

# Pharmacokinetic and Biodistribution Analysis of 5-Fluorouracil- and Celecoxib-loaded Eudragit S100-coated Chitosan Microspheres Intended for Colon-specific Delivery

Vikas Bansal<sup>1,2</sup>, Anjoo Kamboj<sup>1</sup>, Jitender Madan<sup>1</sup>

<sup>1</sup>Department of Pharmaceutics, Chandigarh College of Pharmacy, Mohali, Punjab, India, <sup>2</sup>I.K. Gujral Punjab Technical University, Jalandhar, Punjab, India

## Abstract

**Background and Objectives:** Colon cancer is one of the leading causes of deaths around the world in human beings. In the present investigation, pharmacokinetic and biodistribution analysis of eudragit-coated chitosan microspheres bearing 5-fluorouracil (5-FU) and celecoxib (Cb) combination (5-FU-Cb-Ch-ES-MSs) was carried out in Wistar rats. **Materials and Methods:** The tailored microspheres and a suspension of 5-FU and Cb (5-FU-Cb-PS) at the dose of 15 mg/kg of 5-FU and 3.3 mg/kg of Cb, respectively, were administered through oral route of administration. **Results and Discussion:** 5-FU-Cb-PS exhibited  $C_{max}$  of  $36.71 \pm 3.23$   $\mu\text{g/ml}$  at  $t_{max}$  of  $1 \pm 0.17$  h for 5-FU. On the other hand,  $C_{max}$  of  $10.14 \pm 1.16$   $\mu\text{g/ml}$  at  $t_{max}$  of  $10.26 \pm 0.58$  h was noticed for 5-FU released from 5-FU-Cb-Ch-ES-MSs. 5-FU-Cb-PS showed  $C_{max}$  of  $22.48 \pm 5.32$   $\mu\text{g/ml}$  at  $t_{max}$  of  $8 \pm 0.00$  h for Cb. Correspondingly,  $C_{max}$  of  $3.18 \pm 0.16$   $\mu\text{g/ml}$  at  $t_{max}$  of  $12.39 \pm 0.62$  h was observed for Cb released from 5-FU-Cb-Ch-ES-MSs. Moreover, at the end of 8 h, 5-FU-Cb-Ch-ES-MSs delivered a substantial amount of 5-FU (56.3  $\mu\text{g/g}$ ) and Cb (78.6  $\mu\text{g/g}$ ) in the colon, the site of action. **Conclusion:** This remarkable therapeutic concentration of both the drugs in the colon may be associated with pH-dependent solubility of eudragit S100 and subsequent degradation of Ch core in the colonic milieu due to the presence of polysaccharidase enzyme. Therefore, it can be postulated that the 5-FU-Cb-Ch-ES-MSs is a potential candidate for further *in vivo* study.

**Key words:** 5-fluorouracil, celecoxib, colon targeting, pH-sensitive microspheres

## INTRODUCTION

Colon cancer or colorectal cancer (CRC) is the third most commonly detected neoplasm worldwide in human beings, both men and women. It is considered as a lifestyle disease.<sup>[1]</sup> It is also known as bowel cancer and it starts from the epithelium of colon and rectum. In contrary to CRC, occurrence of anal cancer is rare.<sup>[2]</sup> Occurrence of CRC varies worldwide and more cases are reported in countries such as North America, Australia, Japan, Western Europe, and New Zealand and it is closely associated with western lifestyle or prosperity.<sup>[1]</sup> A small number of cases of CRC are linked with genetic disorders.<sup>[3]</sup> It usually starts from adenomas, a precancerous growth, non-cancerous, or benign polyps.<sup>[4]</sup> These benign polyps can progress into adenomatous polyps slowly and approximately 85% of all

colorectal neoplasm is developing from these adenomatous polyps. Most CRCs are adenocarcinomas.

