Multiparticulate Colon Targeted Drug Delivery and In Vitro Evaluation of 5-Fluorouracil for Colorectal Cancer

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ABSTRACT

One of the most prevalent tumours in the world is colorectal cancer, which is also thought to be the second largest cause of mortality from cancer. Colon cancer can be treated with 5-fluorouracil (5-FU, also known as 5-fluoro-2,4-pyrimidinedione) or in conjunction with chemotherapy treatments. The present study aimed to create a Eudragit S100 coated colon-targeted microbead method to enhance colon targeting of 5-Fluorouracil by enhancing its local action in the colon.A technique called ionotropic gelation was used to create the formulations. Formulations were assessed based on their surface phenomenon, bead shape, entrapment effectiveness, drug loading, and in vitro drug release. Moreover, Eudragit S100 was enteric coated on Calcium alginate beads with an improved core.The particle size of the optimized formulation was 895 µm, drug entrapment efficiency was 89.91%, drug loading was 38.64%, and in vitrorelease was 79.35% after 6 hours in phosphate buffer at pH 6.8. The drug release was 55.12±1.23% after 10 hours following enteric coating of optimized calcium alginate microbeads with Eudragit S100 (10% weight increase). Regression coefficient values for the zero order, first order, and Higuchi models were determined to be 0.9790, 0.7529, and 0.9887, respectively. Based on percent cell viability, the 50 percent inhibitory concentration value found was 3.252.

Conclusion: The formulated enteric-coated calcium alginate microbeads may be taken orally to deliver 5-Fluorouracil specifically to the colon to effectively treat colorectal cancer.

Keywords:5-Fluorouracil, Cell Viability, Eudragit S100, Pectin, Release Kinetic.

1. INTRODUCTION

In the United States and other Western nations, colorectal cancer is a major cause of morbidity and mortality among the different types of cancer. More than 6 million people die from cancer each year, making it one of the world's most feared and dangerous diseases. The second most common cause of mortality is lung cancer, which is followed by colorectal cancer. [1,2]

Colorectal cancer is treated with a variety of cytotoxic medications, such as 5-fluorouracil, Oxaliplatin, and Cisplatin.5-fluorouracil (5-FU, also known as 5-fluoro-2,4-pyrimidinedione), has a broad spectrum of effectiveness against solid tumours (tumours of the gastrointestinal tract, pancreas, ovary, liver, brain, breast, colon, etc.).

The advantage of utilizing cytotoxic medications over others is that they are poisonous to fast dividing cells, which is justified by the fact that cancer cells grow and proliferate quickly. Even though the majority of these medications are given intravenously to achieve maximum bioavailability, most cytotoxic medications show treatment failure.The drug's inability to work specifically at the target site, which results in a lack of site-specificity and adverse effects on both healthy cells and malignant cells, is the primary cause of therapy failure. Anticancer medications also have hydrophobic properties, poor biodistribution, and a tendency to develop drug resistance.[3]

Due to their potential advantages, such as increased bioavailability, decreased risk of systemic toxicity, decreased risk of local irritation, predictable gastric emptying, and retention in the ascending colon for a considerable amount of time, multiparticulate dosage forms are currently receiving much more attention than single unit systems. Less inter- and intrasubject variability results from these systems' smaller particle sizes than single-unit dose forms' ability to readily move through the GI tract [4].

Some of the current approaches available to target the release of drugs to the colon include the development of prodrugs, coating of pH-sensitive polymers, use of colon-specific biodegradable polymers, timed release systems, osmotic systems, and pressure-controlled drug delivery systems. One of the various ways to achieve targeted drug delivery to the colon is to use polymers, especially those that are broken down by colonic bacteria. Polysaccharides, which are substrates for enzymes generated by bacteria in the colon, can be used by medications that aim to treat the colon. [3-6]

The cell walls of plants include a substance called pectin.L-rhamnose residues with 1, 2 links and predominantly -(1-4) linked D-galacturonic acid residues make up the majority of the linear polymer that makes up pectin.Intestinal bacteria thoroughly degrade it, but the upper GIT does not digest it. Pectin's solubility is an issue. However, this shortcoming can be remedied by modifying the methoxylation amount or by producing calcium pectinate. [5,6,7,8]

This work's objective was to utilize the natural polysaccharide pectin to develop a multiparticulate delivery system for the site-specific administration of 5-FU (Eudragit S100). This strategy was developed to prevent drug breakdown in the upper GIT, reduce systemic dose, and alleviate gastrointestinal distress by using Eudragit S100 and accurately delivering 5-FU to the colon.

2.MATERIAL ANDMETHODS

2.1 Materials

5-FU was obtained from Sigma Aldrich, Mumbai, India. Sodium alginate, Pectin,and Calcium chloride were procured from SD fine-chemical Ltd., Mumbai, India. All other reagents and solvents used were of analytical grade.

