ORIGINAL ARTICLE

Colon Targeting of 5-fluorouracil Loaded Dual Cross-linked Multiparticulate System: *In vitro* and *in vivo* Characterizations

Sandeep Kumar Sonkar[1], N. Ganeshan[2], M. Mathur[2], S. K. Lanjhiyana[1]

[1]Institute of Pharmaceutical Sciences, Pharmaceutics Research Division, Guru Ghasidas Vishwavidyalaya (Central University), Bilaspur, Chhattisgarh, India, [2]Research Division, Nuclear Medicine and Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, Madhya Pradesh, India

Abstract

Objective: The present study aimed to develop sustained released 5-fluorouracil loaded chitosan-pectin blended dual cross-linked gel beads system. **Materials and Methods:** Dual cross-linked beads were evaluated for drug content, particle size, swelling degree, scanning elecron microscopy, differential scanning calorimetry, X-ray diffraction, etc., for its suitability for colon targeting. **Results:** The developed systems were appreciably performed during *in vitro* drug releases in simulated gastric (simulated gastric fluid) at pH 1.2, intestinal (simulated intestinal fluid) at pH 6.8, and colonic fluids at pH 7.4 (simulated colonic fluid [SCF]) with and without rat cecal content medium for up to 24 h. Batch formulations were shown lesser releases in acidic dissolution medium, whereas augmented releases in alkaline medium at the end of 24 h studies. It was found with significant drug releases ($P > 0.05$) in SCF containing 2 and 4% w/v rat cecal as compared to control studies. During curve fittings using several models, the R^2 value of Higuchi matrix model confirmed for drug release was followed with anomalous non-Fickian transport mechanisms. Those dual cross-linked gel beads confirmed for its improved mechanical core strength, controlled, and sustained release potentials during the experimental. Gamma scintigraphic imaging during *in vivo* studies confirms for targeting potential of optimized formulations for colon-specific region. It was evident that the ionic gelation based dual cross-linked chitosan-pectin beads with divalents Ca^{2+} and SO_4^{-2} ions exhibited better delayed drug release pattern than single cross-linked beads for colon targeting. **Conclusion:** The prepared dual ionic cross-linked optimized formulations may be potential system for targeting drug to colon for colorectal cancer.

Key words: pH sensitive polymers, ionic cross linking, colon targeting, gamma scintigraphy, rat cecal content, kinetic modeling

INTRODUCTION

Multiparticulates system containing natural polymers were found consisting of biodegradable, biocompatible, and pH sensitive properties. These have met with considerable attention since past few decades for development of novel, controlled, and sustained release dosage forms. Multiparticulates based dual cross-linked microbeads are reported for superior reproducible pharmacokinetic behavior for colon-specific drug targeting as compared to conventional formulations. This system lowers intra and inters individual variability in plasma levels and bioavailability. The colon has longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. With the help of those systems based on combined usage of chitosan and pectin, the drug releases or absorption in the upper stomach and small intestine can be minimized until it reaches up to colonic region. Ionic gelation based inter penetrating polymeric network or polyelectrolyte complexes has combined unique physicochemical and improved biocompatibility properties that have been focused for intensive fundamental and applied research.

Address for Correspondence:S. K. Lanjhiyana, Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (Central University), Bilaspur, Chhattisgarh - 495 009, India. Tel.: 07752 260027, Fax: 07752 260148, Cell: +91-9826252991.
E-mail: sklanjh1975@gmail.com

Received: 31-10-2020
Revised: 28-11-2020
Accepted: 12-12-2020

Pectin also known as pectic polysaccharides are rich in galacturonic acid consists mainly of linear chains of α-1,4 D-galacturonic acid and 1,2 D-rhamnose with D-galactose and D-arabinose side chains having lower viscosities than other plant gums.[1] During last few decades, pectin has been reported to possessing gelling properties to form hydrogel beads due to an ionotropic interaction between the anionic carboxylic acid (COO^-) groups with the cationic divalent and trivalent metal ions, namelt, Ba^{2+}, Zn^{2+}, Ca^{2+}, and Al^{3+},. As due to these properties, it has been extensively investigated in the designing of various oral drug delivery.[2,3] Chitosan is the most widely used natural polysaccharide having excellent properties of non-toxic, biocompatible, mucoadhesion, and easily biodegradable nature. The repeating glucopyranose units of chitosan consist of cationic amino groups ($-NH_2$) on the C-2 position that can electrostatically interact with the anionic carboxylic acid (COO^-) groups of pectin, to form polyelectrolyte complexes.[4] It is having unique properties of gel and film forming due to which it is used in pharmaceutical industries as potential carriers for development of various controlled drug delivery systems especially for protein and peptide drugs.[5]

Among several polymers, the alginate-chitosan based polymeric beads prepared by either single cross linking or dual crosslinking beads had been widely investigated by those researchers, but their potential stability, kinetic modeling or *in vivo* performances have not been adequately researched.[6,7] In the present study, the multiparticulates system based on ionic gelations were prepared using natural matrix chitosan and pectin polymers.[8-12] Perhaps in this study it was approached to display both the *in vitro* and *in vivo* correlations, while at the same time tried to justify the reasons behind modified mechanical and release retardant properties of the developed systems. Conventional formulation containing 5-fluorouracil (5FU) was suffering from problems of severe side effects due its rapid absorption and metabolism at the upper gastrointestinal (GI) tract, due to which a delayed release system is presently designed for management of colorectal cancer. The objective of present investigation was to design and evaluate stable pH-dependent and microbial triggered based dual cross-linked microbeads systems using natural polymers for targeting of 5-FU to colon-specific region for possible management of colon cancer. This may be achieved by targeting drug directly into the vicinity of the desired colonic region while, by passing the assaults of the upper GI tract. These may help to overcome those limitations of single cross-linked beads and conventional marketed preparations.

MATERIALS AND METHODS

The drug 5FU was provided as a gift sample from Avra Synthesis Pvt Ltd., Hyderabad. Chitosan (MW ~ 3.0×10^5) was received from Central Institute of Fisheries Technology (Cochin, India). LM pectin (MW ~30,000–100,000, Loba-Chemie, India), calcium chloride, and sodium sulfate (HiMedia Pvt Ltd., India) were used in studies. All of the other chemicals and reagents were of analytical grade and were used without additional purification.