Studies show that approximately 1.2 million people are diagnosed with CRC every year and account for more than 6 lakhs deaths per year due to this deadliest disease.<sup>[5]</sup> As per the report of the World Health Organization, cancer: Fact sheet, cancer is one of the second leading causes of death globally and was responsible for 8.8 million deaths in 2015. CRC is at the third foremost reason of death in all kinds of

### Address for correspondence:

Vikas Bansal, Department of Pharmaceutics, Chandigarh College of Pharmacy, Mohali - 140 307, Punjab, India.  
Tel: +91-01723984267. Fax: +91-01723984209.  
E-mail: vikasbansal16@gmail.com

**Received:** 19-09-2018

**Revised:** 25-11-2018

**Accepted:** 08-12-2018

cancer after lung and liver cancer followed by stomach and breast cancer. It is responsible for approximately 7.74 lakhs deaths. As per the reports of American cancer society, in 2017, approximately 95,520 recent cases of colon cancer and 39,910 cases of rectal cancer were diagnosed in America Alone.<sup>[6]</sup>

5-fluorouracil (5-FU), a synthetic pyrimidine antimetabolite, is the first-line chemotherapeutic agent for the treatment of CRC.<sup>[7,8]</sup> 5-FU is extensively metabolized in liver and blood by dihydropyrimidine dehydrogenase into dihydrofluorouracil. Moreover, 5-FU has invariable oral bioavailability which necessitates the administration of drug as continuous intravenous infusion, to maintain the therapeutic plasma concentration.<sup>[9,10]</sup> Moreover, continuous intravenous infusion of 5-FU is also associated with serious gastrointestinal, hematological, dermatological, and cardiotoxicity including angina pectoris, myocardial infarction, and arrhythmia.<sup>[11,12]</sup> As a monotherapy, 5-FU has a very poor response rate of 20% as compared to the response rate of 45% when combined with other anticancer drugs.<sup>[9]</sup>

5-FU and its prodrug capecitabine both have cardiotoxic nature, leading to angina pectoris, myocardial infarction, arrhythmias, heart failure, and cardiogenic shock.<sup>[11,13]</sup> 5-FU also diminishes the heart pump capacity and consequently leads to heart insufficiency due to scattered necrosis with loss of cardiomyocytes. Cardiotoxicity induced by 5-FU may be associated with dose, route of administration, and plan of chemotherapy.<sup>[13]</sup>

Numerous epidemiological, clinical, and laboratory statistics have manifested the role of nonsteroidal anti-inflammatory drugs in prevention and retardation of gastrointestinal tumor including CRC.<sup>[10,14]</sup> Moreover, circumstantial evidence supported the potential role of cyclooxygenase-2 (COX-2) in CRC, having increased level of prostaglandins.<sup>[15]</sup> High COX-2 expression is often discerned in 85% of human colorectal adenocarcinomas.<sup>[16,17]</sup> USFDA established the role of Cb, a selective COX-2 inhibitor in halting the growth and progression of CRC in the patients of familial adenomatous polyposis.<sup>[18,19]</sup>

A therapeutic combination can potentially more effective in the management of CRC in addition to the reduction of side effects as compared to single-drug therapy. Clinical observations established the possible synergism between Cb and 5-FU to improve the chemotherapy of CRC.<sup>[10]</sup>

Therefore, we have fabricated eudragit S100 (ES)-coated chitosan microspheres of 5-FU and Cb combination for colon targeting. In the development of site-specific drug delivery system, the polymer plays a key role as it acts as a carrier to transport the drug at the desired site of action. Chitosan (Ch) is a natural polysaccharide made up of copolymers of glucosamine and N-acetyl glucosamine and employed as a core material for the development of microspheres (MSs).