2.2 Method

2.2.1 Preparation of Calcium Alginate Microbeads

By using the ionotropic gelation process, calcium alginate microbeads were produced. The 5FU was added to a 5 ml (2% w/v) aqueous solution of sodium alginate. This solution was manually injected using a hypodermic syringe and a needle with size no. 22G into a 25ml (2% w/v) solution of $CaCl_2$. The prepared beads were kept in the medium for 20 minutes while being constantly stirred at 500 rpm; following that, they were filtered out, cleaned in distilled water, and dried.

2.2.2 Fabrication of Calcium Alginate-Pectinate Microbeads

Calcium alginate-pectinate microbeads were produced by ionotropic gelation. 5 ml (2% w/v) of sodium alginate in an aqueous solution, 5 ml without pectin (FP_1), 0.5% (FP_2), and 1.0% w/v (FP_3) of pectin in 25 ml (2% w/v) of $CaCl_2$ solution, and a 5FU were all combined. Before being filtered out, rinsed with distilled water, and dried, the prepared beads were placed in the medium and agitated at 500 rpm for 20 minutes. Each of the three batches was analyzed using release studies (Table 1)

Table 1 Composition of Microbeads.

Formulation code	Sodium Alginate Concentration (%w/v)	Pectin Concentration (%w/v)	Curing Time (minute)
FA_1	1	0.5	20
FA_2	2	0.5	20
FA_3	3	0.5	20
FP_1	2	0	20
FP_2	2	0.5	20
FP_3	2	1	20
FC_1	2	0.5	10
FC_2	2	0.5	20
FC_3	2	0.5	30

2.2.3 Coating of Calcium Alginate Microbeads

An enhanced calcium alginate bead formulation covered in Eudragit S100 was produced using the solvent evaporation procedure. Beads were added to various solutions of Eudragit S100 in the presence of acetone for weight gains of 5%, 10%, and 15%. The solvent was evaporated using a spinning evaporator with a vacuum applied at 300 mm Hg and 50 rpm. The beads were vacuum-dried in a desiccator for an additional 12 hours to ensure that any solvent that might have been left behind was evaporated. [9.10]

2.3 Characterization of 5 Fluorouracil Microbeads

2.3.1 Measurement of bead size

The bead size and size distribution of formulations containing pharmaceuticals were assessed using an optical microscope fitted with an ocular and stage micrometer. At least 50 microbeads from a batch were examined for estimation, and tests were carried out in triplicate. [11]

2.3.2 Shape and surface study

SEM images taken with the JSM 5600 scanning microscope (Japan) were collected to examine the beads' morphology and surface organization. The beads were set

atop a brass base that had been thinly vacuum-sputter-coated with gold. With an acceleration voltage of 20 kV, the secondary electron served as a detector [12].

2.3.4 Drug Entrapment Efficiency and Drug Loading:

Encapsulation efficiency is the proportion of additional medication that is encapsulated in the formulation of the beads (EE). 100 mg of the bead mixture was carefully measured out and dissolved in 100 mL of 0.1NHCl. The sample was ultrasonically treated three times for five minutes each, followed by a five-minute break. It was allowed to equilibratefor 24 hours at room temperature. The solution was diluted appropriately, filtered, and the absorbance was measured using a spectrophotometer (UV Shimadzu) at 266 nm in comparison to the appropriate blank. The amount of drug contained in the microbeads was calculated using the formula below.

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

$$\% \text{ Drug loading} = \frac{\text{Weight of drug in microbeads}}{\text{The weight of microbeads recovered}} \times 100$$

2.3.5 In-vitro drug release study from core microbeads

The in-vitro drug release study of core sodium alginate pectinate microbeads and release was carried out using the USP Dissolution Apparatus II (Veego DA, 6DR Japan) at 37± 0.5^0C at 100 rpm in simulated intestinal fluid pH 6.8. Muslin fabric was placed on top of the basket to prevent the beads from falling out. Spectrophotometric measurements were made using a UV-Visible spectrophotometer (UV-1700 Pharma Spec, Shimadzu) at 266 nm after a predetermined volume of 5 ml of the sample solution was taken out, filtered using Whatman filter paper, and then properly diluted. The percentage cumulative drug release was calculated immediately after the test sample was removed and an equivalent volume of new dissolving solution was added.