Preparation of chitosan-pectin blended beds system by dual ionic gelation method

5FU loaded chitosan-pectin microbeads were formulated using polyelectrolyte complexation based ionotropic gelation method. First, the blend mixture containing 2% (w/v) chitosan-sodium alginate poly electrolytes solutions (100 ml) was prepared following mass proportions of 6:6, 4:8, and 2:10, respectively. The pectin powder was accurately weighed and dissolved in deionized water and then, 5FU (20% w/w of dry polymer weight) was homogeneously dispersed into it using homogenizer for 30 min. Chitosan was separately dissolved in deionized water that were previously added with 1% (v/v) acetic acid at ambient temperature. Then, both the above prepared poly electrolytes containing polymeric solutions were added together with different mass proportions and thoroughly homogenized for 40 min at 45°C to obtain uniform blend mixture. The blend solution containing drugs were ultrasonicated for another 10 min for complete debubbling and then was adjusted to pH 5 using NaOH (0.1 mol/l). Thereafter, the bubble-free blend solution was extruded through 0.45 mm inner diameter needle using hypodermic glass syringe at dropping rate of 1 ml/min and falling distance of 6 cm into calcium chloride (2% w/v) solution. This resulted into the formation of Ca^{2+} ions containing smooth and spherical single cross-linked microbeads, but mechanically weak. The obtained composite gel beads were cured into the same calcium chloride solution for 60 min for hardening and then subsequently decanted and washed thrice using de-ionized water. Following curing process, the beads were immediately double cross linked by exposing to different concentration of sodium sulfate solution (2, 4, and 6% w/v) to form SO_4^{2-} cross linkages which were cured for another 60 min. Then, the resultant double cross-linked beads with improved mechanical properties were removed and washed with deionized water; subsequently dried at room temperature for 24 h and then furthermore oven dried at 45°C for 12–15 h. The obtained stable dried products were stored at room temperature into suitable container till further characterizations. The optimization, core compositions, and independent variables that were used for preparation are shown in Table 1. All batches were prepared in triplicate.

Characterizations of ionic dual cross-linked beads

Scanning elecron microscopy (SEM) analysis

The external surfaces and cross-sectional morphological of micro beads were performed by means of scanning electron microscope (JSM-5800, JEOL, Japan) facility. Before imaging, the samples were prepared by lightly sprinkling the

Table 1: Different variables used in the preparation of 5-FU loaded microbeads system

Formulations	Drug (% w/w)	Chitosan:pectin ratio (% w/v)	Calcium chloride (%w/v): Sodium sulfate (%w/v) solution	Curing time (min)
$5F_UCP_{B1}$	20	6:6	2:2	60
$5F_UCP_{B2}$	20	6:6	2:4	60
$5F_UCP_{B3}$	20	6:6	2:6	60
$5F_UCP_{B4}$	20	4:8	2:2	60
$5F_UCP_{B5}$	20	4:8	2:4	60
$5F_UCP_{B6}$	20	4:8	2:6	60
$5F_UCP_{B7}$	20	2:10	2:2	60
$5F_UCP_{B8}$	20	2:10	2:4	60
$5F_UCP_{B9}$	20	2:10	2:6	60

beads on a double-sided adhesive tape that stuck on brass stub. This stub was then coated with gold layer under vacuum using sputter coater to a thickness of (~300Å) for 150 s and at 20 kV and then viewed under the electron microscope with different magnifications.[13]

Particle size and size distribution

Measurement of particle size distribution and mean diameter of those randomly selected 100 dried beads were carried out with the help of optical microscope (Olympus, Germany). Those randomly selected beads from batch formulations were mounted on the clean glass slide (sterilized) and their Feret's diameters were determined under 4 × with accuracy, as shown on Table 2.

Estimation of drug content, drug entrapment efficiency, and percent yield

Quantitative drug estimation was performed with accurately weighed amount of 100 mg beads. Those beads were then placed into 100 ml of phosphate-buffered saline (pH 7.4) for 24 h at 37 ± 0.5°C with occasionally shaking using graduated flask. These samples were ultrasonicated and continued for 20 min till it gets completely dissolved. The drug content in the filtered supernatant obtained through Whatman filter paper (# 41) was assayed spectrophotometrically (Shimadzu UV-1800) at 258 nm against an appropriate blank following suitable dilutions [Table 2]. The entrapment efficiency and percent yields were measured using the following formula:

$$\text{DEE}(\%) = \frac{\text{Acutal content}}{\text{Theoretical content}} \times 100$$

$$\text{Percent yield} = \frac{\text{Total weight of beads}}{\text{Total weight of drug and polymer}} \times 100$$

Equilibrium swelling behavior in various pH media

Dried beads (50 mg) among batch formulations were accurately weighed and placed into separate USP dissolution apparatus II (basket type) containing 500 ml swelling media of various pH values, namely, simulated gastric (simulated gastric fluid [SGF]) (pH 1.2), simulated intestinal fluid (SIF) (pH 6.8), and simulated colonic fluid (SCF) (pH 7.4). That bead was allowed to swell at 37 ± 2°C up to constant equilibrium weight for 4 h time intervals and maintained under 50 rpm speed. At predetermined time intervals, those swollen beads from swelling medium were removed, wiped with filter paper for moisture elimination, followed with weighing on an electronic balance for weight variations.[14] The dynamic percent swelling degree of beads with respect to time was calculated accordingly with the below equation:

$$\text{S.D.}(\%) = \frac{Ws - Wi}{Wi}$$

Whereas, W_s is equilibrium weight of wet beads in the medium, and W_i is absolutely initial weight of dried beads [Table 2].

Differential scanning calorimetry (DSC) thermal analysis

The thermal behavior of different bead components was characterized using differential scanning calorimeter (DSC 4000, Perkin Elmer, Germany) which was calibrated against indium as reference. Approximately, 2-2.5 mg of dried powdered samples were weighed out and hermetically sealed inside 40-µl aluminum pans. The measurement was performed over the temperature range from 30°C to 350°C at the constant heating rate of 10°C/min to obtain thermograms of those samples.

Qualitative X-ray diffraction (XRD) analysis

X-ray powder diffraction patterns of pure drug, physical mixture of bead components, and drug loaded beads were performed using a wide angle X-ray diffractometer (Ultima-III, Reganku SmartLab, Japan). Those samples were separately irradiated with monochromatized Cu K-α rays (1.54056 Å). The voltage and current used into the system were 30 kV and 30 mA, respectively. Finally that was scanned at the rate of 5°/ min, over the diffraction angle (2θ) range of 20°–80° and the count range of 3000 cps.[15]

Table 2: Effect of variable polymer ratio and dual cross linking agents on particle size, percent yield entrapment efficiency, and degree of swelling of different batches of bead formulation containing 5-fluorouracil

