery systems. Coating with different polymers is done to target the drug tithe site. (Table: 1)

Table 1: pH in different parts of Colon

Parts of GIT	pH
Stomach	1.5-2
Fasted state	
Fed state	2-6
Small intestine	6.6- 7.5
Colon	6.4
Ascending colon	
Transverse colon	6.6
Descending colon	7.0

Colonic microflora and enzymes

The GIT contains a variety of microorganisms that produces many enzymes need for metabolism. Growth of this microflora is controlled by the GIT contents and peristaltic movements. The enzymes released by different microorganisms E. coli, Clostridia, Lactobacilli, Eubacteria, Streptococci are responsible for the various metabolic reactions that take place in the GIT. (Table: 2)

Table 2: Different microflora, enzymes released and action

Microorganism	Enzyme	Metabolic reaction
E.coli, Bacteroids	Nitroreductase	Reduces aromatic & heterocyclic nitro compounds
Clostridia, Lactobacilli	Hydrogenase	Reduces carbonyl groups & aliphatic double bonds
Clostridia, Eubacteria	Glucosidase	Cleavage of By glycosidase of alcohols & phenols
Eubacteria, Clostridia, Streptococci	Sulfatase	Cleavage of O sulphates& Sulfamates

Pharmaceutical factors

Drug candidates

Due to high retention time of colon, colon causes an increase in the absorption of poorly absorbed agents like peptides, etc. drugs used for treatment of inflammatory bowel diseases, etc. are suitable for colon targeted drug delivery system.

Drug carriers

The selection of carrier for CDDS depends on the nature of the drug, disease for which the drug is used. The various physicochemical factors of drug that effect the carrier selection includes chemical nature, stability, partition coefficient, functional groups of drug molecule etc.

POLYMERS USED IN COLON TARGETING [15]

Polymer contain a large number of structural unit joined by same type linkage, form into a chain like structure. These are nowadays used in formulating various pharmaceutical products. Naturally found polymer, which include gummy exudates, proteins, enzymes, muscle fiber, polysaccharides. In olden days natural polymers are widely used in pharmacy but a variety of synthetic polymer are used nowadays for pharmaceutical and cosmetic development, using these polymer many therapeutic system of body namely controlled drug delivery systems, are achieved [12,15,19].

Natural polymer

Guar gum, Insulin, Pectin, Cyclodextrin, Dextran, Amylase, Chitosan, Chondrotin sulphate, Locust bean gum.

Synthetic polymer

Shellac, Ethyl cellulose, Cellulose acetate phthalate, Hydroxypropyl methyl cellulose, Eudragit, Poly vinyl acetate phthalate.

APPROACHES FOR COLON TARGETED DRUGDELIVERY [15, 19]

- Primary approaches for colon targeted drug delivery
    - pH sensitive polymer coated drug delivery system
    - Delayed release drug delivery system
    - Microbial triggered drug delivery
        - Prodrug approach
        - Polysaccharide based system
- New approaches for colon targeted drug delivery
    - Pressure controlled drug delivery system (PCDDDS)
    - CODE
    - Osmotic controlled drug delivery system (OROS-CT)
    - Pulsatile colon targeted drug delivery
        - Pulsincap system
        - Port system
    - Azo hydrogels
    - Multiparticulate system based drug delivery

pH sensitive polymer coated drug delivery system

The pH varies in different parts of the gastrointestinal tract. The pH in stomach ranges between 1 and 2 during fasting. The pH in the proximal part of small intestine is 6.5 and in distal part of small intestine it is 7.5. The pH is6.4 in caecum, 5.7 in ascending colon, 6.6 in transverse colon and 7.0 in descending colon. The pH dependent drug delivery system is based on the solubility of different

polymers at different pH ranges. The polymers are insoluble at lower pH values and get solubilized as the pH increases. As the polymers are insoluble at lower pH values the polymer can protect a formulation in stomach and to some extent in small intestine. In this way by altering the polymers used the release of drug from the formulation can be controlled [18].

Delayed or time controlled release drug delivery system

Time controlled drug delivery system includes sustained or delayed release systems. In this system the delayed release or colon targeted drug delivery is attained by prolonging the lag time. The transit time varies in different parts of gastrointestinal tract. This transit time is responsible for the delayed release of drug. The main drawbacks of this delivery system are that the transit time varies from one person to other and amount of food intake. It also varies with the peristalsis or contraction in the gastrointestinal tract.

