International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 5, 2020

Original Article

DEVELOPMENT OF pH-DEPENDENT CHRONOMODULATED DELIVERY SYSTEMS OF 5-FLUOROURACIL AND OXALIPLATIN TO TREAT COLON CANCER

JASWANTH GOWDA B. H.[1], S. J. SHANKAR[1*], MURALI MUNISAMY[2], AKSHATHA R. S.[3], V. S. SAGAR[4]

[1]Department of Pharmaceutics, Vivekananda College of Pharmacy, Dr. Rajkumar road, Rajajinagar 2nd Stage, Bengaluru, India 560055, [2]Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal, India 576104, [3]Department of Pharmaceutics, PES College of Pharmacy, 50 feet Road, Hanumanth nagar, Srinagara, Bengaluru, India 560050, [4]Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram, India 608002
Email: sjjyothi@gmail.com

Received: 04 May 2020, Revised and Accepted: 17 Jun 2020

ABSTRACT

Objective: To develop two different oral formulations such as 5-fluorouracil (5-FU) tablets and oxaliplatin (OX) microspheres which were further filled into capsules and coated with pH-sensitive polymer (eudragit S-100) for the chronotherapeutic treatment of colon cancer (Fluorouracil: Oxaliplatin regimen) to perform as a substitute for intravenous (IV) route based chronomodulated chemotherapy.

Methods: The 5-FU tablet formulation was prepared with alginate and guar gum polymers in varied concentrations using wet granulation technique in two varieties such as granules coated and tablet coated formulations using eudragit RSPO as coating material to achieve controlled drug release. Alongside OX microspheres were formulated using the ionotropic gelation methodology in combination with alginate and chitosan polymers in varying concentrations to accomplish a time-controlled drug release. Prepared formulations were evaluated for pre-compression and post-compression parameters, percentage yield, percentage drug entrapment, Fourier transformed infrared spectroscopy (FT-IR), Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM), *In vitro* and *Ex vivo* dissolution studies.

Results: Pre-compression and post-compression parameters for 5-FU tablets were satisfied with Indian pharmacopeia specifications. The entrapment efficiency of OX microspheres were increased due to the elevated concentration of polymers up to a certain level as seen in A7M, further greater the concentration of polymer resulted in a decline of entrapment efficiency as seen in A4M and A8M. The optimized formulations A14T and A14M were shown *in vitro* drug release of 90.36 % by 24 h and 79.63 % by 9 h respectively.

Conclusion: The two different oral formulations of 5-FU (Tablets) and OX (Microspheres) were found to be successful in controlled drug release. Therefore they can be efficiently used to control the rate of drug release to the colon in synchronization with the circadian timing system in the belief of improved therapeutic efficacy, tolerability and overall survival rate of cancer patients. Hence it is promised to be a better alternative for intravenous route based chronomodulated chemotherapy.

Keywords: Colon cancer, Chronopharmaceutics, pH-dependent drug delivery, 5-fluorouracil, Oxaliplatin, Eudragit S-100, Eudragit RSPO, FOLFOX regimen, Microspheres

DOI: http://dx.doi.org/10.22159/ijap.2020v12i5.38156. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Colon cancer is one of the three most widespread categories of cancers, that accounts for more than 1.5 million cases, along with 8,62,000 deaths in the year 2018 [1, 2]. The patients in advanced phase of colorectal cancer require resection surgically along with adjuvant chemotherapy, even then patients lie at elevated menace for metastasis and recurrence with a 6% survival rate [3, 4]. It has been demonstrated that leucovorin (LV), 5-fluorouracil (5-FU) and oxaliplatin (OX) regimen offers better therapeutic efficacy, tolerability and insignificant adverse effects than 5-FU alone or other regimens to treat progressive colorectal cancer [5].

Most often these chemotherapeutic drugs are administered via the intravenous route, which in turn accumulates on healthy cells/tissues owing to non-specific dispersal of chemotherapeutic drugs [6, 7]. The oral route of delivering drugs is extensively adopted and the most readily accepted route of administration, with the dominance of being patient compliance, convenience, and invulnerability [8]. It also regards as a secure and more logical administration route for the treatment of colorectal conditions such as colon cancer. An ideal oral drug delivery system (DDS) should be able to accurately deliver the drug in a required manner at the preferred site without undergoing premature release and degradation in the GI tract. Henceforth, patients developing chemotherapy-related side effects can be negligible, accompanying enhanced bioavailability on specific target sites and reduced unnecessary amount of dose [9]. The pH-sensitive DDS is best suited for colon targeting due to different pH levels of the GI tract in various physiological conditions. The formulation has to be made in such a way that it releases drugs specifically in colonic pH [10-12].

Nowadays, the most burdensome challenge in the pharmaceutical industries for many scientists is to attune the drug release kinetics according to the required therapeutic range [13]. Adopting chronotherapy can possibly help in improving the efficacy of the treatment, thereby decreasing the side effects associated with treatment [14]. Chronopharmaceutics is a part of pharmaceutics committed to the design and evaluation of DDS that release medication at a rhythm that preferably matches the biological requirement of given disease therapy. Ideally, chronopharmaceutical DDS should consolidate site-specific and time-dependent DDS [15, 16]. The changes in tumor blood flow and cancer cell growth as per circadian rhythm are similar either tumors are tiny and growing expeditiously or tumors are larger and growing more slowly. The growth rate of tumors and the flow rate of blood into tumors are extensive up to three times in the course of the daily activity stage of the circadian cycle than during the daily rest stage [17, 18]. The toxicity induced by 5-FU during DNA synthesis in the target tissue is negligible during the night and extensive during day time [19]. On that account, the ratio of healthy cells possibly destroyed by 5-FU is lesser at night due to an increase in whole-body clearance of 5-FU [20, 21]. The ideal chronomodulated time corresponding to peak delivery ranges from 12:00 am to 4:00 am for 5-FU/lV and 1:00 pm to 6:00 pm for OX. The delivery of drug in this window period will help in strengthen tolerability by avoiding unnecessary toxicity associated with normal healthy cells, which can further benefit in an overall survival rate of patients compared to conventional chemotherapy [22, 23].

Thus, we are concentrating on the conversion of chronomodulated chemotherapy-treated through IV route into the oral route. Hereby

the present study aims to formulate pH-dependent, controlled release mini-tablets of 5-FU and OX microspheres which are further filled in capsules and thereafter coated with pH-sensitive polymer for delivering a drug into colon region at a specified concentration to treat colon cancer by adopting chronomodulated schedules to obtain patient compliance along with increased tolerability and finally to overcome unnecessary toxicity associated with the 5-FU and OX.