2.3.6 In-vitro drug release study from coated microbeads

The drug release study of coated alginate microbeads was conducted using the USP Dissolution Apparatus II (Veego DA, 6DR Japan). The dissolving solution was a phosphate buffer with a pH of 7.4 for the final three hours and a simulated stomach fluid with a pH of 1.2 for the first two hours. After the beads were changed to phosphate buffer pH 6.8, the drug release was then observed for up to 5 hours. A predetermined volume (5 ml) of

sample solution was taken out and examined spectrophotometrically to determine the amount of drugs released. [14,15,16]

2.3.7 Preparation of rat caecal contents

According to the CPCSEA's guidelines (HIPER/IAEC/66/2021/04), the work was completed. Two Wistar albino rats were maintained on a standard diet and had similar body weights (150–200g). One Wistar albino rate of them received 1ml of a 1% w/v dispersion of pectin in water every day for seven days to start the induction of microbial enzymes. Before the investigation, each rat was killed, and the abdomen was cut open. The caecum was dissected, ligated at both ends, cut loose, and then placed in the simulated colonic fluid that contained carbon dioxide bubbles and had a pH of 6.8. A final rat caecal concentration of 2% w/v was achieved by weighing, pooling, and suspending the caecal contents of each rat individually in phosphate buffer pH 6.8.[17]

2.3.8 In vitro drug release study in the presence of rat caecal contents

The in vitro percentage cumulative drug release of Eudragit S100 coated alginate-pectin microbeads in rat caecal content was evaluated in a USP Dissolution Apparatus II (Veego DA, 6DR Japan) with 200 ml of dissolution medium (PBS pH 6.8) at 100 rpm and 37 ± 0.5^{0}C while maintaining sink conditions throughout the study. With rat caecal and a steady stream of carbon dioxide added to the dissolving media to replicate the colonic environment, studies on the cumulative percentage release of drugs were conducted using this simulated colonic fluid. [18]

2.3.9 Mechanism of drug release

Data from in vitro percentage cumulative drug release studies were fitted into several kinetic models to study the release kinetics. These models included Korsmeyer Peppa's equation and Higuchi's model, which plot the cumulative percentage of drugs released against the square root of time and the cumulative percentage of drugs released against log time, respectively. The best-fit model was supported because the correlation coefficient value was close to 1. The data were sent to the most appropriate model [19].

2.3.10 Cytotoxicity Study by MTT Assay

A cell viability assay measures the absolute or relative amount of living cells in the sample. Viability is impacted by cellular growth and death. The semi-automatic 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT, Sigma) assay was used to assess the viability of the cells by the manufacturer's instructions. The cells were essentially

plated in a 96-well dish and left to develop there for the night. 10 to 1000µg/ml doses of 5-FU were administered to the cells. After 48 hours, the medium was supplemented with MTT (0.5 mg/mL), and the cells were incubated for 4 hours. The formazan precipitate was dissolved in 200 µl of dimethyl sulfoxide (DMSO), and the absorbance at 490 nm was measured using a benchmark microplate reader (Bio-Rad, CA). Three different assays were conducted. GraphPad Prism 9.0 software was used to generate cytotoxicity curves by graphing the determined cell viability (%) by MTT test against 5-FU concentrations. [20]

3. RESULTS

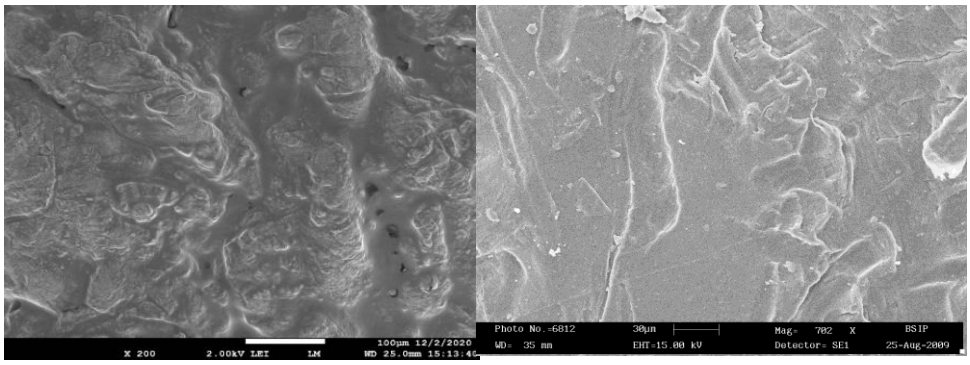
Based on bead size, bead shape, entrapment effectiveness, and in-vitro % cumulative drug release, several formulation parameters, such as sodium alginate, pectin, and curing time, were optimized. These microbeads had a spherical shape, an ideal size, good entrapment efficiency, and a high percentage cumulative drug release when made with 2% w/v sodium alginate and 0.5% w/v pectin in filtered water for a cure time of 20 minutes. (Table 2)

Table 2. Characteristics of 5-FU loaded uncoated microbeads.