Microbial triggered drug delivery system

The various microfloras of the colon are Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria and Ruminococcus, etc. This microflora of gut depends on fermentation of undigested materials in the small intestine for their energy requirements. The microflora performs fermentation by producing a large number of enzymes like glucoronidase, xylosidase, arabinosidase, galactosidase, nitroreductase, and deaminase and urea dehydroxylase. These biodegradable enzymes are capable of degrading the polymers used for targeting the drug delivery to colon. Different polymers aroused for preventing the release of drug in the stomach and small intestine. When the coated formulations reach the intestine the biodegradable polymers gets degraded by the enzymes produced by the microbial flora and the drug gets released in the targeted region. Prodrug is the main approach of microbial triggered drug delivery system in which the drug release from the formulation is triggered by the microflora present in the gut. Prodrug is the inactive form of an active parent drug that undergoes enzymatic transformation to release the active drug. The produrgs are prepared by linking the active drug with hydrophobic moieties like amino acids, glucoronic acids, glucose, galactose, cellulose, etc. These prodrug molecules get hydrolysed in the presence of the enzymes released by the microflora.

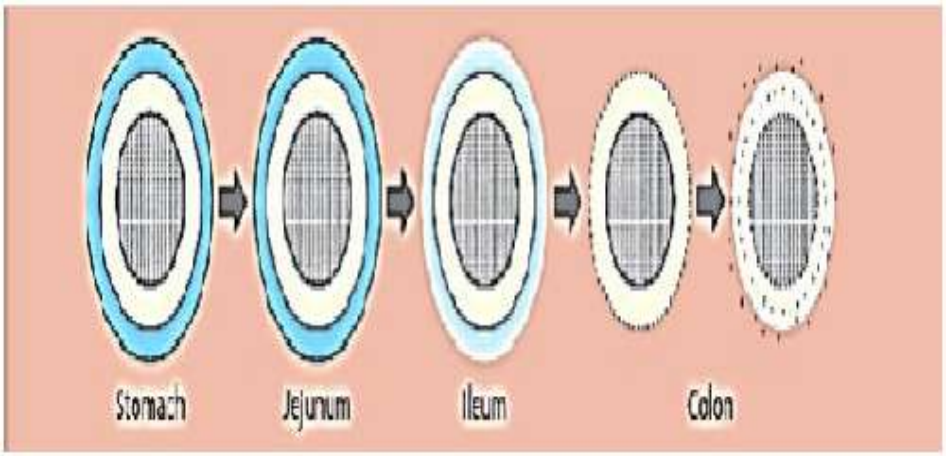

Figure 2: Drug release pattern of a multilayer coated system at different pH conditions in GIT[12]

The main drawback of this approach is that the formulation depends on the functional groups available on drug moiety for chemical linkage. The prod rugs formed upon linkage results in the formation of new chemical entities that need a lot of evaluation before using themes carriers. The most widely used prodrug approaches the metabolism of azo compounds by intestinal bacteria. Polysaccharide based delivery system is the other form of microbial triggered drug delivery system. Naturally occurring polysaccharides like guar gum, xanthan gum, chitosan, alginates, etc. are used in targeting the drug delivery. These are broken-down by the colonic microflora to simple saccharides.