Batches	Particle size (μm) (mean±S.D.)	Percentage yield (mean±S.D.)	DEE (% w/w) (mean±S.D.)	Drug content (% w/w) (mean±S.D.)	Degree of swelling in pH 1.2 (4 h)	Degree of swelling in pH 6.8 (4 h)	Degree of swelling in pH 7.4 (4 h)
$5F_UCP_{B1}$	985.14±0.24	70.40±0.14	60.54±0.32	80.20±0.58	0.10±0.02	0.12±0.02	0.16±0.02
$5F_UCP_{B2}$	988.20±0.52	68.20±0.28	58.23±0.32	81.68±0.63	0.08±0.01	0.13±0.05	0.15±0.05
$5F_UCP_{B3}$	980.36±0.40	72.12±0.11	63.25±0.42	80.36±0.60	0.10±0.03	0.15±0.08	0.16±0.03
$5F_UCP_{B4}$	970.25±0.24	75.45±0.58	62.10±0.52	85.25±0.70	0.07±0.02	0.18±0.06	0.22±0.08
$5F_UCP_{B5}$	960.34±0.54	81.53±0.52	69.65±0.52	88.40±0.25	0.07±0.01	0.25±0.06	0.28±0.08
$5F_UCP_{B6}$	958.36±0.56	80.88±0.34	69.89±0.47	86.50±0.54	0.06±0.01	0.25±0.08	0.26±0.04
$5F_UCP_{B7}$	951.10±0.48	81.16±0.26	70.10±0.25	89.30±0.42	0.06±0.01	0.27±0.05	0.28±0.08
$5F_UCP_{B8}$	948.33±0.15	82.20±0.43	72.40±0.60	90.20±0.42	0.05±0.006	0.28±0.04	0.26±0.04
$5F_UCP_{B9}$	947.44±0.25	83.98±0.32	72.98±0.42	88.56±0.25	0.05±0.005	0.26±0.04	0.29±0.05

Each value represents the mean±S.D.; (n=3)

In vitro drug release studies

USP type-II dissolution apparatus (paddle type) was used to carry out *in vitro* release studies for drug loaded batch formulations. An equivalent weight of 100 mg beads was placed into 900 ml of acidic SGF (pH 1.2) that was stirred at the speed of 50 rpm for 2 h intervals and maintained at constant 37 ± 0.5°C temperature. After that, the release tests were then continued in SIF at pH 6.8 for next 3 h and in SCF at pH 7.4 for until 24 h at similar conditions. The collected sample liquids were then filtered suitably diluted and analyzed at fixed λ_{max} value of 258 nm for absorbance against blank solution using a ultraviolet (UV)–vis spectrophotometer.[16]

Kinetic modeling and analysis of drug release mechanism

To analyze the *in vitro* release data different mathematical kinetic models were used for the purpose to discover the possible drug release mechanism(s) through polymeric matrix based beads formulations, as shown in Table 3. Microsoft Excel software was applied to perform curve plotting and simulation studies. Those well-known exponential equations were used for model fittings such using zero-order equation, first-order equation, Higuchi's model, and Hixson-Crowell model. The accuracy and prediction ability of these models were compared with calculation of squared correlation coefficient (R^2) values.[17]

In vitro drug release studies containing rat cecal content medium (2 and 4% w/v RCCM)

The study protocol was approved by Institutional Animal Ethics Committee (IAEC) (994/a/GO/06/CPCSEA) at Guru Ghasidas Vishwavidyalaya, Bilaspur, India. Drug release studies containing rat cecal content in the dissolution medium were also carried out for the purpose of simulating with human microbial environment of colon. The optimized formulation ($5F_UCP_{B5}$) was selected, since it was comparatively better performed for optimum release rate, drug entrapment, percent yield, good swelling properties, etc. The experiment was performed using USP dissolution rate test apparatus of paddle type (50 rpm, 37 ± 0.5°C) in sealed anaerobic conditions. The experiments were carried out using a 250 ml beaker that was immersed in the jars of the dissolution test apparatus. Initially, the studies were carried out in 187.5 ml of 0.1N HCl (pH 1.2) for 2 h. Thereafter, 62.5 ml of 0.2 M trisodium phosphate and 2% w/v cecal content were added into the same dissolution media, pH was adjusted to 6.8 and 7.4, respectively, and then continued for up to 24 h period. The same study was also performed into the medium containing 4% w/v cecal content for 24 h period. Then, release studies were also performed without rat cecal content (control) in SCF at pH 7.4 for another 24 h. Then, without delay, those 5-ml samples were filtered through 0.45 μm membrane filter, appropriately diluted and analyzed for drug contents using UV-vis spectrophotometer against suitable blank solution.[18,19]

Table 3: Results of curve fitting of the *in vitro* 5-fluorouracil release data from chitosan-pectin based bead formulations containing 5-fluorouracil

Formulation code	Zero order	First order	Higuchi	Hixson-Crowell
$5F_UCP_{B1}$	0.8375	0.8954	0.9184	0.8706
$5F_UCP_{B2}$	0.8415	0.8983	0.9039	0.8758
$5F_UCP_{B3}$	0.8193	0.8897	0.9174	0.8683
$5F_UCP_{B4}$	0.7956	0.9655	0.9878	0.9358
$5F_UCP_{B5}$	0.8035	0.9153	0.9260	0.8843
$5F_UCP_{B6}$	0.8273	0.9475	0.9502	0.9215
$5F_UCP_{B7}$	0.8056	0.9615	0.9621	0.9223
$5F_UCP_{B8}$	0.8116	0.9329	0.9491	0.8966
$5F_UCP_{B9}$	0.8033	0.9375	0.9407	0.9067

Gamma scintigraphic imaging of bead formulation

The preparation steps for uptake of sodium pertechnetate ($99^{m}Tc$) were carried out as per our previous studies.[20] The *in vivo* gamma scintigraphic study was carried out which was approved by Institutional Animal Ethics Committee (IAEC) (994/a/GO/06/CPCSEA). During the study, six healthy male rabbits weighing 1.5–2 kg were used to monitor the *in vivo* transit behavior of optimized formulation. Animals were divided into two groups of three animals each (one control group). The animals were fasted for 12 h before the commencement of each experiment for the reason to standardize the condition of GI motility. Thereby, capsules containing optimized formulation were orally administered to the animals of the first group with the help of a feeding tube, followed by sufficient volume of drinking water. All the four legs of the rabbit were tied over a piece of plywood and the location of the formulation in the GI tract was monitored at every 1 h by keeping the subject in front of the gamma camera. The gamma camera was arranged with a field view of 40 cm and was fitted with a medium energy parallel-hole collimator. The 140 keV gamma rays emitted by 99m Tc were imaged. Specific GI tract site (anterior) were imaged by E-cam Single Head Gamma Camera (Siemens, Germany) after a definite time interval. The gamma images were recorded using an online computer system and stored on magnetic disk and analyzed to determine the distribution of activity in the GI tract.

Statistical analysis

All the experimental data have been represented as mean ± standard deviation (S.D). Mean values of particle size, percent yield, and entrapment efficiency were compared using the Student's *t*-test. Differences are considered statistically significant at level of $P < 0.05$, respectively.

RESULTS

Preparation of 5-FU loaded chitosan-pectin microbeads and optimization

During beads preparation process, when blend mixture of poly electrolytes containing drug-polymer dispersion (20%, w/w of polymer concentration and polymer-to-polymer ratio of 6:6, 4:8, and 2:10) was drop-wise added at fixed flow rate into the cross linking $CaCl_2$ solutions, then immediately spherical cross-linked networks of gel beads or "egg-box" model system was formed. Furthermore, optimization for two independent variables for ratio of polymer(s) and cross linker(s) that were varied at three low, medium, and high levels resulted into overall nine batch formulations. On other hand, the concentration of drug and cross linking times was taken at fixed level throughout the experimental [Table 1].