Code	Particle Size (µm)	Entrapment Efficiency (%)	%Drug Loading	%Cumulative Drug Release	Shape and Surface morphology
FA_1	775.21±6.22	68.40±1.75	25.11±1.18	91.23±1.28	Regular and rough
FA_2	895.45±8.54	89.91±3.69	38.64±1.53	79.35±1.65	Regular and smooth
FA_3	998.19±5.61	96.10±3.87	42.79±1.77	62.12±2.36	Irregular and smooth
FP_1	854.45±8.54	84.91±3.69	37.64±1.13	91.12±2.56	Regular and rough
FP_2	895.45±8.54	89.91±3.69	38.64±1.53	79.35±1.65	Regular and smooth
FP_3	828.21±6.22	72.40±1.75	28.10±1.18	82.12±2.16	Irregular and smooth
FC_1	647.56±5.34	69.75±2.41	24.76±3.12	89.36±3.07	Regular and rough
FC_2	895.45±8.54	89.91±3.69	38.64±1.53	79.35±1.65	Regular and smooth
FC_3	970.19±5.62	92.10±3.87	41.79±1.77	64.59±1.72	Irregular and smooth

3.1 Shape and Surface Morphology

According to the SEM analysis, formed uncoated microbeads had spherical shapes, a rough surface, and a sand-like appearance, whereas coated microbeads had spherical shapes, features, and smooth surfaces. Alginate microbeads' surface revealed fissures (Fig. 1a and 1b), which may have been caused by sodium alginate's low degree of etherification, whereas calcium alginate-pectinate microbeads were discovered to have a smooth surface. Moreover, 5-FU dispersion as tiny crystalline particles were seen on the surface of the microbeads.



(a) **(b)**

Figure 1 (a) Surface of calcium alginate microbeads **(b)** Surface of calcium alginate pectinate microbeads

3.2 Determination of Particle size

Alginate microbeads were observed to range in micrometersize from 775.21±6.22 to 998.19±5.61. The mean particle size of microbeads was found to increase with sodium alginate concentration as a result of increased cross-linking between alginate and calcium ions, resulting in larger particles, but the irregular shape and in vitro cumulative drug release were hindered, so the ideal size was determined to be 895.45±8.54. (Table 2)

3.3 Drug entrapment efficiency

Alginate microbeads were reported to have a percentage drug entrapment efficiency ranging from 72.40±1.75 to 96.10±3.87. Alginate microbeads were reported to have a drug entrapment effectiveness of 89.91±3.69. The drug was evenly distributed throughout the mixture. It was found that the polymer matrix effectively caught almost all of the drugs, leading to greater entrapment efficiency (Table 2). Alginate microbeads without pectin or with a shorter curing time were found to have a low entrapment efficiency, which may contribute to the possibility of drug leakage from calcium alginate microbeads with surface pores.

3.4*In-vitro* Cumulative Percentage Drug Release Study from Core Microbeads

As indicated in Table 2, after 6 hours in phosphate buffer pH 6.8, the maximal in vitro cumulative percentage drug release of alginate microbeads was discovered to be 62.12±2.36 to 91.23±1.28. Alginate microbeads were shown to have an ideal in vitro cumulative drug release percentage of 79.35±1.65. Microbeads mimicked the drug release process of the polymer matrix swelling and erosion. As sodium alginate concentration was increased, the cross-linking between alginate and calcium ions also increased. This caused a denser cross-linked cage structure to form, which in turn caused microbeads to become denser and smaller beads. This increased entrapment efficiency and decreased cumulative percentage of drug release are depicted in Figure 2.