Ch is a biodegradable and biocompatible polymer and widely used in the development of controlled drug delivery system. Ch has good biological properties such as biocompatibility, biodegradability, non-toxicity, and antibacterial characteristics which make it suitable to be employed as a favorable drug carrier for many potential routes of administration. Due to some physicochemical properties of Ch, for example, cationic charge in acidic medium and inter- and intramolecular hydrogen bonding, this natural polymer can be a more promising carrier for the design of various novel drug delivery systems. Chitosan can be used for a variety of purposes such as a coating agent, mucoadhesive agent, gel former, permeation enhancer, and controlled-release matrix. Chitosan is not degraded in the stomach and small intestine, but it is degraded in the colon due to the presence of gut flora, anaerobic bacteria which degrade polysaccharides in the presence of enzyme.<sup>[20]</sup>

On the other hand, eudragit S100 (ES), a pH-sensitive polymer is used for the coating of microspheres to prevent the release of the therapeutic moiety in upper gastrointestinal tract. It is a copolymer of methacrylic acid and its esters and has a considerable role in pH-dependent site-specific drug delivery. ES releases the drug in the colon as it dissolves in the colonic environment (pH 7.0). In the present investigation, we have reported the pharmacokinetic and biodistribution of 5-FU-Cb-PS as well as 5-FU-Cb-Ch-ES-MSs. Both were introduced separately to rats at the dose of 15 mg/kg of 5-FU and 3.3 mg/kg of Cb, respectively.

## MATERIALS AND METHODS

### Materials

5-FU (98–99% pure) was purchased from CDH-Laboratory Chemicals, India. Cb was gifted by Cadila Pharmaceutical, Ahmadabad, India. Ch (M.W. 75,000–150,000 Da) and ES were procured from HiMedia Laboratories, Mumbai, India. Acetonitrile and water (HPLC grade) were purchased from Fisher Scientific, Mumbai, India. All other reagents and chemicals used were of analytical grade.

### *In vivo* study

*In vivo* study was executed as per the standard guidelines laid down by CPCSEA, Ministry of Social Justice and Empowerment, Government of India. The Institutional Animal Ethics (IAEC) had approved the study (Protocol No. IAEC/Feb17/19). The *in vivo* study was carried out in male healthy Wistar rats (250–300 g). 48 animals were randomly divided into two groups (comprising 24 in each) which were designated as standard and test. The rats were housed in standard laboratory conditions at an ambient temperature of  $25 \pm 3^\circ\text{C}$  and a relative humidity of  $65 \pm 5\%$  on a 12:12 h light:dark cycle. The rats were subjected to 12 h fasting before dosing with free access of water.

### Pharmacokinetic and biodistribution analysis

The first group of rats served as “standard” comprising eight groups of rats ( $n = 3$ ) was orally administered with 5-FU-Cb-PS of combination of 5-FU (15 mg/kg) and Cb (3.3 mg/kg) prepared in PBS (pH 7.4). The second group, termed as “test,” received 5-FU-Cb-Ch-ES-MSs at identical dose.

The blood samples were withdrawn by rupturing the heart of the rat at predetermined time points of sampling, i.e., 0, 0.5, 1, 2, 3, 4, 8, and 24 h. The blood samples were collected in the heparinized tubes and promptly centrifuged at 5000 rpm for 30 min to separate the plasma and subsequently stored at  $-20^{\circ}\text{C}$  until further analysis.<sup>[21]</sup>

Stomach, small intestine, and colon were also isolated from the rat. The luminal contents were separated and weighed. The isolated organs were washed with normal saline solution and longitudinally cut to remove any traces of luminal content. The isolated organs were homogenized with 5 ml of normal saline to prepare tissue suspension. Next 1 ml of tissue suspension was mixed with 5 ml of acetonitrile to precipitate the proteins. Following this, the mixture was vortexed for 30 min to extract the drugs. Furthermore, the