SEM

The surface morphological of optimized 5-FU loaded chitosan-pectin containing single and double cross-linked beads (dried) systems was carried out with help of SEM micro analysis, as depicted in Figure 1. These photographs evident that those single cross-linked beads with Ca^{2+} ions were found with larger sized, spherical, and smooth surfaced. Whereas, on the other hand, the surface of double cross-linked beads is found with compact sized, quasi-spherical, roughness, wrinkled, and minor cracks after drying which might be due to reason for shrinkage and/ or partly collapsing of polymeric gel network during the second steps of dual cross-linked with SO_4^{2-} ions.

Particle size

The average particle diameter of beads was carried out using optical microscopy method for beads having variable mass ratios of core chitosan-pectin polymers as presented in Table 2. Here, it is clear that those average diameters of particles increased significantly ($P < 0.05$) with increased concentration of chitosan polymers, whereas on increments for mass proportion of pectin resulted into decreased particle sizes. The decreased particle sizes with pectin proportions suggested that strong cross-linked networks of pectin attributed to more compactness in comparison to chitosan proportions.[21] In addition, it was found that the mean size of double cross-linked beads was lower than that of single cross-linked beads, which suggested for shrinkage of beads had occurred during the second step of cross linking process.[6]

Percentage yield, entrapment efficiency, and drug content

The batch formulations containing 5-FU loaded chitosan-pectin beads were evaluated for micromeritic properties, as shown in Table 2. The values were found increased significantly ($P < 0.05$) with the increase mass proportions of pectin as compared to chitosan in the core compositions. The significant increments of yield and entrapment values were due to that of ionic cross-linked networks of pectin were more strong and compact in comparison to chitosan network. Analysis for drug content revealed for the values increased significantly ($P < 0.05$) from 82.05 ± 0.62 to 89.92 ± 0.36% with increased concentration of pectin polymers and varying ratio of curing agents.[22]

Swelling behaviors at various acid and alkaline media

Swelling degree studies was carried out for batches using various GI fluids (SGF at pH of 1.2, SIF at pH 6.8, and SCF

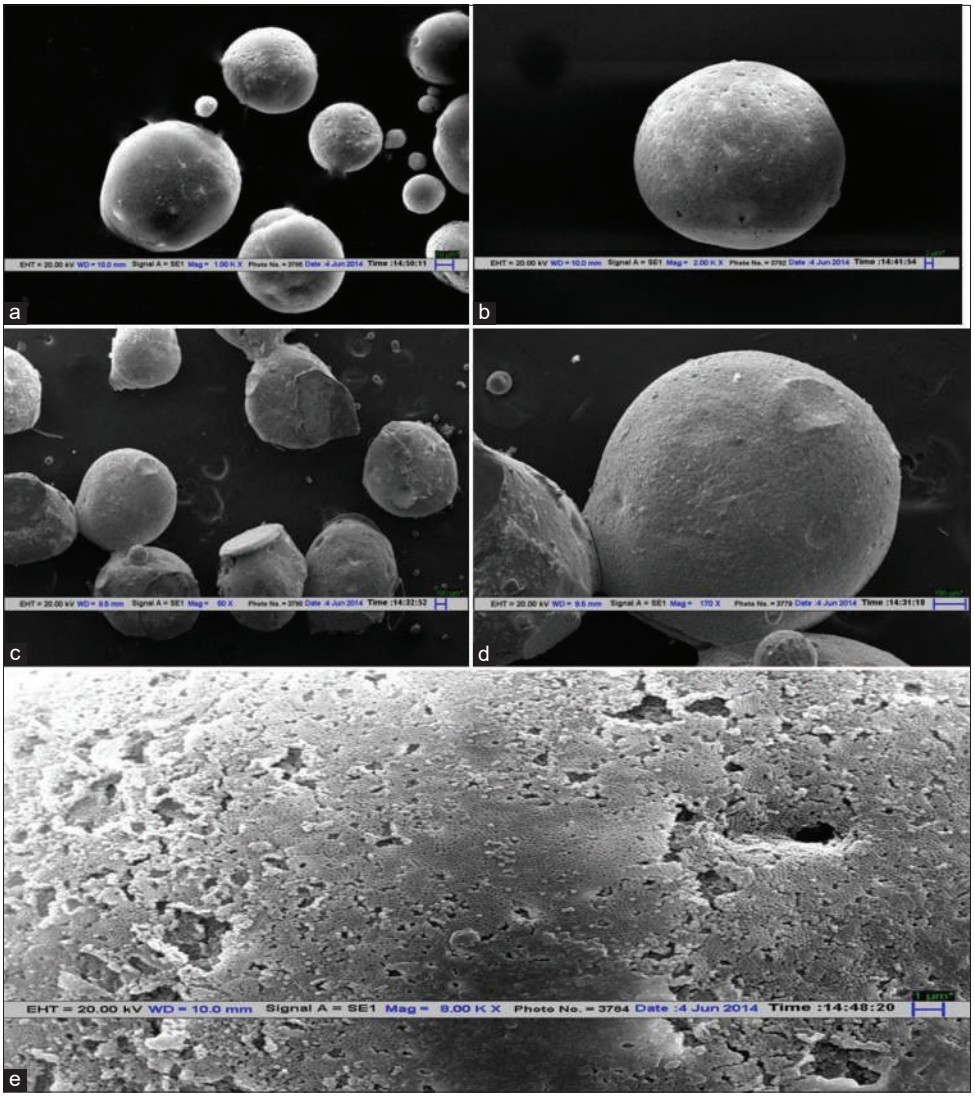

Figure 1: Scanning electron microphotographs of 5-FU loaded 4:8 ratios containing chitosan/pectin based (a) single cross-linked microbeads cured with 2% w/v of calcium chloride, surface magnified at 1.00 kx (b) single cross-linked microbeads, surface magnified at 2.00 kx (c) double cross-linked microbeads, cured with 2 and 2% w/v of calcium chloride and sodium sulfate, surface magnified at ×170 (d) double cross-linked microbeads, cured with 2 and 6% w/v of calcium chloride and sodium sulfate, surface magnified at ×60 and (e) double cross-liked microbeads with rough, fibrous and small pores surface, highly magnified at 8.00 kx

at pH 7.4), as shown in Table 2. These result shown that swellings degrees for most of batches were seem to be almost negligible in SGF at pH 1.2, except with slight raised values observed with those formulations consisted of increased mass proportions of chitosan polymer. It revealed for overall acid resistant nature of beads into acidic environment due to ionic cross-linking among inter-penetrating polymeric networks. However, on the other hand, those batch formulations had shown variable swelling degree in simulated fluids at pH 6.8 and 7.4. These result attributed that with the increments of percent pectin followed with increased swelling degree at alkaline medium (pH 6.8 or 7.4) while, at the same time, it gets slightly decreased with increased concentration of cross linkers used during dual cross-linking steps. In addition, when there was increments of percent chitosan were found followed with slight increased swelling degree at acidic medium (pH 1.2).