MATERIALS AND METHODS

Materials

5-Fluorouracil (5-FU) was purchased from Ultra Labs (Karnataka, India). Oxaliplatin (OX) was obtained as a gift sample from Strides Arco Labs (Karnataka, India). Sodium alginate was purchased from Otto chemicals (Mumbai, India). Chitosan was purchased from Sigma-Aldrich (Bengaluru, India). Eudragit S-100, eudragit RSPO were purchased from Evonik. Calcium chlorides, zinc sulfate, polyvinylpyrrolidone k-30, acetone, acetic acid were purchased from SRL chemicals (Mumbai, India).

Preparation of 5-fluorouracil tablets

The 200 mg of 5-FU and a specified quantity of polymer sodium alginate were mixed thoroughly. The 10% w/v PVP K-30 in isopropyl alcohol was used as a binder in this preparation. The dough was prepared by adding an appropriate amount of binding solution to the previously prepared drug-polymer mixture. The dough mass was passed through sieve no. 16 to obtain granules and allowed it to dry on tray drier for 30 min at 40 °C. Finally compressed using 5 mm punch besides 1% w/w magnesium stearate as a lubricant. In the case of granule coating, the granules obtained after passing through sieve no. 16 were coated with the coating solution in a fluidized bed processor. The dried granules after coating were allowed to pass through sieve no. 12 and subsequently compressed with less than 5 mm punch using 1% w/w magnesium stearate as a lubricant. For tablet coating, already prepared 5-FU tablets were coated in a coating pan with the coating solution until the required amount of the eudragit RSPO gets uniformly coated on to the tablet. The compositions of prepared 5-FU tablets were shown in table 1, 2.

Table 1: Compositions of granule coated 5-FU formulation

Formulation code	Amount of drug in mg	Amount of alginate in mg	Amount of guar gum in mg	% w/v of eudragit RSPO coating sol	% w/v of eudragit S-100 coating sol
A1G	200	50	-	10	10
A2G	200	50	-	15	10
A3G	200	-	50	10	10
A4G	200	-	50	15	10
A5G	200	60	-	10	8
A6G	200	60	-	15	8
A7G	200	100	-	10	8
A8G	200	100	-	15	8

Table 2: Compositions of tablet coated 5-FU formulation

Formulation code	Amount of drug in mg	Amount of alginate in mg	Amount of guar gum in mg	% w/v of eudragit RSPO coating sol	% w/v of eudragit S-100 coating sol
A1T	200	50	-	10	10
A2T	200	75	-	10	10
A3T	200	-	50	10	10
A4T	200	-	75	10	10
A5T	200	75	-	15	10
A6T	200	100	-	15	10
A7T	200	50	-	15	8
A8T	200	50	-	20	8
A9T	200	100	-	20	8
A10T	200	100	-	25	8
A11T	200	40	-	20	8
A12T	200	30	-	20	8
A13T	200	20	-	20	8
A14T	200	10	-	20	8

Table 3: Compositions of OX microspheres

Formulation code	% w/v of drug	% w/v of alginate	% w/v of calcium ions	% w/v of zinc ions	% w/v of chitosan	% w/v of eudragit S-100 coating sol
A1M	0.25	1.3	0.65	0.65	0.2	10
A2M	0.25	1.3	1.3	1.3	0.2	10
A3M	0.25	2.6	1.3	1.3	0.2	10
A4M	0.25	3.9	1.3	1.3	0.2	10
A5M	0.25	1.3	0.65	0.65	0.1	10
A6M	0.25	1.3	1.3	1.3	0.1	10
A7M	0.25	2.6	1.3	1.3	0.1	10
A8M	0.25	3.9	1.3	1.3	0.1	10
A9M	0.25	0.65	0.65	0.65	0.025	8
A10M	0.25	0.33	0.33	0.33	0.013	8
A11M	0.25	0.33	0.17	0.17	0.05	8
A12M	0.25	0.65	0.65	-	0.025	8
A13M	0.25	0.33	0.33	-	0.013	8
A14M	0.25	0.33	0.17	-	0.05	8

Preparation of oxaliplatin microspheres

The microspheres of OX were prepared by ionotropic gelation technique [24-26]. OX (0.25% w/v) was added to an aqueous solution of sodium alginate and stirred thoroughly for 15 min. This solution was dropped using a hypodermic syringe into a solution containing Calcium ions, Zinc ions and chitosan in acetic solution (0.5% v/v). Microspheres formed immediately were left for 24 h in the same solution to ensure internal jellification. Further filtered, washed and dried at room temperature [27, 28]. The detailed formulation components were shown in table 3**.**

Preparation of coating solution

The 10, 20, 30 and 50% w/v solution of eudragit RSPO were prepared in acetone using 20% w/w of di-butyl phthalate in ethanol for coating granules and tablets of 5-FU to achieve time-controlled release, further 8% and 10% w/v solution of eudragit S-100 were prepared in acetone using 20% w/w of di-butyl phthalate in ethanol for coating 5-FU tablets filled capsules and microspheres filled capsules. The different concentrations of eudragit RSPO coating solutions used to coat tablets and granules of 5-FU, further eudragit S-100 coating solutions used to coat capsules filled by tablets and microspheres were displayed in table 1**,** 2, 3.

Evaluation of granule and tablet coated 5-fluorouracil tablets

Pre-compression parameters

Bulk density (D_b)

The bulk density of the formulated granules was evaluated using a bulk density apparatus. It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a graduated measuring cylinder and the volume was noted [29]. It is expressed in g/ml and is given by,

$$D_b = \frac{M}{V_b}$$

Where M-Mass of the powder.

V_b-Bulk volume of the powder.

Tapped density (D_t)

It is the ratio of the total mass of powder to the tapped volume of powder. The tapped volume was measured by tapping the powder to a constant volume [29]. It is expressed in g/ml and is given by,

$$D\_t = \frac{M}{V_t}$$

Where, M-Mass of the powder.

V_t-Tapped volume of the powder.

Compressibility index (I) and hausner's ratio

Carr's index and Hausner's ratio measure the propensity of granule to be compressed and the flow ability of granule. Carr's index and Hausner's ratio were calculated using the following formula [29].

$$I = \frac{D_t - D_b}{D_t} * 100$$

Where, D_t-Tapped density of the powder.

D_b-Bulk density of the powder.

Hausner's ratio

$$\frac{D_t}{D_b} = \frac{V_b}{V_t}$$

Angle of repose

The frictional forces in a loose powder can be measured by the angle of repose. This is the maximum angle possible between the surface of a pile of powder and the horizontal plane. Sufficient quantities of granules were passed through a funnel from a particular height (2 cm) onto a flat surface until it formed a heap, which touched the tip of the funnel. The height and radius of the heap were measured. The angle of repose was calculated using the formula [29].

Angle of repose $\phi = \tan^{-1}(h/r)$

Where, h–Height of the pile in cm.

r–Radius of the pile in cm.

Post-compression parameters

Hardness test

The prepared 5-FU tablets were subjected to hardness test. It was carried out by using Labgo hardness tester type 04 and expressed in kg/cm^2.