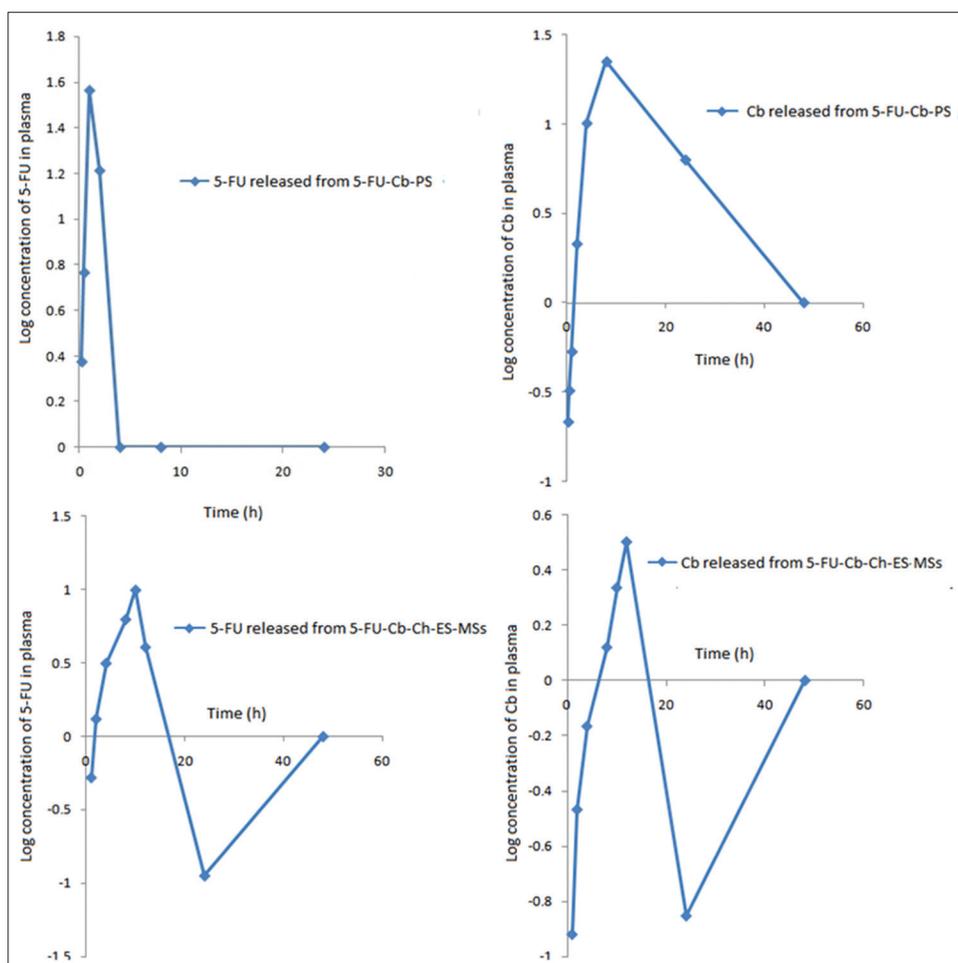
mixture was centrifuged at 5000 rpm for 20 min at  $4^{\circ}\text{C}$ . The supernatant was filtered through  $0.22\ \mu\text{m}$  membrane filter. A  $20\ \mu\text{l}$  of the filtrate was injected into HPLC to determine the 5-FU and Cb content.<sup>[22,23]</sup>

### HPLC determination of 5-FU and Cb

The quantitative estimation of 5-FU and Cb in rat plasma and different parts of GI tract was performed by HPLC. The system used for this purpose was HPLC ([1220 Infinity LC], Agilent Technologies, Germany) equipped with UV detector and EZ Chrome Elite software. Zorbax eclipse C18 column was used as the stationary phase, whereas the combination of acetonitrile and water (50:50) was used as the mobile phase. The flow rate of 1 ml/min was maintained at which mobile phase was pumped into the column. The detection of the eluents was carried out by UV-vis detector at 260 nm.<sup>[12]</sup>

## RESULTS AND DISCUSSION

Colon targeting through oral route of administration is more convenient as compared to parenteral route of administration in terms of improved therapeutic index, higher bioavailability



**Figure 1:** Pharmacokinetic profile of a combination (5-FU-Cb-PS, 5-FU - 15 mg/kg and Cb - 3.3 mg/kg) and 5-FU-Cb-Ch-ES-MSs in rats following oral administration