DSC study

DSC thermogram of 5-FU drug showed a sharp melting endothermic peak at 288.07°C, indicating for its purity as depicted in Figure 2. Thermograms of chitosan pectin polymers were found with broader endothermic peaks (Tg) at 86.54 and 91.90°C and corresponding exothermic peaks (Tc) at 249.62 and 312.07°C, respectively. In thermal curve of formulation, an early diminished melting endothermic peak (Tm) at 82.30°C was observed due to moisture evaporation or dehydration. The thermogram of formulation was further followed with

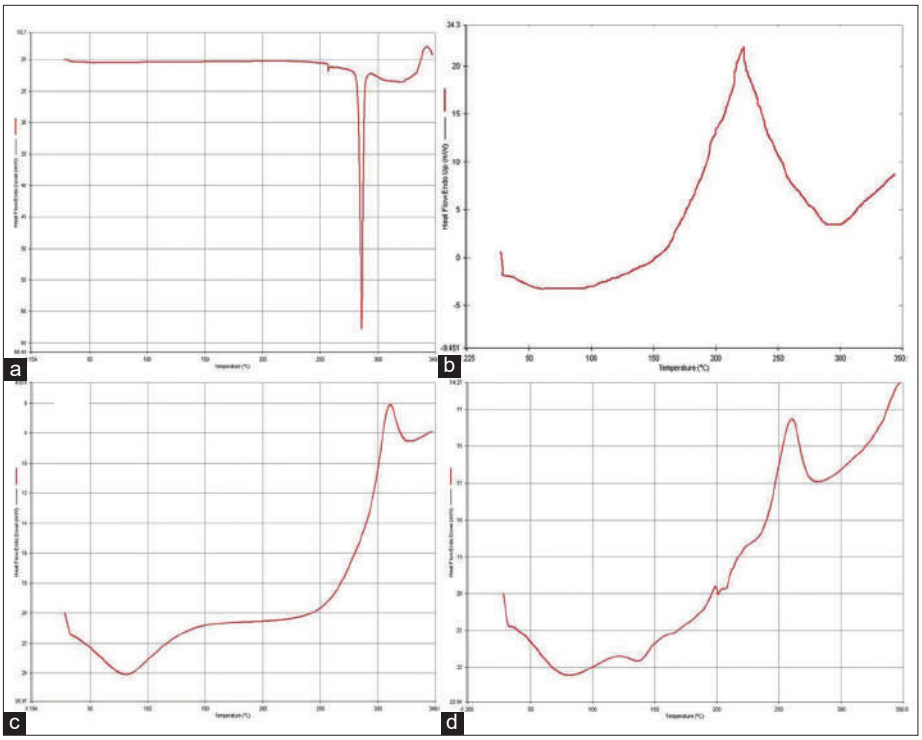

Figure 2: Differential scanning calorimetry thermogram of (a) pure 5-fluorouracil, (b) chitosan polymer, (c) pectin polymer, and (d) chitosan-pectin based 5-fluorouracil loaded dual cross-linked optimized microbead formulation

diminished melting endothermic peaks (Tm) of matrix drug at 202.15°C and sharp exothermic peak at 261.31°C, respectively. Thereafter, the formulation was found to be oxidized or degraded at 328.06°C as shown in the thermogram.

XRD study

The P-XRD diffractograms of pure 5-FU, chitosan, pectin, and drug-loaded beads were analyzed over the diffraction angle (2θ) range of 20°–80° with different signal intensities as demonstrated in Figure 3. The 5-FU had shown its important crystallographic characteristic sharp peaks at 20.66, 21.93, 23.97, 24.87, 25.90, 28.76, 31.22, 33.12, 33.99, 36.41, 37.25, 39.32, 51.07, 54.43, 59.29, and 61.79 due to its crystalline nature. While, in case of diffractograms of chitosan and pectin, no intense peaks were observed due to its inherent amorphous properties. Furthermore, the formulation was also shown to be consisted with diminished diffraction pattern of drug which suggested for drug dispersed homogeneously at molecular level into polymeric matrices of chitosan and pectin, respectively.

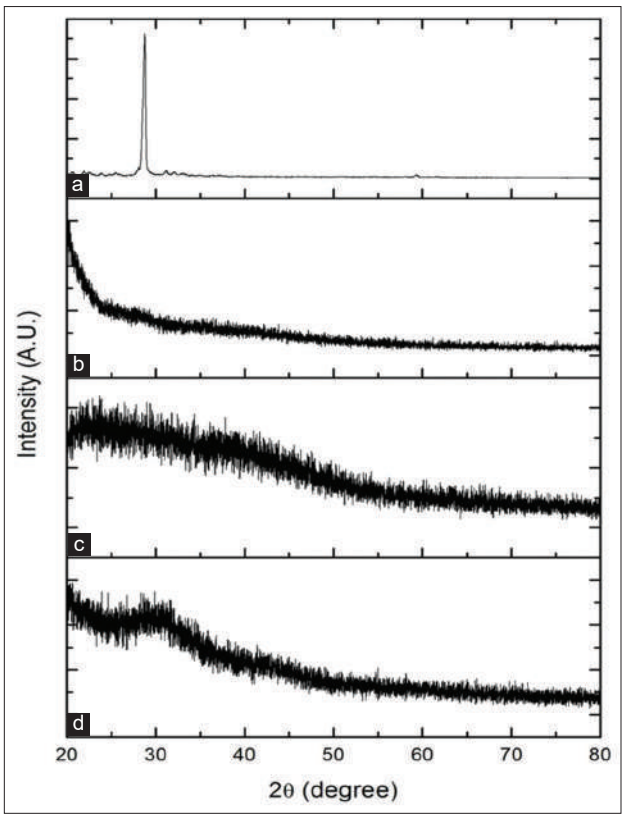

Figure 3: X-ray diffractogram of (a) 5-fluorouracil drug, (b) chitosan polymers, (c) pectin polymers, and (d) chitosan-pectin based optimized 5-fluorouracil loaded microbeads

In vitro drug release studies

Effect of polymer concentration

5-FU loaded batch formulations were carried out for *in vitro* release studies for the purpose to confirm the potential of for resistance against the assaults of upper GI tracts, as shown in Figure 4. The cumulative drug released in SGF at acidic medium was found slow, as merely 3.06 ± 0.09–5.02 ± 0.05% loaded drug was released during 2 h interval. Whereas