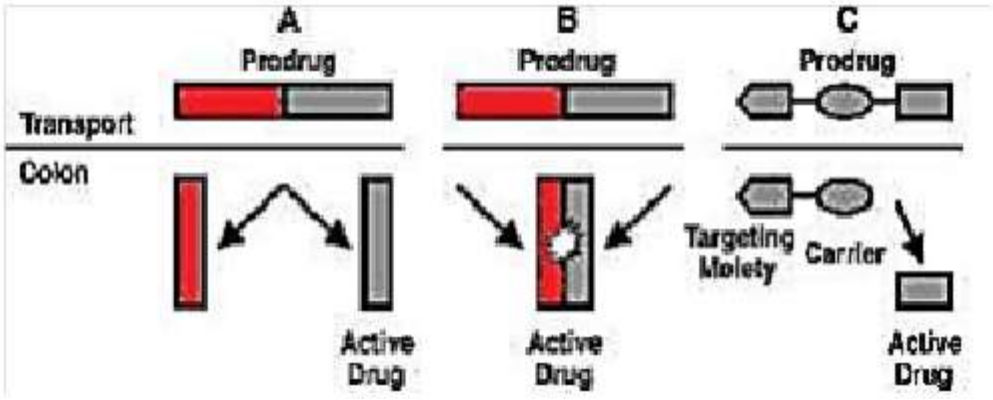

Figure 3: Prodrug approach for CDDS

New approaches for colon targeted drug delivery

Pressure controlled drug delivery system

Digestion mainly occurs due to the contractility of the stomach and peristaltic movement of the intestine. The contractility movement of stomach leads to the digestion or breakdown of larger particles to smaller ones which are then transferred to intestine. The peristaltic movement of

intestine is responsible for the passage of bolus from one part of GIT to the next part. The peristaltic movement of ascending colon transfers the bolus to transverse colon called as mass peristalsis. These peristaltic movements occur in limited number. Three to four times a day. These peristaltic movements of intestine results in an increase in the luminal pressure. This increase in luminal pressure is the key point in the development of pressure controlled drug delivery system.

The pressure controlled drug deliverysystem25 consists of a capsule in which the drug is present. These gelatin capsules are coated with water insoluble polymer like ethyl cellulose on their inner side. The drug is introduced into the capsule along with suppository base. The thickness of ethyl cellulose coating determines the disintegration capacity of the capsule. After administration the suppository base dissolves at body temperature. The water from intestinal contents is absorbed resulting in increased viscosity which leads than increase in the pressure in the capsule. The pressure in the capsule expels the drug into the colon. The intestinal pressure developed varies with the circadian rhythms, state of body, food administration, etc.

CODES technology

This method is developed to minimize the problems associated with the pH and time dependent drug delivery systems. In this system the pH sensitive polymers are used along with the polysaccharides that are degraded only by specific bacteria present in the intestine. This system consists of a core tablet coated with three layers of polymer coatings[27].

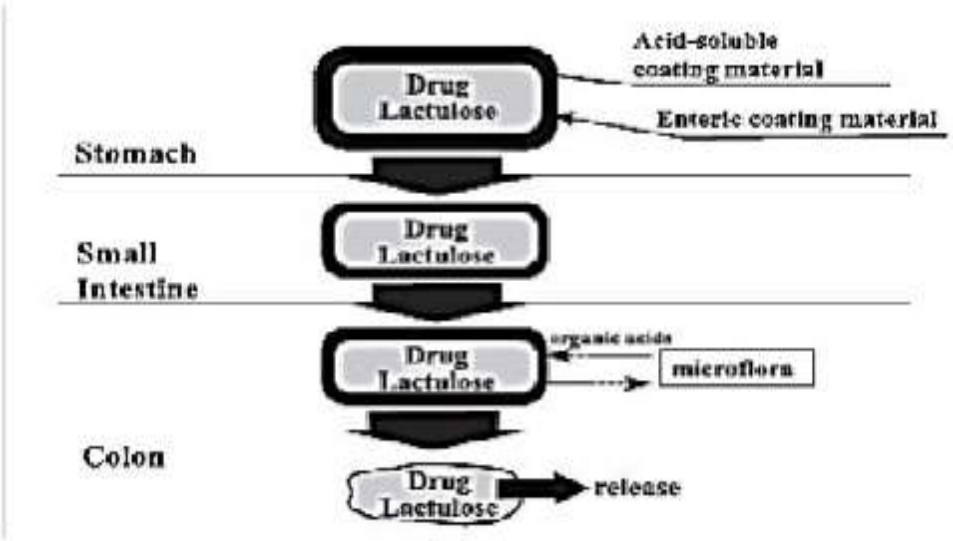

Figure 7: CODES system

The outer coating is composed of the polymer Eudragit L. This coating gets dissolved once the tablet passes though the pyloric and duodenum and exposes the next coating. The next coating is composed of Eudragit E. This layer allows the release of lactulose present in the inner core. This

released lactulose gets metabolized into short chain fatty acids that lower the surrounding pH where the Eudragit Layer dissolves. The dissolving of eudragit results in the exposure of the drug. The other polysaccharides that are used along with the drug in the core tablet are mannitol, maltose ,etc. The bacteria present in the colon are responsible for the degradation of polysaccharides that are released from the core tablet. The degradation of polysaccharides results in organic acids formation that lowers the pH of the contents surrounding the tablet.