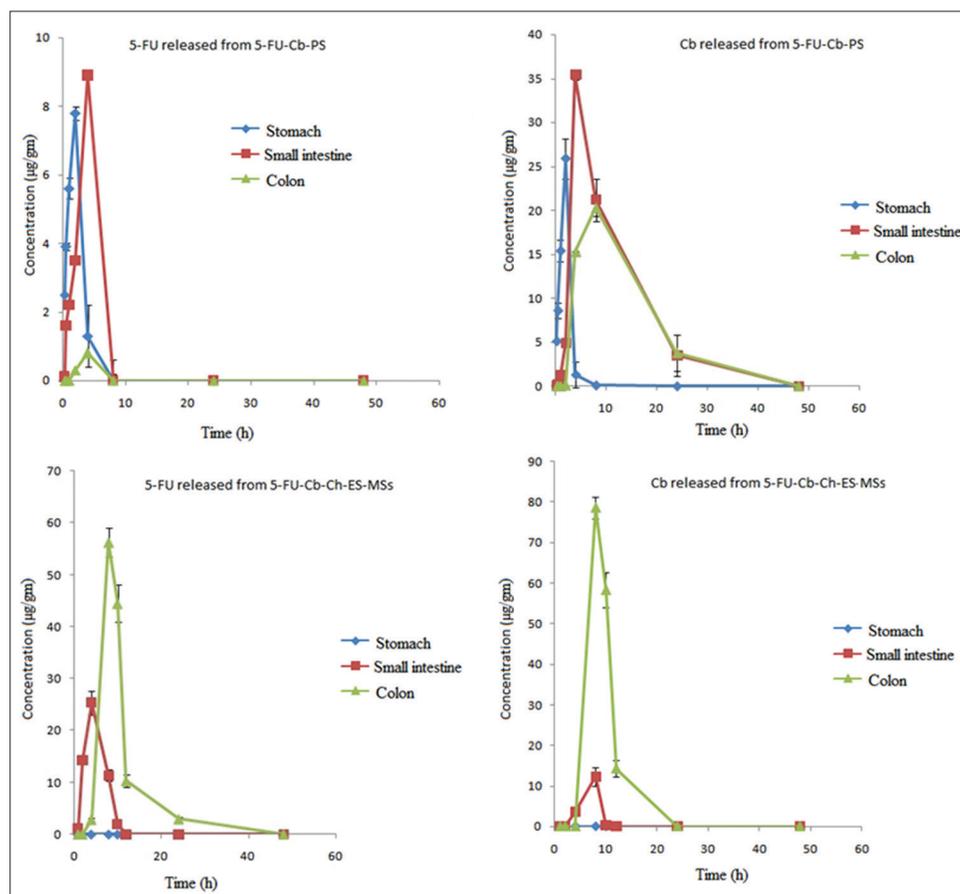
at the target site, preferred route of administration, and increase in the quality of life of patients. Pharmacokinetic parameters of 5-FU and Cb, after oral administration of 5-FU-Cb-PS and 5-FU-Cb-Ch-ES-MSs, are presented in Tables 1 and 2, respectively. The plasma concentration-time profile depicted that 5-FU appeared promptly, but a lesser amount of Cb was absorbed in systemic circulation following oral administration of 5-FU-Cb-PS [Figure 1]. The results of non-compartmental pharmacokinetic study indicated that the  $t_{1/2}$  ( $10.26 \pm 0.58$  h) and MRT ( $13.78 \pm 1.32$  h) of 5-FU released from 5-FU-Cb-Ch-ES-MSs were significantly (unpaired *t*-test,  $P < 0.05$ ) higher as compared to  $t_{1/2}$  ( $1 \pm 0.17$  h) and MRT ( $1.72 \pm 0.58$  h) of 5-FU released from 5-FU-Cb-PS. The  $t_{1/2}$  ( $16.5 \pm 0.87$  h) and MRT ( $21.56 \pm 1.42$  h) of Cb released from 5-FU-Cb-Ch-ES-MSs were notably increased (unpaired *t*-test,  $P < 0.05$ ) in contrast to  $t_{1/2}$  ( $7.7 \pm 0.32$  h) and MRT ( $14.3 \pm 1.42$  h) of Cb released from 5-FU-Cb-PS. The higher  $t_{1/2}$  and MRT of 5-FU and Cb released from tailored microspheres may be credited to unavailability of the 5-FU and Cb in the upper GI tract for absorption, and subsequently, pH dependent targeted delivery in colon. The augmented MRT signifies that the average residence time of 5-FU and Cb loaded in the 5-FU-Cb-Ch-ES-MSs is longer than that of 5-FU-Cb-PS. The study indicated that the drug stays for longer duration in GI tract. The area under the curve ( $AUC_{0-t}$ )