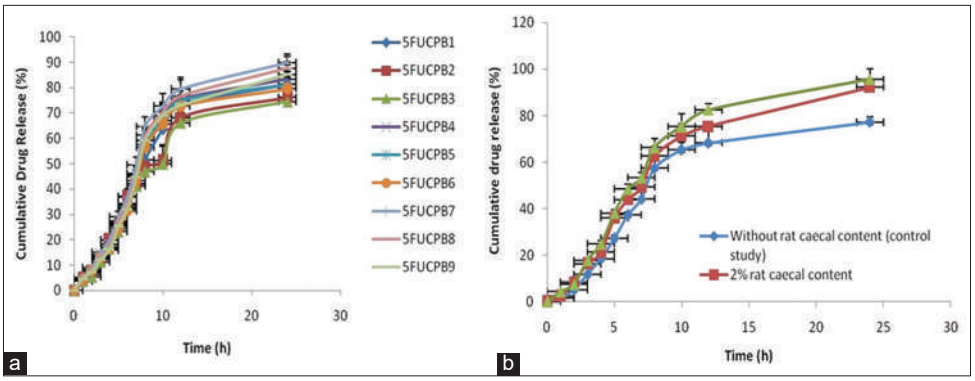

Figure 4: Cumulative percent release profile of 5-fluorouracil loaded chitosan-pectin based (a) various batch formulations with different polymer ratio and dual cross-linking agents in various simulated gastrointestinal (GI) fluids at pH 1.2, 6.8 and 7.4 for up to 24 h period and (b) optimized microbead formulations in various simulated GI fluids at pH 1.2, 6.8 and 7.4 containing 2 and 4% rat cecal content and in absence of cecal content (control) medium for up to 24 h period; Mean ± S.D.; *n*=3; S.D. denoted by error bars

cumulative drug released in SIF at pH 6.8 was found with 15.22 ± 1.26–20.30 ± 2.07% during next 3 h interval studies. The drug released in SCF at pH 7.4 was achieved with 63.42 ± 3.24–85.84 ± 3.90% during up to 24 h studies.

Effect of concentration of cross linking agents

The drug release was also affected with variable concentrations of the cross-linkers and fixed curing time used during beads preparations. Drug released from batches consisting of variable mass ratios of chitosan: Pectin within a group was found decreased with increase in cross-linking concentration irrespective of those simulated dissolution medium (acidic and alkaline).

Mechanism of drug release

Data obtained from *in vitro* release studies were treated for best fit using various mathematical kinetic models such as zero-order, first-order, Higuchi, and Hixson-Crowell, respectively. This was carried out for the purpose to determine the mechanism of drug released from various ionically gelled formulations. The accuracy and prediction aptitude of these models were determined using regression coefficient (R^2) values and those curve fitting results. The results of curve fittings established that in most cases the drug release had followed Higuchi kinetic model, as shown in Table 3. Since the r^2 value was observed to be the highest and closer to unity (r^2 = 0.9039–0.9878) for best-fit and the plot revealed for linearity. The drug release data fit linearly to Higuchi's square root kinetic equation for all those sample formulations.

In vitro drug release studies in simulated cecal fluid containing 2 and 4% w/v rat cecal content

The optimized $5F_UCP_{B5}$ formulation was selected for further studies on the basis of better performances shown during experimental. It was observed that there was augmented cumulative percent drug released in the presence of rat cecal content at the end of 24 h studies when compared to control study conducted in absence of cecal content. Drug released from optimized formulation was found with 67.5 ± 0.09 and 92.2 ± 0.09% correspondingly in 2 and 4% RCCM, when compared to control formulation that released only 33.2 ± 0.09% in absence of cecal content at pH 7.4 during 24 h dissolution studies as shown in Figure 4.

Gamma scintigraphic imaging

During experimental, animal groups were orally administered with radio labeled $5F_UCP_{B5}$ formulations were found intact in stomach at 2.0 ± 0.5 h with gamma tracer, but shown very lesser amount of released tracers due to improper tagging within the formulation [Figure 5]. After 3 h interval, again lesser amount of tracers was found released when particulate reaches into small intestine at 5.0 ± 0.5 h. Furthermore, those scintigraphy images indicated that the systems were began to disintegrate in colon after 6.0 ± 0.30 h, and then within 12–24 h the formulation got completely collapsed in colon region.

DISCUSSION

The SEM results provided proof for deviation in particle's size, shapes, and surface shrinkage after drying might have occurred during the second cross-linking steps of single cross-linked beads. These results are found in conformity with other report of Pasparakis and Bouropoulos and Claesson and Ninham,[23,24] respectively. Concomitantly factors governing swelling of those pH-sensitive gel systems were based on theories of ionization and/ or ion-exchange mechanism associated with charged poly cations (chitosan) and poly anions (pectin) as poly electrolytes. Such theories were equally effective for swelling degrees of those bead systems in addition to cross linking networks theory. The ionizations processes are exhibited with protonation, deprotonation, and

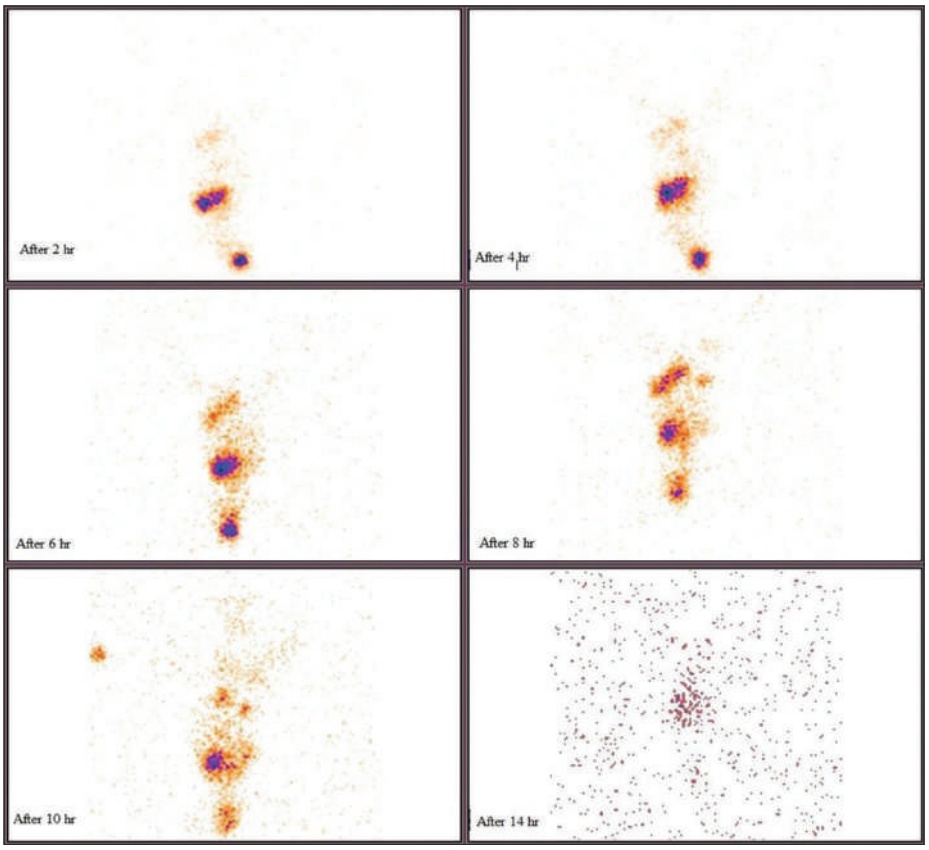

Figure 5: Gamma scintigraphy showing gastrointestinal transit and *in vivo* release of loaded radioactive tracer (^{99m}TC-DTPA) from optimized $5F_UCP_{B5}$ formulation at different time intervals of 2, 4, 6, 8, 10, and 14 h (n = 3)

chelation of poly ions that were also one of the responsible phenomenon for swelling degree at specific acidic and/ or alkaline media. As per Donnan equilibrium also called as Gibbs-Donnan effect, the swelling degree took place due to concentration gradient of ionic charges (Donnan potential) across the gel phase boundaries or diffusion barriers for those bead systems.[25-27]