Friability test (F)

The friability was determined using Roche friabilator and expressed in percentage. The 20 tablets of 5-FU from each batch were weighed separately ($W_{initial}$) and placed in the friabilator, which was then operated for 100 revolutions at 25 rpm. The tablets were reweighed (W_{final}) and the percentage friability was calculated for each batch by using the following formula.

$$F = \frac{W\,initial - W\,final}{W\,initial} * 100$$

Weight variation test

The 20 tablets OF 5-FU were selected at random from the lot, weighed individually and the average weight was determined. The percent deviation of each tablet weight against the average weight was calculated as per the official method [30].

Drug content uniformity

The tablets of each 5-FU formulation were weighed and powdered. The quantity of powder equivalent to 500 mg of 5-FU was transferred into 100 ml volumetric flask and extracted with (pH-7.4) phosphate buffer and kept aside for 2 h. Then the solution was filtered and absorbance was measured by using Double Beam UV-Vis spectrophotometer (Shimadzu-1700, JAPAN) at 267 nm after suitable dilution [10].

Evaluation of oxaliplatin microspheres

Surface morphology and particle size analysis of microspheres

The scanning electron microscope (SEM) was adopted to analyze the size and surface morphology of the OX microspheres. The microspheres were set up on stubs with the help of a two-sided adhesive strip and placed on a respective slot. Then they looked under SEM (JOEL, JSM-5600 LV, JAPAN) to find the size and surface characteristics of OX microspheres [31].

Percentage yield of microspheres

The prepared OX microspheres were collected, dried and further weighed. The obtained weight was then divided by the whole quantities of the OX and the excipients that were incorporated into the formulation of OX microspheres [32, 33].

$$\%\ yield = \frac{total\ weight\ of\ microspheres}{total\ weight\ of\ drug\ and\ polymer} * 100$$

Percentage drug entrapment efficiency of microspheres

The evaluation of drug residence inside microspheres was carried out by an indirect method in which the aliquots were taken after crushing and filtering microspheres to remove broken beads and thereon were assayed spectrophotometrically at 240 wavelengths. The amount of OX entrapped was calculated from the difference between the whole quantity of OX involved in the formulation and the quantity of the OX traced in the filtered solution to that of the whole quantity of OX added [34].

$$\%\ Drug\ Entrapped = \frac{total\ amount\ of\ drug - amount\ of\ the\ drug\ in\ filtrate}{total\ amount\ of\ drug} * 100$$

Fourier-transform infrared spectroscopy (FTIR)

The compatibility of 5-FU and OX with adopted polymers was analyzed by using FT-IR in which the spectra for pure drug, physical mixture of polymers, 5-FU tablets, and OX loaded microspheres were recorded in a Fourier-Transform Infrared Spectrophotometer (PERKIN ELMER, USA) in the scanning range of 4000-400 cm^{-1} with the help of potassium bromide pellets.

Differential scanning calorimetry (DSC)

The thermal behaviors of 5-FU, OX, physical mixture of polymers, 5-FU tablets, and OX loaded microspheres were examined with an automatic thermal analyzer system (DSC 60, SHIMADZU, JAPAN) using TDS tread line software. In this study, sealed aluminum-lead pans were adopted for all the samples and were run over a temperature range from 30 to 450 °C at a heating rate of 10 °C/min under an inert environment flushed with nitrogen at a rate of 20 ml/min.

***In vitro* dissolution study for 5-FU tablets under the simulated gastric and intestinal condition**

In vitro drug release study was carried out using a type 2 dissolution apparatus. To study the comparative release of 5-FU under different pH conditions, the experiment was performed in a variety of medium such as an acidic buffer (pH 2.0) for 2 h to simulate fasted stomach, phosphate buffer (PO_4) (pH 4.5) to simulate duodenum, PO_4 buffer (pH 6.8) to simulate mid jejunum and PO_4 buffer (pH 7.4) to simulate ileo-colon conditions for almost 24 h. Samples (10 ml aliquots) were withdrawn at particular time intervals for 24 h and were centrifuged at 1500 rpm for 10 min. The amount of drug was quantified spectrophotometrically (UV 1700, SHIMADZU, JAPAN) at 266 nm for 5-FU release. All dissolution tests were run in n=4 and the results were plotted as the percentage cumulative drug content released into dissolution medium versus time [35].

***In vitro* dissolution study for OX microspheres under the simulated gastric and intestinal condition**

The *in vitro* release of drug was conducted using USP type 1 dissolution apparatus to accurately find the OX release. The different dissolution fluids of different pH values were adopted in this study. In 1st hour simulated gastric fluid of pH 1.2 was used, during the 2ndhour mixture of simulated gastric and intestinal fluid of pH 4.5, further in 4-5th hour simulated intestinal fluid of pH 7.5 and finally in 6-8th hour simulated colonic fluid of pH 7.0. *In vitro* release of drug from microspheres into 900 ml of simulated dissolution medium at 37±1 °C with stirring under perfect sink conditions, further dissolution fluids were changed timely. Samples (10 ml aliquots) were withdrawn at a specified time and replaced with the same volume of fresh simulated fluid, and the amount of drug present was analyzed spectrophotometrically (UV 1700, Shimadzu, JAPAN) at 240 nm for OX release. All dissolution tests were run in n=4 and the results were plotted as the percentage cumulative drug content released into dissolution medium versus time [36].

***Ex vivo* drug release study using rat caecal content**

The everted colon method was used to perform *ex vivo* studies. An intestinal segment of the rat was isolated, the colon part was everted and tied at one end. Further, into the sac-like everted colon, 7.4 phosphate buffer, 5-FU tablets, and OX microspheres were added, which acts as a donor compartment. This everted colon was placed inside a beaker containing 7.4 phosphate buffers that act as a receptor compartment. Under aeration, the study was carried out for 20 h. At 37 °C, samples were withdrawn at a 1 h time interval and were analyzed for the amount of drug released. All dissolution tests were run in n=4 and the results were plotted as the percentage cumulative drug content released into dissolution medium versus time [34].

RESULTS AND DISCUSSION

Pre-compression parameters of 5-FU tablets

The granules were evaluated for angle of repose, compressibility index, and hausner's ratio and the obtained results were listed in table 4 and 5. The angle of repose less than 30 degree indicates good flow properties. The carr's index of granules shown in table 4 and 5 was obtained between 5-15%, that comes under an excellent category [37]. Thus the pre-compression properties of granules exhibited good results.

Table 4: Pre-compression parameters of granule coated formulations

Formulation code	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Carr's index (%)	Hausner's ratio
A1G	27.68±4.8	0.484±0.09	0.515±0.02	6±0.7	1.06±0.01
A2G	27.35±3.9	0.490±0.04	0.524±0.04	6.4±0.9	1.06±0.01
A3G	27.24±4.4	0.502±0.07	0.537±0.01	6.5±0.9	1.06±0.03
A4G	27.21±2.8	0.513±0.07	0.540±0.07	5.4±0.1	1.05±0.01
A5G	27.09±2.1	0.520±0.08	0.555±0.08	6.3±0.8	1.06±0.02
A6G	27.22±3.6	0.525±0.04	0.560±0.07	6.3±0.4	1.06±0.01
A7G	27.88±4.2	0.532±0.07	0.566±0.07	6.1±0.2	1.06±0.02
A8G	28.89±2.1	0.533±0.07	0.574±0.08	7.1±1.1	1.07±0.01

*All values are expressed as mean±SD, (n=3).