is a key parameter which reflects the extent of absorption of the drug. A significant decrease (unpaired *t*-test,  $P < 0.05$ ) in the  $AUC_{0-t}$  ( $8.19 \pm 1.25$   $\mu\text{g}\cdot\text{h}/\text{ml}$ ) was observed for 5-FU released from 5-FU-Cb-Ch-ES-MSs in comparison to  $AUC_{0-t}$  ( $52.32 \pm 3.44$   $\mu\text{g}\cdot\text{h}/\text{ml}$ ) of 5-FU released from 5-FU-Cb-PS.

**Table 1:** Pharmacokinetic parameters of 5-FU after oral administration of 5-FU-Cb-PS and 5-FU-Cb-Ch-ES-MSs in rats

Parameters	5-FU-Cb-PS	5-FU-Cb-Ch-ES-MSs
Ke ( $\text{h}^{-1}$ )	$1.230 \pm 0.45$	$0.048 \pm 0.004$
$t_{1/2}$ (h)	$0.56 \pm 0.31$	$14.23 \pm 0.17$
$C_{\text{max}}$ ( $\mu\text{g}/\text{ml}$ )	$36.71 \pm 3.23$	$10.14 \pm 1.16$
$T_{\text{max}}$ (h)	$1 \pm 0.17$	$10.26 \pm 0.58$
$AUC_{0-t}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	$52.32 \pm 3.44$	$8.19 \pm 1.25$
$AUC_{0-\infty}$ ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	$57.62 \pm 4.32$	$8.45 \pm 1.78$
MRT (h)	$1.72 \pm 0.58$	$13.78 \pm 1.32$

5-FU: 5-Fluorouracil, 5-FU-Cb-PS: 5-FU and Cb suspension, 5-FU-Cb-Ch-ES-MSs: Eudragit-coated chitosan microspheres loading 5-FU and Cb combination, Ke: Elimination rate constant,  $t_{1/2}$ : Half-life,  $C_{\text{max}}$ : Peak plasma concentration,  $T_{\text{max}}$ : Time taken to reach the maximum concentration,  $AUC_{0-t}$ : Area under the curve up to last sampling point,  $AUC_{0-\infty}$ : Area under the curve infinity, MRT: Mean residence time



**Figure 2:** Biodistribution profile of a combination (5-FU-Cb-PS, 5-FU - 15 mg/kg and Cb - 3.3 mg/kg) and 5-FU-Cb-Ch-ES-MSs in rats following oral administration

**Table 2:** Pharmacokinetic parameters of Cb after oral administration of 5-FU-Cb-PS and 5-FU-Cb-Ch-ES-MSs in rats

Parameters	5-FU-Cb-PS	5-FU-Cb-Ch-ES-MSs
Ke (h <sup>-1</sup> )	0.09±0.08	0.042±0.03
t <sub>1/2</sub> (h)	7.7±0.32	16.5±0.87
C <sub>max</sub> (µg/ml)	22.48±5.32	3.18±0.16
T <sub>max</sub> (h)	8±0.00	12.39±0.62
AUC <sub>0-t</sub> (µg-h/mL)	322.21±62.17	53.23±3.76
AUC <sub>0-∞</sub> (µg-h/mL)	367.42±84.43	55.18±2.12
MRT (h)	14.3±1.42	21.56±1.42