The DSC curves were shifted to lower melting region strongly suggested for conversion of crystalline drug into amorphous form due to ionic gelations of interpenetrating cross-linked networks of pectin and chitosan. This behavior might be depending on the strength of electrostatic cross linking reactions taken place between those divalent cross linkers Ca^{2+} and carboxylic groups of pectin-chitosan. It shows that there was homogeneous drug dispersion into the polymeric matrix network.[28,29] During the process of bead formation by ionic gelation method, the molecular interactions and entrapment of drug takes place of which shifting of characteristic peak of thermal curves at lower melting values was reported.

The XRD had provided ground reasons for drug transformation from crystalline to amorphous form and drugs entrapment into complex network matrices due to ionic gelation process. Thus both X-ray crystallographic data coupled with thermal behavior studies (DSC) demonstrated for attenuation of characteristics diffraction peaks and endothermic peaks for the reason of drug transformation from crystalline to amorphous forms during bead formations. Similar results were also reported by other researchers Bhattachary *et al.* and Bera *et al.*[21,30] These drug transformations into the matrices improve the performances of poorly soluble drug and there then increases the overall absorption and bioavailability many folds. DSC and XRD analysis revealed for amorphous state (or semi crystalline) nature of drug after drug entrapment or encapsulation. Hence, those drug loaded beads prepared using crystalline drug and amorphous polymer(s) helped to obtain optimized properties of improved stability and bioavailability at the same time.

An *in vitro* drug release study was carried out to observe those effects of polymer concentration. During experimental it was found that the loaded drug released into the various simulated mediums was found highly depended on independent variables of mass polymer concentration and cross linker used into the batch formulations. With the increase of core pectin concentration in comparison to chitosan had resulted into diminished drug releases into the acidic medium at pH 1.2. The increased core pectin proportion provided with lesser swelling degree of the polymeric chains due to that it remains unionized and anhydrite at acidic medium.

Similarly, the *in vitro* study was as well carried out to monitor those effects of concentration of cross linking agents. Xu *et al.*

had previously reported for dual cross-linked BSA loaded alginate/chitosan beads with fewer drug releases at SGF (pH 1.0) during 4 h followed with augmented released at SIF and SCF (pH 6.8 and 7.4) during each 3 h studies.[6] Our results are in agreement with such findings with fewer drug releases at SGF (2 h) followed with sustained released at SIF (3 h) and SCF (up to 24 h) containing medium. In our studies, those differences in sustained drug release pattern at SIF and SCF medium were obtained may be due to additional usage of higher mass proportions of 4 and 6% w/v sodium sulfate as cross linkers (other than lower 2% w/v) during second cross linking steps for those batch formulations. Hence, it may be predicted for maximum quantity of dose may be delivered into the colonic region thereby maximum therapeutic efficacy and minimum drug losses will be meeting up on other hand.

Mechanism of drug release studies revealed that drug release from these systems was followed with anomalous non-Fickian transport mechanisms referring to combination of swelling controlled and diffusion controlled type. It was also suggested that those optimized core concentrations of cross linkers and polysaccharides (independent variables) used during formulation development has greatly affected and modified the drug release mechanism in the given dissolution medium. Those possible reason behind may be that the dual cross linkages of polymeric matrices were provided the beads system with more compactness, stiffness, and less porosity. This, in turn, finally provided for controlled swellings rate and drug diffusion of beads formulations. Our results were found in concordant with the reports of Maiti *et al.*[14]

Results of *in vitro* drug release studies in simulated 2 and 4% w/v RCCM revealed that the percent cumulative amounts of drug released in the dissolution medium were found significantly increased ($P < 0.05$), when 2% concentration was replaced with higher 4% concentration respectively. Our results were found concordant with the reports of Sinha *et al.* and Chaurasia *et al.,* respectively.[18,31] Hence, the present study has confirmed for susceptibility of those anaerobic bacteria population present in rat cecal content for degradation/digestion of matrix polysaccharides of bead formulations and found responsible for augmented drug release profiles in the dissolution medium.

Gamma scintigraphic imaging results shown that release with more amount tracers in colon confirmed for degradation of matrix chitosan-pectin of formulation that may be due to combined actions of pH based ionization and microbial enzymatic degradation properties. The scintigrams confirmed for colonic arrivals at about 6–7 h and uniform distribution of the released tracer during 24 h intervals across the entire colonic region. These results are found similar with those studies of Sharma *et al.* Krishnaiah *et al.* and Lai *et al.* that were performed using various other human and animal subjects.[32-34] This technique had provided "proof of concept" for transit time and location for disintegration and dispersion of our administered particulates. Hence, this *in vivo* pharmaco-scintigraphy study had demonstrated that those developed formulations may be able to utilize as potential carrier for targeting of anticancer drug(s) into the colonic region for possible local and topical actions against colon cancer disease.

CONCLUSION

In the present study, the 5-FU loaded matrix chitosan-pectin based gel microbead systems were successfully optimized and prepared using dual cross linkages by Ca_2^+ and SO_4^{2-} ions as per earlier reports of Xu *et al*. and Lanjhiyana *et al*.[6,20] The drug content, entrapment, and loading were achieved successfully in beads systems that confirm its potential for oral dose administrations. The *in vitro* release in 2 and 4% w/v rat cecal content medium and *in vivo* gamma scintigraphic studies revealed the sustained release and targeting efficiencies of our prepared systems. The drug releases from developed beads were followed with anomalous non-Fickian transport mechanisms referring to combination of swelling controlled and diffusion controlled type. The *in vivo* results were found to support and correlate with *in vitro* release profile data. At present, the combined effects of both pH based ionizations and microbial degradation phenomenon were tried here to explain for possible better understanding on mechanisms drug release properties. Hence, it can be concluded that those laboratory developed dual cross-linked bead system may be suitable carriers for targeting of 5-FU to colon specific region.

ETHICS APPROVAL

The performed *in vivo* study was approved by Institutional Animal Ethics Committee (IAEC/994/a/GO/06/CPCSEA) under the strict guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) respectively.

ACKNOWLEDGMENT

The authors are grateful to the Head of Department, Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, for providing necessary research amenities for work completion. Additionally thankful to the Department of Nuclear Medicines, IIT, Delhi, India, for providing sophisticated analytical instrumentation facilities.