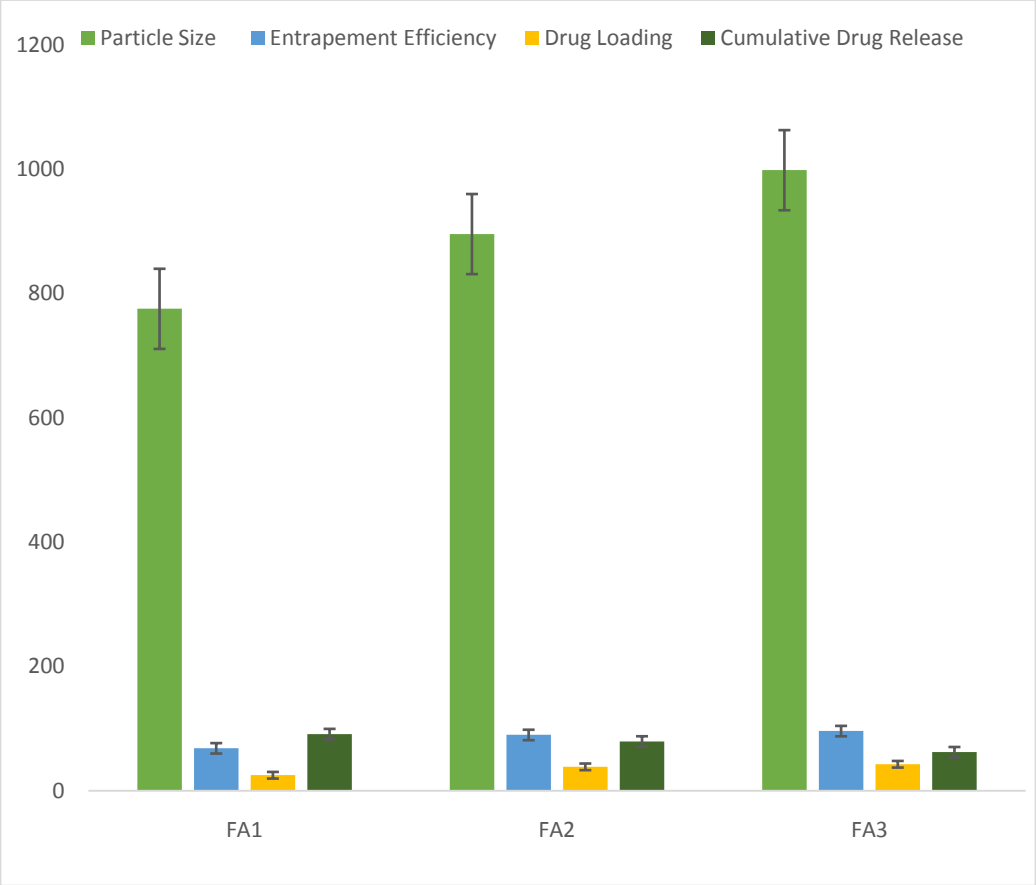


Figure 2 Effect of sodium alginate concentration on particle size, entrapment efficiency, drug loading, and percentage cumulative drug release

3.5 Optimization of coating thickness and *In-vitro*percentage cumulative drug release study from coated microbeads

After coating the beads with various concentrations of the Eudragit S100 solution, the coating thickness was optimized in terms of the total weight gain (TWG) of the beads. In simulated GIT fluids, the impact of coating thickness on in vitro drug release was investigated. The findings of the current investigation clarified how the drug was completely

protected from gastric juice's acid and enzymes. With its carboxyl groups that ionize from neutral to alkaline media, enteric coating by Eudragit S100 provided a stable environment for the drugs in the acidic and enzymatic medium.The ionization that occurs in phosphate buffer saline disturbs the film's integrity, causing the medicines to release at pH 7.4 (the small intestine), the coating to dissolve, and the microbeads to become exposed to the colonic environment. At 5%, 10%, and 15% TWG, respectively, the drug release was found to be 75.23%, 55.12%, and 31.26% after 10 hours (Fig. 3). It was also noted that as the percentage of total weight gain increased, the percentage drug release in the stomach fluid reduced due to the thickness of the coated layer.Due to its optimal drug release through the coating's ionization, disruption, and dissolution, the formulation with a coating thickness of 10% TWG was found to be the most suitable, as opposed to beads with a 15% TWG coating that showed hindered drug release.

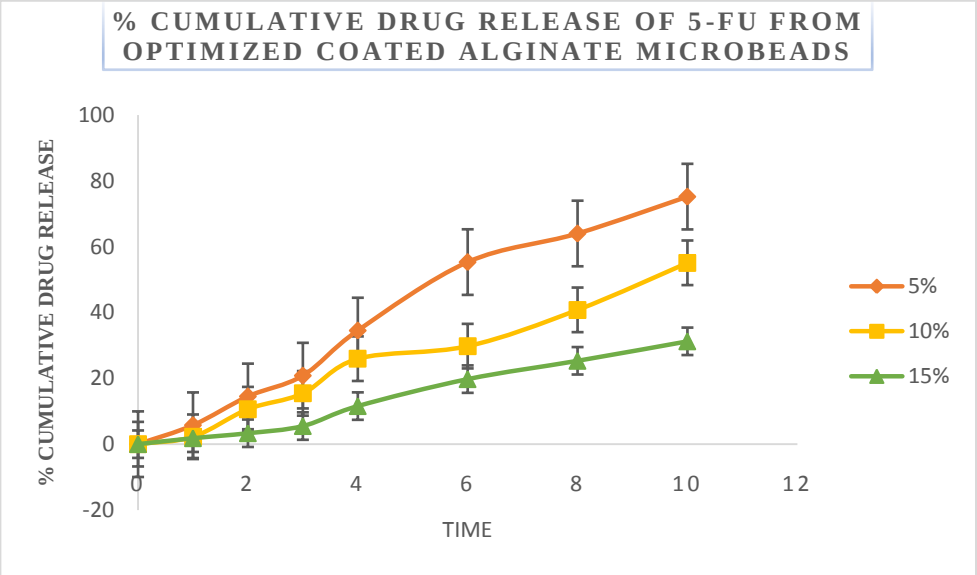


Figure 3 Percent cumulative drug release of 5-FU optimized formulation

3.6*In vitro* Drug Release Study in the Presence of Rat Caecal Contents

Moreover, rat caecal content investigations on in vitro drug release were carried out in simulated colonic fluid (pH 6.8). Without enzyme induction of rat caecal content, 50.73±3.15% of the drug was found to be released from the formulation. The rat caecal content medium's (2% w/v) colonic bacterial action may be insufficient to break down the inflated microbead's gel barrier. As a result, 1 ml of a 1% w/v aqueous solution of pectin was given to the rats daily for seven days to induce the enzymes necessary for pectin degradation.Tests were conducted in the simulated colonic fluid that contained 2% w/v of rat caecal content and was collected after 7 days of enzyme induction. After 24 hours, an

enhanced release was seen, reaching 78.96±2.89%. It is possible to conclude that the colonic bacteria found in rat caecal content, as shown in Fig. 4, are responsible for the sodium alginate and pectin degradation that leads to the drug's release in the physiological environment of the colon.