Osmotically controlled colon targeted drug delivery system

This system30 consists of osmotic units. The osmotic units are used either singly or as many as 5-6 push pull units that are encapsulated in a hard gelatin capsule. The push pull units are bilayered with outer enteric impermeable membrane and inner semi permeable membrane. The internal or central part of the push pull consists of the drug layer and push payer. The semi permeable membrane which is present next to the drug layer consists of an orifice through which the drug contents are expelled during the course of time.

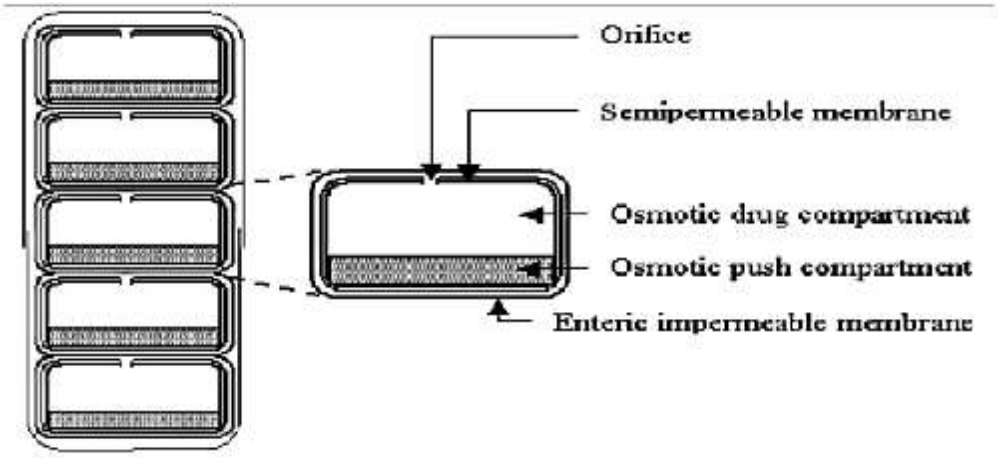

Figure 8: Osmotically controlled CDDs [12]

The capsule body enclosing the push pull units gets dissolved immediately after administration. During the passage of the push pull units through the GIT the enteric impermeable membrane prevents the water absorption into the unit. The coating gets dissolved once it reaches the small intestine due to higher pH (>7). Water enters the unit through the semi permeable membrane causing the push layer to swell. The swelling of the push compartment forces the drug into the surrounding environment through the orifice. These osmotic controlled drug delivery systems deliver the drug at a constant rate for up to 24hr.

Pulsatile colon targeted drug delivery

Pulsincap system

In this system the formulation is developed in a capsule form. The plug placed in the capsule controls the release of the drug. Swellable hydrogels are used to seal the drug contents. The capsule27 gets swelled when it comes in contact with the dissolution fluid and after a lag time the plug gets pushed off from the capsule and the drug will be released. Polymers such as different grades of hydroxyl propyl methyl cellulose (HPMC), poly methyl methacrylate and polyvinyl acetate are used as hydrogel plugs. The lag time is controlled by the length and points of intersection of the plug-in the capsule body.