Table 5: Pre-compression parameters of tablet coated formulations

Formulation code	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Carr's index (%)	Hausner's ratio
A1T	26.32±3.1	0.452±0.07	0.485±0.02	6.8±0.3	1.06±0.01
A2T	25.83±6.1	0.459±0.04	0.497±0.02	7.6±0.9	1.07±0.01
A3T	25.71±2.2	0.467±0.02	0.501±0.03	6.7±0.6	1.06±0.01
A4T	25.68±1.7	0.484±0.02	0.515±0.07	6.01±0.2	1.06±0.03
A5T	27.35±2.9	0.490±0.03	0.524±0.04	6.4±0.4	1.06±0.02
A6T	27.24±3.3	0.502±0.04	0.537±0.02	6.5±0.7	1.06±0.01
A7T	27.21±4.8	0.513±0.04	0.540±0.08	5.4±0.4	1.05±0.01
A8T	27.09±2.6	0.520±0.04	0.555±0.04	6.3±0.3	1.06±0.01
A9T	27.22±2.4	0.525±0.07	0.560±0.04	6.25±0.9	1.06±0.01
A10T	26.88±1.2	0.532±0.07	0.566±0.07	6±0.4	1.06±0.03
A11T	25.89±1.7	0.533±0.06	0.574±0.04	7.1±1.1	1.07±0.01
A12T	25.98±3.2	0.546±0.04	0.582±0.04	6.1±0.8	1.06±0.01
A13T	25.66±3.9	0.554±0.04	0.588±0.09	5.7±0.4	1.05±0.02
A14T	25.75±1.9	0.566±0.04	0.602±0.02	5.5±0.2	1.05±0.01

*All values are expressed as mean±SD, (n=3).

Post-compression parameters of 5-FU tablets

The compressed 5-FU tablets were evaluated for hardness, friability, weight variation and drug content uniformity. The hardness of all compressed 5-FU tablets was in the range of 8.1 to 9.1 kg/cm^2. The prepared 5-FU tablets were shown decent variability in weight under 5% (w/w) and also shown good friability ranging between 0.11 to 0.2%, which is in prescribed limits [38]. Further, the 5-FU tablets were shown acceptable variation in weight and excellent uniformity in drug content of 265±40 mg and 97%, respectively as shown in the table 6 and 7.

Table 6: Post-compression parameters of granule coated formulations

Formulation code	Average weight of tablet (mg)	Weight variation (%)	Hardness (kg/cm^2)	Friability (%)	Drug content (%)
A1G	255±21	2±1.6	8.6±2.6	0.13±0.01	99±2.1
A2G	275±37	1.7±0.9	8.2±3.1	0.20±0.04	97.8±1.7
A3G	257±29	1.1±1.2	8.1±1.2	0.17±0.03	98.8±1.4
A4G	275±23	1.7±0.4	8.7±1.8	0.11±0.01	98.4±1.3
A5G	255±27	2±0.4	8.6±2.7	0.13±0.04	99±1.3
A6G	275±33	1.7±0.7	8.2±0.8	0.20±0.06	97.8±2.6
A7G	305±39	1.6±0.1	8.8±0.9	0.16±0.02	98.2±2.2
A8G	320±38	3±1.2	8.1±0.4	0.15±0.01	97.4±4.1

*All values are expressed as mean±SD, (n=3)

Table 7: Post-compression parameters of tablet coated formulations

Formulation code	Average weight of tablet (mg)	Weight variation (%)	Hardness (kg/cm^2)	Friability (%)	Drug content (%)
A1T	275±22	1.7±0.5	8.8±0.9	0.15±0.03	99.2±4.2
A2T	300±31	1.6±0.7	8.4±1.6	0.20±0.01	97.8±2.7
A3T	270±14	3.5±0.3	8.3±2.2	0.19±0.02	98.8±3.3
A4T	300±29	1.6±0.9	8.4±1.4	0.13±0.01	98.4±6.6
A5T	285±17	3.3±0.7	8.7±1.2	0.15±0.02	98.8±1.2
A6T	310±40	3.2±1.2	9.1±0.8	0.20±0.01	97.8±7.1
A7T	265±19	1.8±0.2	9.3±2.1	0.16±0.02	98.2±4.6
A8T	295±35	1.6±0.7	8.2±0.6	0.17±0.05	97.4±3.7
A9T	310±22	3.2±1.6	8.4±1.3	0.15±0.04	98.9±3.3
A10T	340±39	2.9±0.9	8.7±1.8	0.20±0.01	97.8±3.8
A11T	250±14	3.8±1.4	8.9±1.1	0.19±0.03	98.8±5.7
A12T	245±29	2±0.4	8.8±1.9	0.13±0.02	98.4±2.4
A13T	230±11	4.3±1.3	8.4±0.6	0.15±0.03	99±3
A14T	220±16	4.5±1.7	8.6±1.1	0.20±0.02	98.8±4.4

*All values are expressed as mean±SD, (n=3).

Surface morphology and particle size analysis of microspheres

The surface morphology of the microspheres was demonstrated from SEM micrographs in fig. 1. It was found that prepared OX microspheres were smaller in size and more uniform in shape. The surface morphology of microspheres showed fuller structure with fewer surface wrinkles; according to the SEM observation, the alginate and chitosan were formed a successful matrix by cross-linking with ca^{+2} and zn^{+2}. The sizes of the OX microspheres were ranged from 22-27 µm.

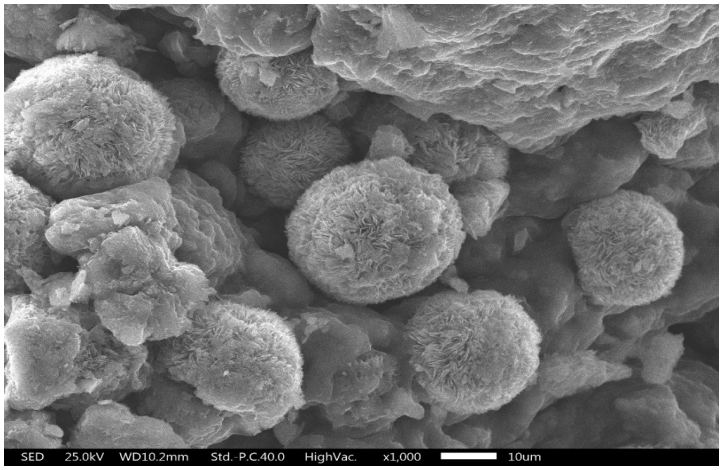

Fig. 1: SEM observation of OX microspheres

Percentage yield and entrapment efficiency of microspheres

OX microspheres were formulated by iontropic gelation technique and the percentage yield of the entire microsphere formulations were said to be in the range of 42.4-68.5% whose polymer concentration varied from low to high. The practical yield mainly depends on the concentration of polymer added into the formulation, as the concentration of polymer increases, the percentage yield will also be greater. The maximum yield obtained is 68.5% for formulation A8M.