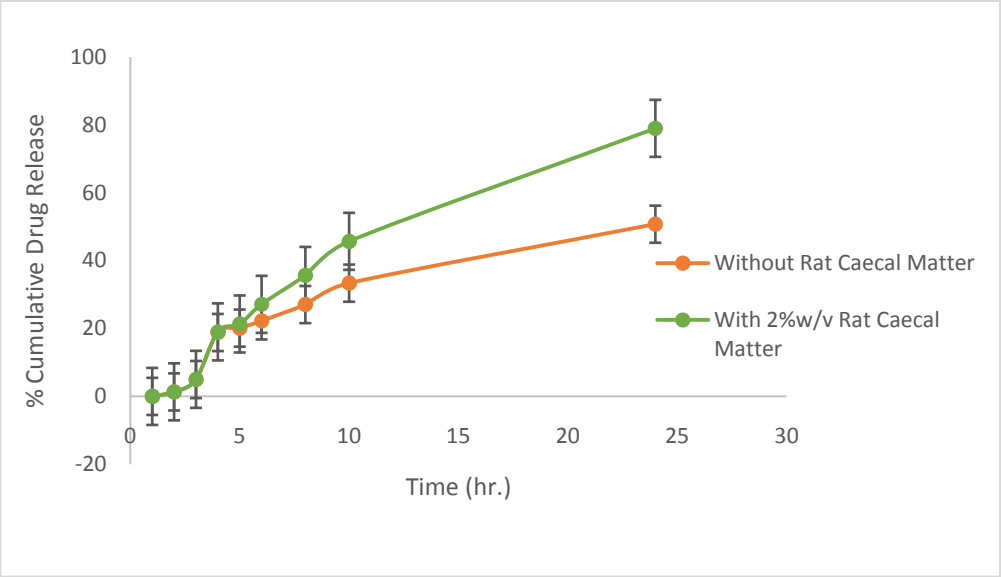


Figure 4 Comparative percent drug release in simulated colonic fluid (pH 6.8) from Eudragit S 100 coated microbeads with and without rat caecal content

3.7Analysis of drug release data of optimized formulation

The calculated regression coefficients for zero order, first order, and Higuchi models were 0.9790, 0.7529, and 0.9887. The in vitro drug release of alginate pectin microbeads was best explained by the Higuchi model as the plot showed the highest linearity, followed by zero order. The Korsemeyer-Peppas release exponent n for the optimized formulation was 1.849 indicating that the release was governed by Non-Fickian Super Case II Transport. (Table 3)

Table 3: Drug Release Kinetics:

Formulation	Zero order (r^2)	First order (r^2)	Higuchi kinetic (r^2)	Korsmeyer-Peppa's (n)
5-FU	0.979	0.7529	0.9887	1.849

3.8 Cytotoxicity study by MTT assay

The MTT assay is currently the most popular technique for determining the cell growth rate and toxicity of the culture. The viability of cells in drug samples at various concentrations was discovered by cytotoxicity tests using MTT. For 24 hours, cells

proliferated in medication solutions at various concentrations. The percentage of cell viability was calculated using the absorbance values. The HCT-116 cell line was created to study the effects of 5-FU on it. By using the MTT assay, the IC_{50} for 5-FU impact on HCT-116 was determined. According to Fig. 4, the viability rate drastically drops as the 5-FU concentration rises from 10 µg/ml to 1000 µg/ml. The cell viability curve led to the value of IC_{50} being determined as 3.52 µg/ml.