5-FU: 5-Fluorouracil, 5-FU-Cb-PS: 5-FU and Cb suspension, 5-FU-Cb-Ch-ES-MSs: Eudragit-coated chitosan microspheres loading 5-FU and Cb combination, Ke: Elimination rate constant, t<sub>1/2</sub>: Half-life, C<sub>max</sub>: Peak plasma concentration, T<sub>max</sub>: Time taken to reach the maximum concentration, AUC<sub>0-t</sub>: Area under the curve up to last sampling point, AUC<sub>0-∞</sub>: Area under the curve infinity, MRT: Mean residence time

Similarly, notable reduction (unpaired *t*-test, *P* < 0.05) in the AUC<sub>0-t</sub> (53.23 ± 3.76 µg-h/ml) of Cb released from 5-FU-Cb-Ch-ES-MSs in contrast with AUC<sub>0-t</sub> (322.21 ± 62.17 µg-h/ml) of Cb released from 5-FU-Cb-PS. This drastic decrease in the AUC<sub>0-t</sub> may be attributed to the fact that both 5-FU and Cb avoided the well absorptive stomach and small intestine and reached up to less absorptive colon.

Biodistribution study of 5-FU-Cb-Ch-ES-MSs was explored after oral administration to Wistar rats. Post 2 h of administration of 5-FU-Cb-PS, 7.8 µg/g of 5-FU was observed in stomach as compared to reduced drug amount (3.5 µg/g) in small intestine, whereas a negligible amount of 5-FU was detected in the colon. More interestingly, at 8 h, only 20.3 µg/g of Cb reached to the colon in comparison to 25.9 µg/g of Cb in stomach at 2 h and 35.4 µg/mg Cb in small intestine at 4 h, respectively. Following administration of 5-FU-Cb-Ch-ES-MSs, a remarkable increase in the amount of 5-FU (56.3 µg/g) and Cb (78.6 µg/g) in colon was observed; however, upper GI tract received only negligible amount of both the drugs [Figure 2]. This notable increased amount of 5-FU and Cb in the colon may be linked with pH-dependent solubility of ES and consequent protection to the Ch-MSs in gastric and intestinal *milieu*. In addition, the presence of ample number of microflora in the colonic environment is also responsible for biodegradation of chitosan core of the tailored MSs.

## CONCLUSION

In the present investigation, pH modulated 5-FU-Cb-Ch-ES-MSs were fabricated to deliver the combination of drugs in colon. Ch, a carbohydrate polymer, was used as the core material which degrades in the presence of polysaccharidase enzyme, localized in the colon. Hence, Ch is widely used for tailoring the micro and nanoparticulate system for colon targeting. ES coating helped the tailored system to release the

drug in the colonic milieu only. Pharmacokinetic parameters of 5-FU-Cb-Ch-ES-MSs in addition to biodistribution pattern were calculated in rats.

In conclusion, our colon targeted formulation is able to deliver a significant amount of 5-FU and Cb in the colon despite its less absorptive surface area with minimal release in stomach and small intestine, having substantial surface area for absorption. Further studies are warranted to test the therapeutic potential of 5-FU-Cb-Ch-ES-MSs in xenograft model of colon cancer under a stringent set of parameters.