REFERENCES

1. Wong TW, Colombo G, Sonvico F. Proximal tumors are associated with greater mortality in colon cancer. J Gen Intern Med 2010;25:1157-63.

2. Munarin F, Tanzi MC, Petrini P. Advances in biomedical applications of pectin gels. Int J Biol Macromol 2012;51:681-9.
3. Patil JS, Kamalapur MV, Marapur SC, Kadam DV. Ionotropic gelation and polyelectrolyte complexation: The novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review. Dig J Nanomater Biostruct 2010;5:241-8.
4. Sonkar SK, Akram W, Lanjhiyana SK. Polysaccharides based novel and controlled released multiparticulate systems for colon-specific delivery: Contemporary scenario and future prospects. Asian J Pharm 2020;14:488-98.
5. Wu C, Lo SL, Boulaire J, Hong ML, Beh HM, Leung DS, *et al*. A peptide-based carrier for intracellular delivery of proteins into malignant glial cells *in vitro*. J Control Release 2008;130:140-5.
6. Xu Y, Zhan C, Fan L. Preparation of dual crosslinked alginate-chitosan blend gel beads and *in vitro* controlled release in oral site-specific drug delivery system. Int J Pharm 2007;336:329-37.
7. Bansal AK, Pandey V. Development and evaluation of dual cross linked pulsatile beads for chemotherapy of rheumatoid arthritis. J Pharm (Cairo) 2013;2013:906178.
8. Giri TK, Thakur A, Alexander A, Ajazuddin A, Badwaik H, Tripathi DK. Modified chitosan hydrogels as drug delivery and tissue engineering systems: Present status and applications. Acta Pharm Sin B 2012;2439-49.
9. Giri TK, Thakur D, Alexander A, Ajazuddin A, Badwaik H, Tripatihi M, *et al*. Biodegradable IPN hydrogel beads of pectin and grafted alginate for controlled delivery of diclofenac sodium. J Mater Sci Mater Med 2013;24:1179-90.
10. Verma VS, Sakure K, Badwaik HR. Xanthan gum a versatile biopolymer: Current status and future prospectus in hydro gel drug delivery. Curr Chem Biol 2017;11:10-20.
11. Giri TK, Choudhary C, Alexander A, Ajazuddin A, Badwaik H, Tripatihi M, *et al*. Sustained release of diltiazem hydrochloride from cross-linked biodegradable IPN hydrogel beads of pectin and modified xanthan gum. Indian J Pharm Sci 2013;75:619-27.
12. Sonkar SK, Lanjhiyana SK. Drug release kinetic modeling and gamma scintigraphic studies of Dual Ca^{2+} and SO_4^{2-} cross-linked microbeads for colon specific targeting. Asian J Pharm 2020;14:661-70.
13. Odeku OA, Okunlola A, Lamprecht A. Microbead design for sustained drug release using four natural gums. Int J Biol Macromol 2013;58:113-20.
14. Maiti S, Ranjit S, Mondo R. Al^{+3} ion cross-linked and acetalated gellan hydrogel network beads for prolonged release of glipizide. Carbohydr Polym 2011;85:164-72.
15. Larosa C, Salerno, M, de Lima, JS. Characterisation of bare and tannase-loaded calcium alginate beads by microscopic, thermogravimetric, FTIR and XRD analyses. Int J Biol Macromol 2018;115:900-06.
16. 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.
17. Ritger PI, Peppas NA. A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J Control Release 1987;5:37-42.
18. Sinha VR, Mittal BR, Bhutani KK, Kumaria R. Colonic drug delivery of 5 fluorouracil: An *in vitro* evaluation. Int J Pharm 2004;269:101-8.
19. Lanjhiyana SK, Dangi JS. Development and *in-vitro* drug release studies of methotrexate from modified pulsatile release guar gum based enteric coated capsules for colon specific delivery. Indian J Pharm Educ Res 2008;42:154-60.
20. Lanjhiyana SK, Bajpayee P, Kesavan K. Chitosan-sodium alginate blended polyelectrolyte complexes as potential multiparticulate carrier system: Colon-targeted delivery and gamma scintigraphic imaging. Expert Opin Drug Deliv 2013;10:5-15.
21. Bera H, Boddupalli S, Nayak AK. Mucoadhesive-floating zinc-pectinate-sterculia gum interpenetrating polymer network beads encapsulating ziprasidone HCL. Carbohydr Polym 2015;131:108-18.
22. Sankalia MG, Mashru RC, Sankalia JM, Sutariya VB. Papain entrapment in alginate beads for stability improvement and site-specific delivery: Physicochemical characterization and factorial optimization using neural network modeling. AAPS Pharm Sci Tech 2005;30:E209-22.
23. Pasparakis G, Bouropoulos N. Swelling studies and *in vitro* release of verapamil from calcium alginate and calcium alginate-chitosan beads. Int J Pharm 2006;323:34-42.
24. Claesson PM, Ninham BW. pH-dependent interactions between adsorbed chitosan layers. Langmuir 1992;8:1406-12.
25. Shu XZ, Zhu KJ. A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery. Int J Pharm 2000;201:51-8.
26. Simsek-Ege FA, Bond GM, Stringer J. Polyelectrolyte complex formation between alginate and chitosan as a function of pH. J Appl Polym Sci 2003;88:346-51.
27. Chen LY, Tian ZG, Du YM. Synthesis and pH sensitivity of carboxymethyl chitosan-based polyampholyte hydrogels for protein carrier matrices. Biomaterials 2004;25:3725-32.
28. Sarmento B, Ferreira D, Veiga F, Ribeiro A. Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. Carbohydr Polym 2006;66:1-7.
29. Michael R, Bhatt SA. Brahmbhatt H. Taste masking of ciprofloxacin by ion-exchange resin and sustain release at gastric-intestinal through interpenetrating polymer network Asian J Pharmaceut sci 2015;10:331-40.
30. Bhattacharya SS, Ghosh AK, Banerjee S, Chattopadhyay P, Amitava G. Al^{3+} ion cross-linked interpenetrating polymeric network microbeads from tailored natural polysaccharides. Int J Biol Macromol

2012;51:1173-84.
31. Chaurasia M, Chourasia MK, Jain NK. Methotrexate bearing calcium pectinate microspheres: A platform to achieve colon specific drug release. Curr Drug Deliv 2008;5:215-9.
32. Sharma BG, Kumar N, Nishad DK. Development of microbial trigger based oral formulation of tinidazole and its gamma scintigraphy evaluation: A promising tool against anaerobic microbes associated GI problems. Eur J Pharm Sci 2016;89:94-104.
33. Krishnaiah YS, Satyanarayana S, Prasad YV. Gamma scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers. J Control Rel 1998;55:245-52.
34. Lai H, Zhu F, Yuan W. Development of multi-unit colon targeted drug delivery system by using alginate: *In vitro* and *in vivo* evaluation. Drug Dev Ind Pharm 2011;37:1347-56.

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