The entrapment efficiency of the prepared microsphere was ranged from 58.3-74.4% depending upon varied polymer concentration. The percent entrapment efficiency increases due to elevated concentration of polymer up to a certain level; further greater polymer concentration results in decline of percent entrapment efficiency as seen in A4M and A8M. This shows that, greater the concentration of polymers than certain limit, bulkier the microspheres resulting in less area present for encapsulation of drug. The formulation A7M showed maximum entrapment efficiency of 74.4%. The detailed values for each preparation of OX microspheres were shown in table 8.

Table 8: Percentage yield and encapsulation efficiency of microspheres

Formulation code	Percentage yield	Entrapment efficiency
A1M	42.42±0.12	73.2±0.34
A2M	43.47±0.78	58.3±0.56
A3M	55.93±0.91	67.8±0.23
A4M	57.97±0.67	59.2±0.45
A5M	43.75±0.89	62±0.12
A6M	35.5±0.56	68.9±0.34
A7M	55.17±0.78	74.4±0.91
A8M	68.57±0.23	64.6±0.45
A9M	42.96±0.89	69.6±0.67
A10M	44.61±0.12	71.8±0.34
A11M	50±0.78	66.93±0.56
A12M	43.75±0.91	65.7±0.23
A13M	52.8±0.67	71.43±0.45
A14M	59.52±0.89	65.6±0.12

*All values are expressed as mean±SD, (n=3).

Fourier-transform infrared spectroscopy (FTIR)

The FT-IR spectra of 5-FU, 5-FU tablet, physical mixture of tablet excipients, OX, OX microsphere and physical mixture of microsphere excipients are shown in fig. 1. N-H stretching vibrations at 3257 cm^{-1}, 3245 cm^{-1}, C-H stretching vibrations at 3062 cm^{-1},3026 cm^{-1},-C-H_2 asymmetric and symmetric stretching vibrations at 2933 cm^{-1}, 2827 cm^{-1}, and 2947 cm^{-1}, 2896 cm^{-1},–C=C stretching vibrations at 1645 cm^{-1}, 1641 cm^{-1}, N-H in plane bending vibrations at 1510 cm^{-1}, 1411 cm^{-1}, Ring stretching vibrations at 1247 cm^{-1}, 1245 cm^{-1}, C-F stretching vibrations at 1224 cm^{-1}, 1220 cm^{-1}, N-H and C-H wagging vibrations at 933 cm^{-1}, 995 cm^{-1}C-F stretching vibrations at 813 cm^{-1}, 817 cm^{-1}, pyrimidine ring vibrations at 754 cm^{-1}, 752 cm^{-1} were observed for 5-FU and 5-FU tablet of A14T respectively.

For the FT-IR spectrum of OX and OX microspheres of A14M, the peaks at 3264 cm^{-1}, 3263 cm^{-1}shows NH stretch, 3508 cm^{-1}, 3514 cm^{-1} for C-N stretch C=O stretch was observed at 1706 cm^{-1}, 1645 cm^{-1}, and the peak at 811 cm^{-1},815 cm^{-1}shows N-H bending respectively.

The FT-IR spectrum of chitosan peaks at 2920 cm^{-1}, 2950 $cm^{-1}$for C-H stretching, the absorption band of carbonyl stretching of secondary amide at 1662 cm^{-1}, 1667 cm^{-1}, the bridge oxygen stretching bands at 1068 cm^{-1} and 1039 cm^{-1}, 1078 cm^{-1} and 1029 $cm^{-1}$for 5-FU excipients and microsphere excipients respectively. Sodium alginate showed the distinctive peaks as a carbonyl, it depicted strong absorption bands at 1602 cm^{-1}, 1380 cm^{-1} due to carboxyl anions. The presence of sharp peak in fig. 2(b) and 2(c) at around 3500-3000 cm^{-1} indicates no physical interaction between chitosan and alginate, moreover, the distinct peaks for sodium alginate were present indicates no interaction between the excipients and the peaks of 5-FU were clearly shown that there was no apparent interaction between 5-FU and polymersas shown in fig. 2(b). The hydrogen bonding between alginate and chitosan were indicated by the broad peaks present in the range of 3500-3000 cm^{-1}. Though OX shows C=O stretching vibrations at 1783 cm^{-1}, sodium alginate is having a characteristic asymmetric stretching vibrations due to carboxyl anions at 1662 cm^{-1} and chitosan is having an absorption band of carbonyl stretching of secondary amide at 1598 cm^{-1} respectively in fig. 2(e), but the OX microparticles showed a peak at 1645 cm^{-1} in fig. 2(d) indicating the entrapment of OX into the ionic complex.