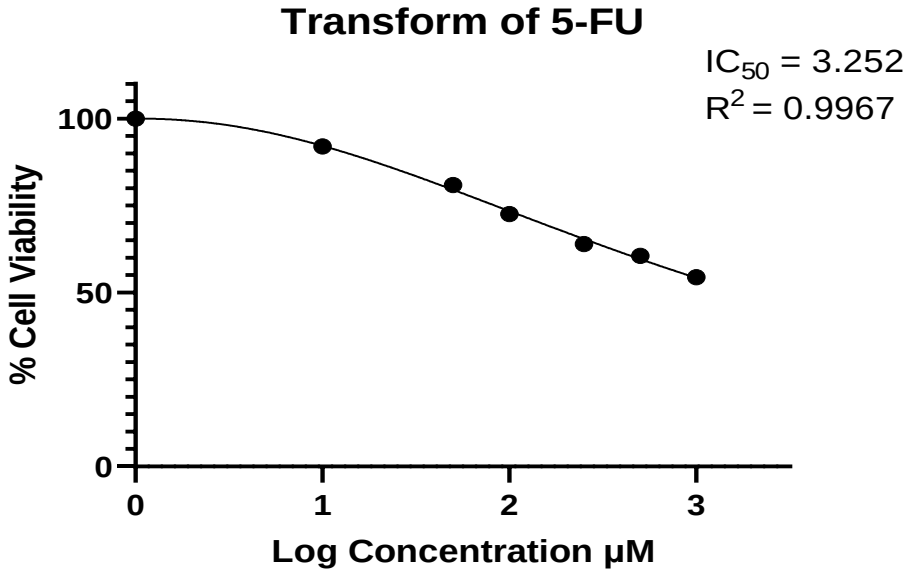


Figure 5 Percent cell viability of 5-FU microbeads in the HCT116 cell line

4. DISCUSSION

By forming ionic crosslinks between the polyionic alginate chains, sodium alginate solidifies in the presence of divalent cations, particularly calcium. Alginate gelation is typically explained in terms of the "egg box" model, in which covalently bonded divalent cations to the carboxyl groups of guluronic acid. The proper spacing between the carboxyl and hydroxyl groups of pectin is provided by the structure of guluronic acid, and the high degree of coordination with calcium cations prevents the first burst release of the medication in an acidic environment.

Alginate microbeads were observed to range in micrometersize from 775.21±6.22 to 998.19±5.61.The mean particle size of microbeads was observed to rise with sodium alginate concentration due to an increase in the cross-linking between alginate and calcium ions, resulting in larger particles, however, the irregular form and in vitro cumulative drug release was hindered. Alginate pectin microbeads had a percentage drug entrapment efficiency ranging from 72.40±1.75 to 96.10±3.87. Alginate microbeads without pectin or a short curing

period were shown to have a low entrapment efficiency, which may be a factor in the possibility of drug leakage from calcium alginate microbeads with surface pores and a low degree of etherification with sodium alginate. Alginate microbead maximal in vitro cumulative percentage drug release was discovered to range from 62.12 ±2.36 to 91.23 ±1.28 after 6 hours in phosphate buffer pH 6.8. To form a more compact structure of beads, the sodium alginate concentration was increased. As a result, a denser cross-linked cage structure was formed, converting the microbeads into denser and smaller ones, which increases entrapment efficiency and decreases the cumulative percentage of drug release.

The optimized formulation, which released 5-FU effectively in colonic formulations, was maintained with minimal drug release in the gastric medium and maximal delivery in the colonic part after coating with various concentrations of Eudragit S 100 based on the optimized batch's total weight gain. After 10 hours, it was discovered that the drug release was 75.23 %, 55.12 %, and 31.26% for 5%, 10%, and 15% TWG, respectively. It was also noted that as the percentage of total weight gain increased, the percentage of drug release in the gastric fluid decreased due to the thickness of the coated layer.Due to the formulation's optimal drug release through coating ionization, disruption, and dissolution, the formulation with a coating thickness of 10% TWG was determined to be the most suited. Tests were conducted in the simulated colonic fluid that contained 2% w/v of rat caecal content and was collected after 7 days of enzyme induction. After 24 hours, an enhanced release was seen, reaching 78.96±2.89%.Some might conclude that the breakdown of sodium alginate and pectin is what causes the medication to release in the physiological environment of the colon. The plot of the release kinetic data indicated that the Higuchi model best explained the 5-FU microbeads, which had the highest linearity and lowest order. The improved formulation's Korsemeyer-Peppas release exponent n was 1.849, indicating that Non-Fickian Super Case II Transport was in charge of the release. According to the low IC_{50} value, 5-FU microbeads in the colonic region are more effective at treating colon cancer than untreated 5-FU.

5. CONCLUSION

The formulations alginatemicrobeads showed better shape and surface morphology, particle size (895µm), entrapment efficiency (89.91±3.69), drug loading (38.64±1.53), and cumulative percentage drug release (79.35±1.65) after 6 hrs in phosphate buffer pH 6.8. 10% TWG of coated beads cumulative percentage drug release found was 55.12±3.45 after 10 hrs. and easily degradable in presence of rat caecal content which makes the formulation more preferable because of a biodegradable polymer. It shows better release in presence of rat

caecal content (78.96±2.89%). IC_{50} revealed that 5-FU is effective on colon cancer cell lines. Studies suggest that formulated colon-targetedenteric-coatedsodium alginate microbeads may be taken orally to deliver 5-FU specifically to the colon because of higher alkalinity due to the presence of sodium alginate for the effective treatment of colorectal cancer.The approach described appears promising for the colonic deliveryof drugs.