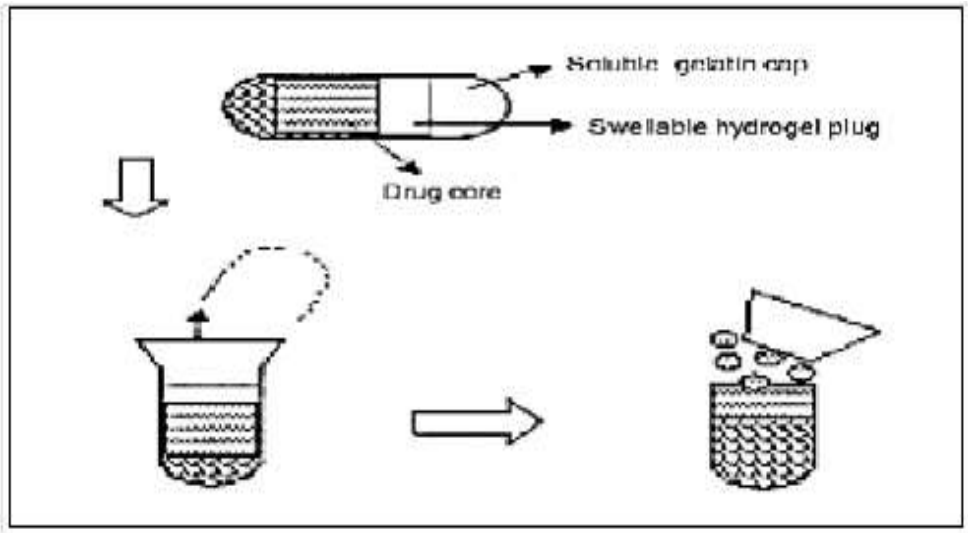

Figure 4: Pulsincap system [12]

Port system

In this system the capsule body is enclosed in a semi permeable membrane. The capsule body consists of an insoluble plug consisting of somatically active agent and drug formulation. When the capsule comes in contact with the dissolution fluid the semi permeable membrane permits the fluid flow into the capsule resulting in the development of pressure in the capsule body which leads to release of drug due to expelling of the plug. The drug is released at regular intervals with time gap between the successive intervals 24 Hrs.

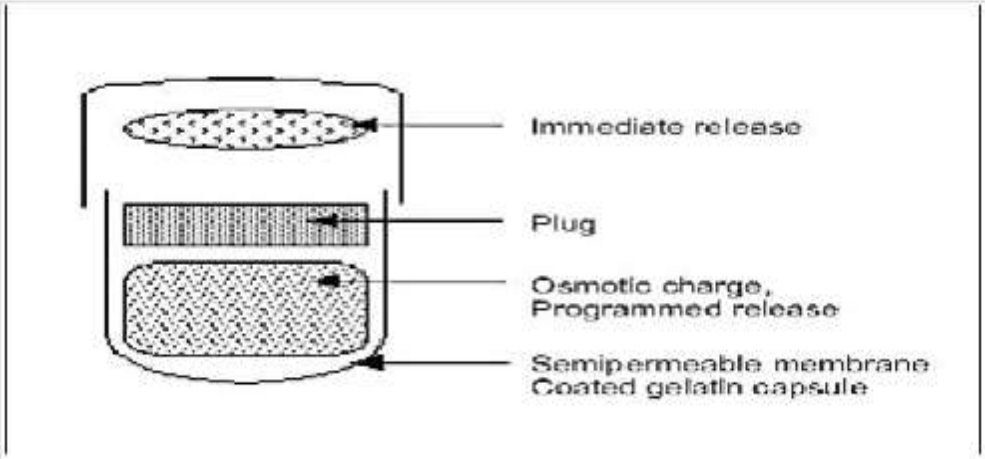

Figure 5: Port system [12]

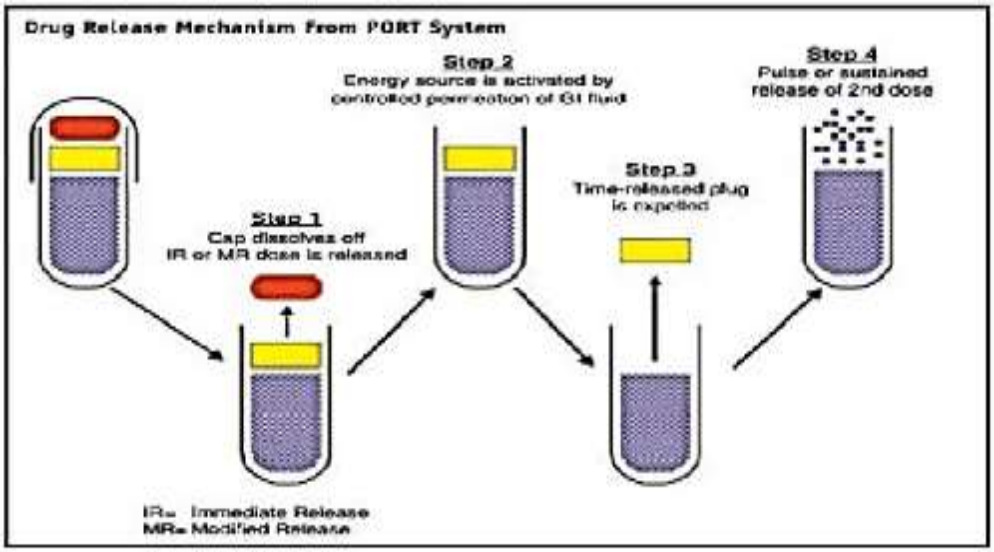

Figure 6: Drug release mechanism of port system [12]