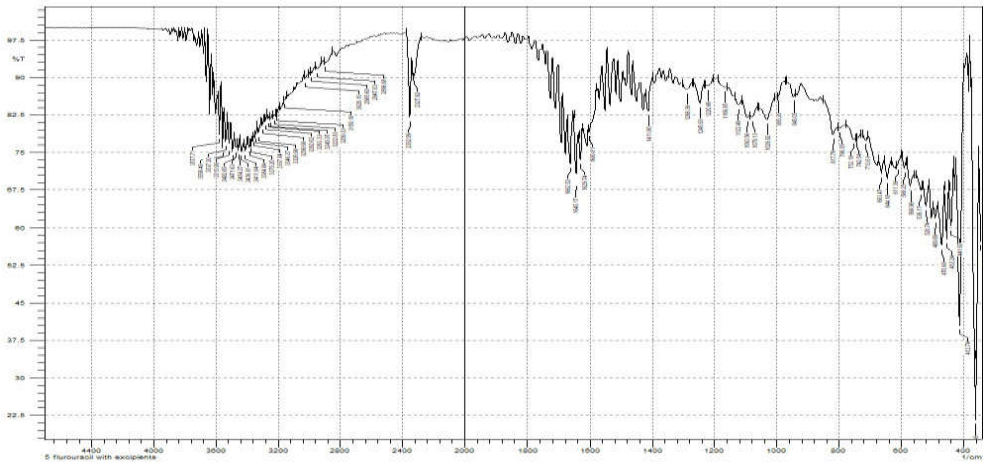

Fig. 2(a): FT-IR of 5-FU

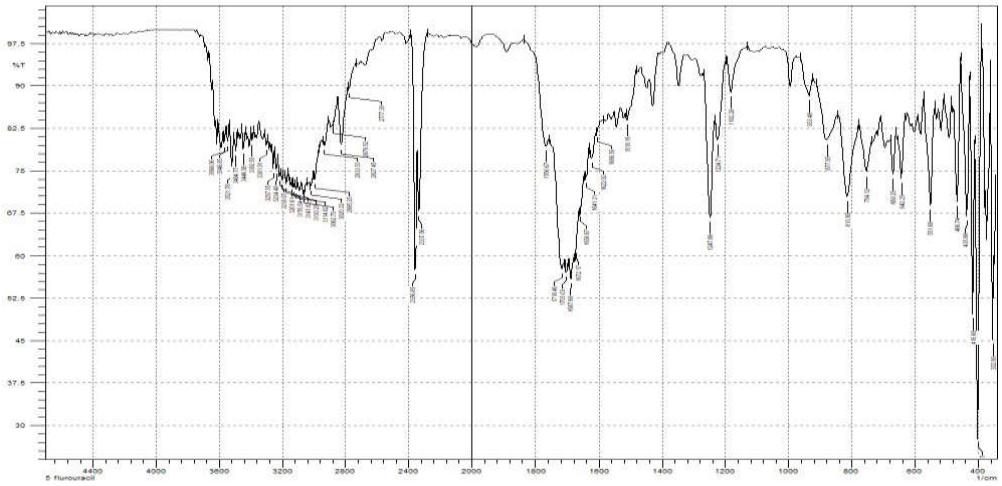

Fig. 2(b): FT-IR of 5-FU tablet (A14T)

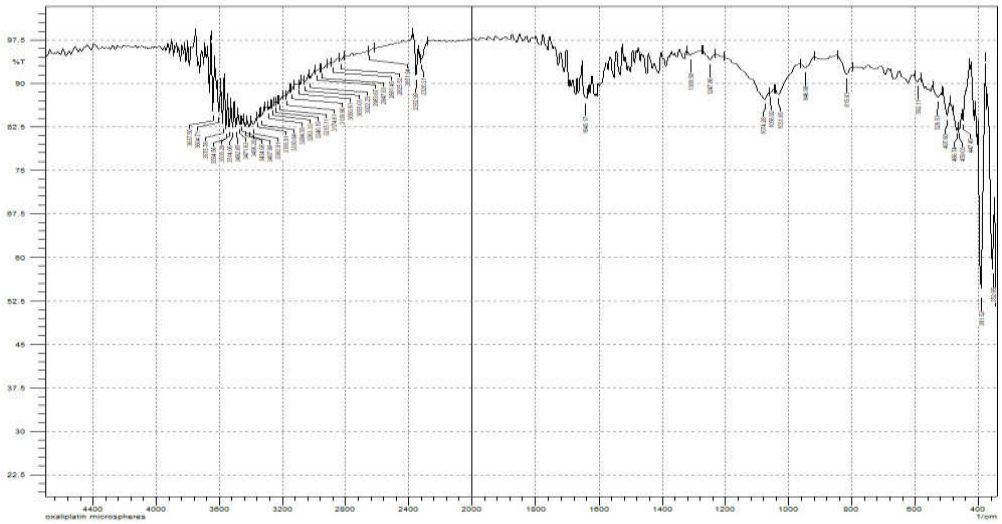

Fig. 2(c): FT-IR of the physical mixture of 5-FU tablet excipients

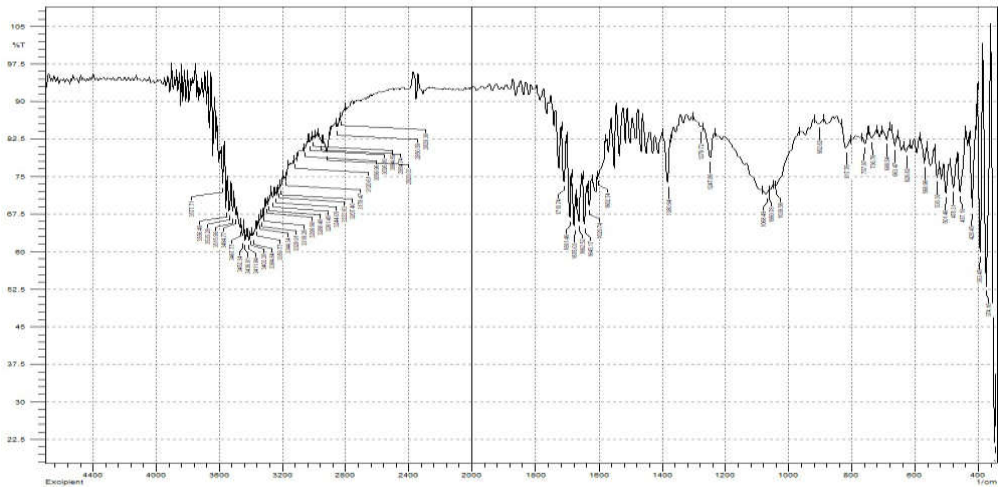

Fig. 2(d): FT-IR of OX microspheres (A14M)

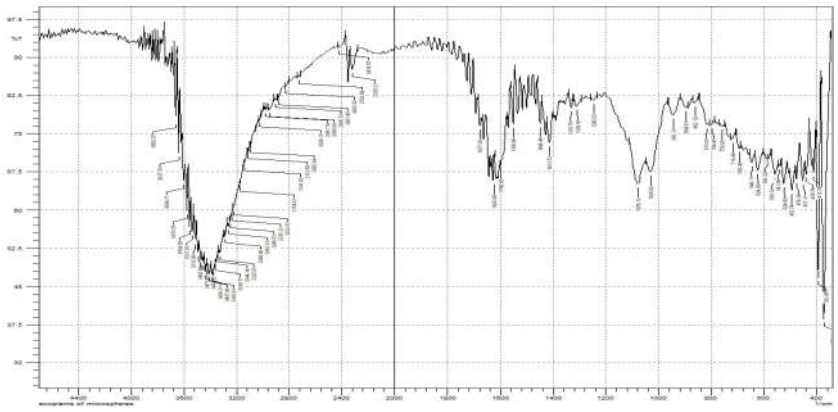

Fig. 2(e): FT-IR of the physical mixture of microsphere excipients

Differential scanning calorimetry (DSC)

The DSC is most crucial during the determination of the thermal behavior of formulation excipients that provides both quantitative and qualitative details about the state of the drug present inside the formulation. If a drug is dispersed in the solid-solution state or molecular dispersion in the polymeric system, there will not be any endotherm detected. In the present study DSC thermograms of pure 5-FU, 5-FU tablet of A14T, physical mixture of tablet excipients, pure OX, OX microspheres of A14M, and physical mixture of microsphere excipients were taken.