Abbreviations:
5-FU:5-Fluoruracil; MTT:3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide; SEM: Scanning electron microscopy; CRC: Colorectal cancer; IC: Inhibitory Concentration; TWG: Total weight gain.

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Ethics approval and consent to participate

The authors assert that all procedures contributing to this work comply with the ethical standards of the Institutional Animal Ethics Committee andthe experiment was granted by CPCSEA(HIPER/IAEC/66/2021/04) Lucknow, India.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

REFERENCES

1. Ramanathan, Ramesh K, Clark, Jeffery W, 2003. Safety and toxicity analysis of oxaliplatin combined with fluorouracil or as a single agent in patients with previously treated advanced colorectal cancer. Am. Soc. Clin. Oncol. 2904–2911.
2. Dhawale SC, Bankar AS, Patro MN, 2010. Formulation and evaluation of porous microspheres of 5-fluorouracil for colon targeting. Int. J. Pharm. Tech. Res. 1112–1118.
3. Wong HL,Bendayan R, Rauth AM, 2007.Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. Adv. Drug Deliv. Rev. .59: 491–504.
4. Michor F, Iwasa Y, Lengauer C, Nowak NA, 2013. Dynamics of colorectal cancer. Semin Cancer Biol. 15, 484–493.
5. Mohideen, B, Ezhilmuthu RP, et al, 2005. Formulation and In Vitro characterization of gliclazide-loaded polymeric nanoparticles. Int. J. Biol. Pharm. Res 4 (7) 533–540.

6. Krishnaiah YSR, Satyanarayan S.2001.Colon-Specific Drug Delivery Systems: Advances in Controlled and Novel Drug Delivery. CBS Publishers and Distributors, 89–119.
7. Levine, D.S, Raisys VA, Ainardi V, 1987. Coating oral beclomethasone dipropionate capsules with cellulose acetate phthalate enhances the delivery of topically active anti-inflammatory drug delivery to the terminal ileum. Gastroenterology .92: 1037–1044.
8. RasmussenSN, Rondesen S, Hvidberg EF,Honore HS, Binder V, Halskov S, 1982. 5-Aminosalicylic acid in a slow-release preparation: bioavailability, plasma level, and excretion in humans. Gastroenterology. 83: 1062–1067.
9. Arias JL, Ruiz MA, LopezViota M, Delgado AV, 2008. Poly (alkyl cyanoacrylate) colloidal particles as vehicles for antitumor drug delivery: a comparative study. Colloids Surf. B Biointerfaces62: 64–70.
10. Malet, Martino M, Martino RL, 2002. Clinical studies of three oral prodrugs of 5-fluorouracil Capecitabine, UFT, S-1): a review. Oncologist. 7: 288–323.
11. Mazumder R, Mahanti B, Majumdar S, Pal R, Chowdhury AD, 2021. Response surface method for optimization of prepared satranidazole powder layered pellet. Future Journal of Pharmaceutical Sciences. 7:190
12. Tamara M, 2004. Drug targeting to the colon with lectins and neoglycoconjugates. Adv Drug Deliv Rev. 56:491-509.
13. Akram W, Navneet G, 2021. Design expert as a statistical tool for optimization of 5-ASA-loaded biopolymer-based nanoparticles using Box Behnken factorial design. Future Journal of Pharmaceutical Sciences. 7:146
14. Kundlik M, Girhepunje, Krishnapiillai, Ranju S et al.2010. Celecoxib loaded microbeads: a targeted drug delivery for colorectal cancer, International Journal of Current Pharmaceutical Research. 2:46-55.
15. Ghosal, k, Das, SK, Ghosh D, 2018.The preparation and evaluation of naringin-loaded polycaprolactone microspheres based on oral suspension using Box-Behnken design. Journal of Molecular liquids, 256:49-57.
16. Yadav P, Rastogi V, Verma A, 2020.Application of Box–Behnken design and desirability function in the development and optimization of self-nano emulsifying drug deliverysystem for enhanced dissolution of ezetimibe. Future Journal of Pharmaceutical Sciences, 6:1-20

17. Khan, M.S., Sridhar, B.K., 2010. Development and evaluation of pH-dependentmicrobeads for colon targeting. Indian Journal of Pharmaceutical Sciences.72;(1):18-23.
18. Pandey AK, Choudhary N, Rai V, Dwivedi H, Kymonil K.M, SarafSA,2012. Fabrication and Evaluation of Tinidazole Microbeads for Colon Targeting. Asian Pacific Journalof Tropical Disease.S197-201.
19. Ramírez-Bergeron DL, Runge A, Adelman DM, Gohil M, Simon MC, 2006.HIF-dependent
hematopoietic factors regulate the development of embryonicvasculature. Dev Cell 11(1):81–92.
20. Mosmann T, 1983.Rapid colorimetric assay for cellular growth and survival: application toproliferation and cytotoxicity assays. Journal Immunol Methods.65:55-63.