## REFERENCES

- Patel MM. Getting into the colon: Approaches to target colorectal cancer. *Expert Opin Drug Deliv* 2014;11:1343-50.
- Wilkes G, Hartshorn K. Clinical update: Colon, rectal, and anal cancers. *Semin Oncol Nurs* 2012;28:e1-22.
- Gulbake A, Jain A, Jain A, Jain SK. Insight to drug delivery aspects for colorectal cancer. *World J Gastroenterol* 2016;22:582-99.
- Lin C, Ng HL, Pan W, Chen H, Zhang G, Bian Z, *et al.* Exploring different strategies for efficient delivery of colorectal cancer therapy. *Int J Mol Sci* 2015;16:26936-52.
- Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet* 2014;383:1490-502.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017;67:7-30.
- Ashwanikumar N, Kumar NA, Nair SA, Kumar GV. Methacrylic-based nanogels for the pH-sensitive delivery of 5-fluorouracil in the colon. *Int J Nanomedicine* 2012;7:5769-79.
- Sasaki K, Tsuno NH, Sunami E, Tsurita G, Kawai K, Okaji Y, *et al.* Chloroquine potentiates the anti-cancer effect of 5-fluorouracil on colon cancer cells. *BMC Cancer* 2010;10:370.
- Udofot O, Affram K, Smith T, Tshabe B, Krishnan S, Sachdeva M, *et al.* Pharmacokinetic, biodistribution and therapeutic efficacy of 5-fluorouracil-loaded pH-sensitive PEGylated liposomal nanoparticles in HCT-116 tumor bearing mouse. *J Nat Sci* 2016;2:e171-8.
- Zhang DQ, Guo Q, Zhu JH, Chen WC. Increase of cyclooxygenase-2 inhibition with celecoxib combined with 5-FU enhances tumor cell apoptosis and antitumor efficacy in a subcutaneous implantation tumor model of human colon cancer. *World J Surg Oncol* 2013;11:16.
- Wacker A, Lersch C, Scherpinski U, Reindl L, Seyfarth M. High incidence of angina pectoris in patients treated with 5-fluorouracil. A planned surveillance study with 102 patients. *Oncology* 2003;65:108-12.
- Dev RK, Bali V, Pathak K. Novel microbially triggered colon specific delivery system of 5-fluorouracil: Statistical optimization, *in vitro*, *in vivo*, cytotoxic and stability assessment. *Int J Pharm* 2011;411:142-51.

13. Jensen SA. On 5-fluorouracil therapy of colorectal cancer. *Dan Med J* 2013;60:B4603.
14. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC, *et al.* Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann Intern Med* 1994;121:241-6.
15. Taketo MM. Cyclooxygenase-2 inhibitors in tumorigenesis (Part II). *J Natl Cancer Inst* 1998;90:1609-20.
16. Goldman AP, Williams CS, Sheng H, Lamps LW, Williams VP, Pairet M, *et al.* Meloxicam inhibits the growth of colorectal cancer cells. *Carcinogenesis* 1998;19:2195-9.
17. Rahman M, Selvarajan K, Hasan MR, Chan AP, Jin C, Kim J, *et al.* Inhibition of COX-2 in colon cancer modulates tumor growth and MDR-1 expression to enhance tumor regression in therapy-refractory cancers *in vivo*. *Neoplasia* 2012;14:624-33.
18. Ruiz JF, Kedziora K, Keogh B, Maguire J, Reilly M, Windle H, *et al.* A double prodrug system for colon targeting of benzenesulfonamide COX-2 inhibitors. *Bioorg Med Chem Lett* 2011;21:6636-40.
19. Finckh A, Aronson MD. Cardiovascular risks of cyclooxygenase-2 inhibitors: Where we stand now. *Ann Intern Med* 2005;142:212-4.
20. Mitra A, Dey B. Chitosan microspheres in novel drug delivery systems. *Indian J Pharm Sci* 2011;73:355-66.
21. Singh G, Pai RS. Pharmacokinetics and *in vivo* biodistribution of optimized PLGA nanoparticulate drug delivery system for controlled release of emtricitabine. *Drug Deliv* 2014;21:627-35.
22. Rai G, Yadav AK, Jain NK, Agrawal GP. Eudragit-coated dextran microspheres of 5-fluorouracil for site-specific delivery to colon. *Drug Deliv* 2016;23:328-37.
23. Sareen R, Jain N, Rajkumari A, Dhar KL. PH triggered delivery of curcumin from eudragit-coated chitosan microspheres for inflammatory bowel disease: Characterization and pharmacodynamic evaluation. *Drug Deliv* 2016;23:55-62.

**Source of Support:** Nil. **Conflict of Interest:** None declared.