An exothermic peak correlate with 5-FU was found to be present at 287.02 °C as shown in fig. 3(a). The exothermic peak correlate with a melting point of 5-FU in the sample of A14T shifted slightly to the 277.1 °C, that may happened because of bound water presence in the sodium alginate and the DSC results indicate the non-existence of possible interactions between 5-FU and sodium alginate and other excipients as shown in fig. 3(b).

Melting endotherms of pure OX, physical mixture of excipients and oxaliplatin microspheres of A14M showed the presence of endothermic peaks at 287.58 °C, 248.92 °C, and 271.68 °C respectively depicted in fig. 3(d) and (e). Besides, considerable exothermic peaks were observed at 104.86 °C, 99.5 °C for microsphere excipients and OX microspheres, respectively, stipulating the crystalline characteristic of the polymers. However, the absence of the OX characteristic peak in OX microspheres, which showed a negligible broad peak at 271.68 °C, indicating that the OX was molecularly dispersed inside microspheres. Due to the presence of polymers in microsphere formulation, the peak sharpness and height reduction is observed.

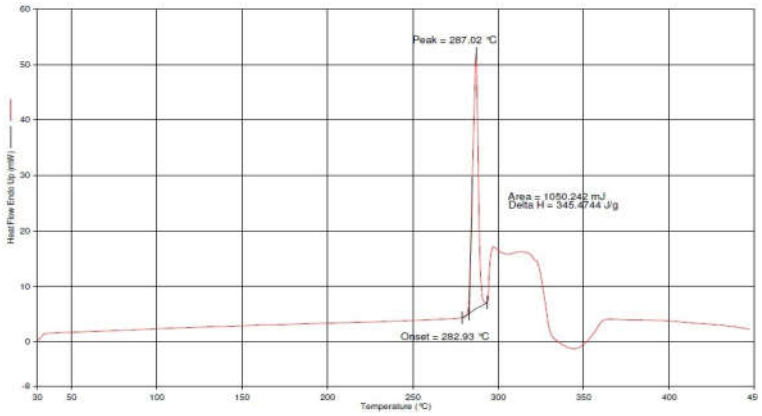

Fig. 3(a): DSC of 5-FU

***In vitro* dissolution study for granules coated 5-FU, tablet coated 5-FU and oxaliplatin microspheres**

To study the effect of various polymers that were used, initially, the dissolution was carried out for 10 h for A1T-A6T and A1G-A4G. Tablet coatings released a lesser amount of 5-FU for 10 h in 7.4 phosphate buffer. But granule coating exhibited promising drug release within 10 h in 7.4 phosphate buffers to proceed further for 24 h study. Guar gum was used in A3T, A4T, A3G, A4G, formulations instead of alginate. Significant differences in the release profiles weren't observed between alginate and guar gum and hence further studies were planned with alginate, owing to the fact that guar gum was used in a lot many formulations for preparing 5-FU tablets previously. The release profiles changed contrastingly in 24 h study with a coated capsule. The very low release profiles of granule coating ruled out the chances of using them for further study. On the other hand, though the tablet coatings too don't have a better release, they have better release enough to continue with them for further. The studies show a direct relationship between the amount of alginate added into the formulation and the amount of eudragit RSPO coated on to the tablet to that of the drug release. The reduction in the coating concentration from 50 mg to 20 mg and alginate from 100 mg to 50 mg in A7T released just 40.9% in 24 h. By gradual reduction in the 50 mg of alginate taken in A7T and keeping 20 mg eudragit RSPO coating constant four formulations were prepared to have 40, 30, 20 and 10 mg of alginate. As the concentration of alginate decreased, the cumulative release of 5-FU was increased to 49.3%, 59.1%, 76.4%, and 90.3% respectively at the end of 24 h. The detailed percentage drug release for tablet coated 5-FU, granule coated 5-FU were shown in fig. 4.

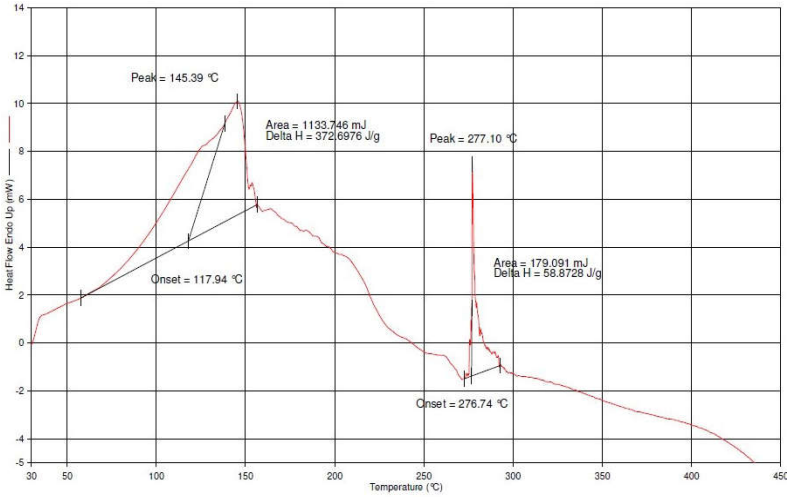

Fig. 3(b): DSC of 5-FU tablet (A14T)

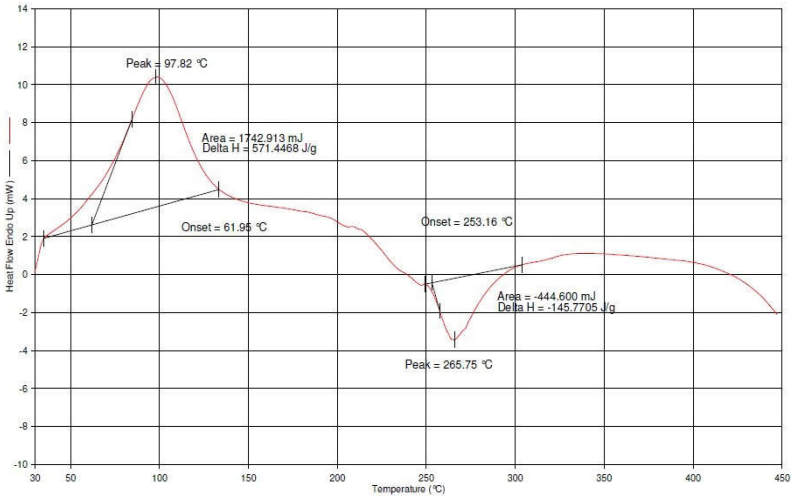

Fig. 3(c): DSC of the physical mixture of tablet excipients

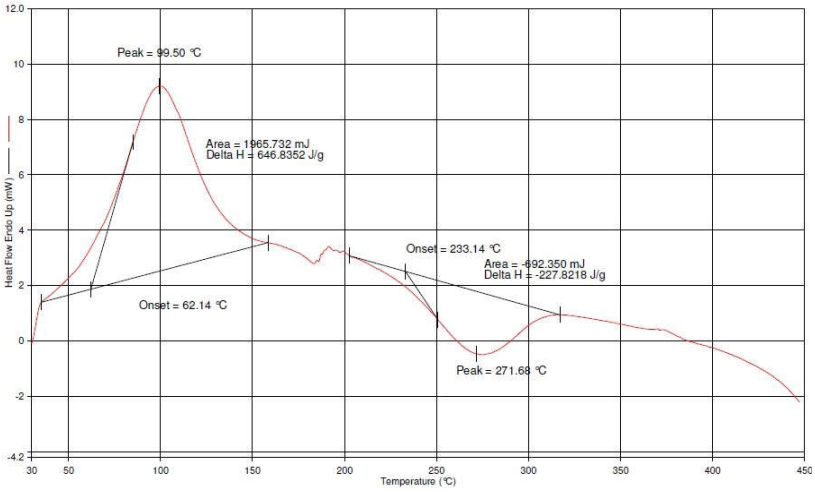

Fig. 3(d): DSC of OX microspheres (A14M)