PHYSICOCHEMICAL CHARACTERIZATION OF NPS [13]

Scanning electron microscopy

The morphology of the NPs was analyzed by scanning electron microscopy. NPs suspended in water were dropped on a carbon tape and air-dried at room temperature in a fume hood or a desiccator. Samples were then coated with platinum for 2 minutes in vacuum and viewed by field emission scanning electron microscopy (S4800, Hitachi, Japan) at an acceleration voltage of 1–5 kV.

Particle size analysis

A qNano size analyzer (Izon Sciences, Christchurch, New Zealand) coupled with an air-based variable pressure module (VPM) was used for the size determination of NPs using 200 nanopore and 200 nm calibration particles. NPs and calibration particles (5 µL) were suspended separately in 1,000 µL of Izon Tris buffer electrolyte and sonicated for at least 30 minutes prior to use. Each

recorded measurement was based on at least 500 particles. Particle sizes were determined using Izon control suite software and were also evaluated using a Zetasizer [13].

Drug loading and entrapment efficiency

The capecitabine entrapped in the NPs was evaluated using high-performance liquid chromatography (HPLC) according to an established method.17 The HPLC system used for the budesonide analysis was an LC-20AT (Shimadzu, Tokyo, Japan) equipped with an auto sampler processor, an SPD-20A UV detector, and a Luna C18 column (5 μm, 150 mm ×4.6 mm, Phenomenex, Torrance, CA, USA). The ultraviolet (UV) detector wavelength was set to 254 nm, and a combination of methanol and water (70:30) at a flow rate of 0.8 mL/min was used as the mobile phase. A linear calibration curve ($R2$ =0.9997) was obtained over the range of 0.125–250 μg/mL using standard budesonide solution. Specific amounts of NPs were dissolved in methanol, and the budesonide content was determined using the calibration curve. Samples were prepared in triplicate, and the drug loading efficiency and encapsulation efficiency (%) were calculated using the following equations:

$$\text{Loading efficiency (\%)} = \frac{\text{Amount of capecitabine in NPs}}{\text{Amount of NPs}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of capecitabine in NPs}}{\text{Amount of capecitabine initially added}} \times 100$$

In vitro drug release study

In vitro drug release was initiated in a buffer system at pH 1.2, and after 2 hours and 6 hours, the pH was changed to 6.5 and 7.4, respectively, corresponding to the pH in the stomach, upper small intestine, and both ileum and colon, respectively. Drug-loaded NPs (10 mg) were suspended in 30 mL of the release medium and incubated in a shaking water bath (60 rpm, 37°C). Tween-80 (0.2% w/v) was added to the release medium to facilitate the solubilization of capecitabine released from NPs. Aliquots of the dissolution medium (150 μL) were withdrawn at predetermined time intervals and centrifuged at 17,000× *g* for 30 minutes. Supernatants containing capecitabine released from the NPs were analyzed using HPLC as described earlier. All experiments were performed in triplicate.

CONCLUSION

We designed and developed novel capecitabine-loaded Time_NPs that can minimize premature drug release in the stomach and small intestine and release the drug in the colon in a sustained manner. An in vitro drug release and in vivo distribution study revealed that Time-NPs exhibited improved colon-specific drug release and distribution. Capecitabine loaded Time-dependent nanoparticles were prepared by using oil-in-water emulsion or nanoprcipitation method.

Nanoparticles having various advantages over tablets or capsules as short gastric residence time, improved bioavailability.

ACKNOWLEDGMENT

Authors are thankful to the management of Amrutvahini College of Pharmacy, Sangamner for providing the necessary service in collecting the several data needed for the preparation of this article.

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