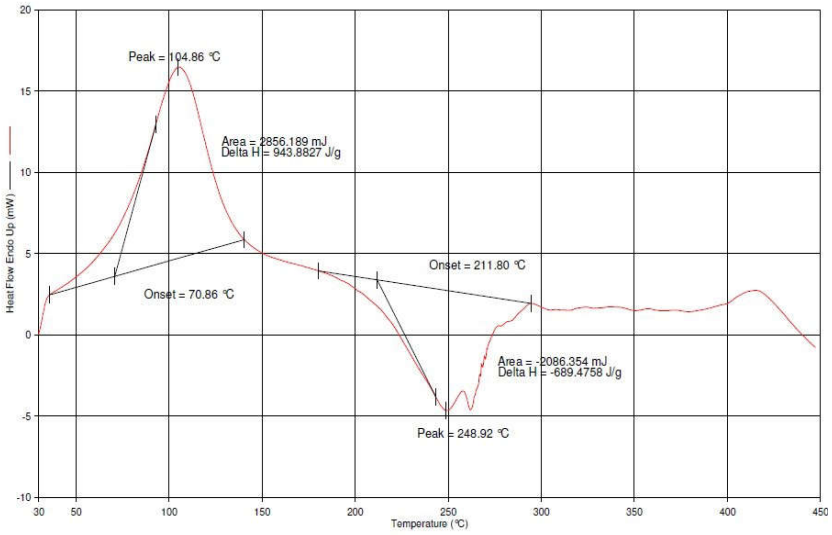

Fig. 3(e): DSC of the physical mixture of microsphere excipients

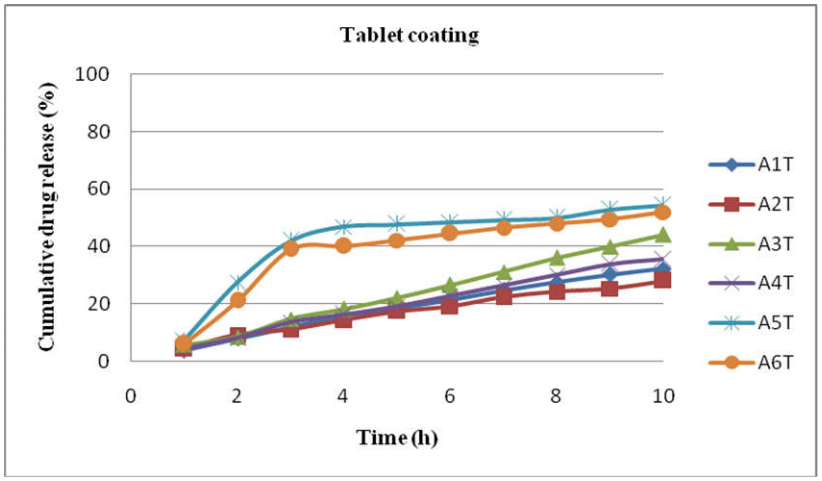

Fig. 4(a): Dissolution profile of A1T-A6T, *results are expressed as mean±SD, (n=4)

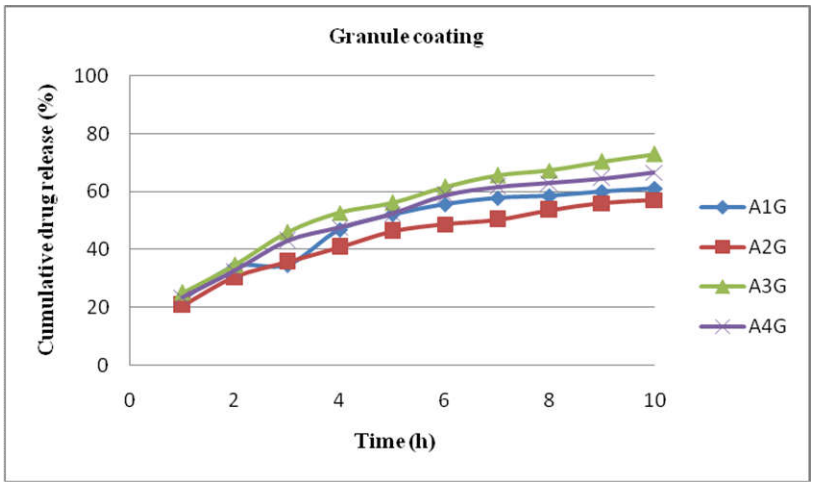

Fig. 4(b): Dissolution profile of A1G-A4G, *results are expressed as mean±SD, (n=4)

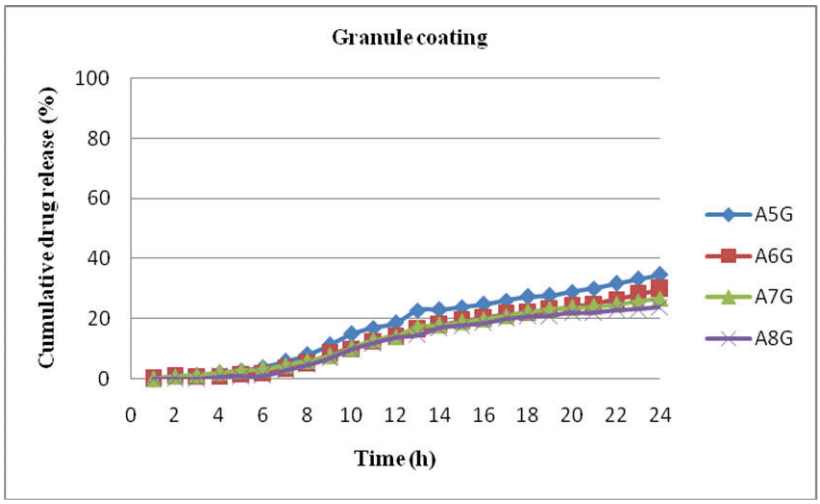

Fig. 4(c): Dissolution profile of A5G-A8G, *results are expressed as mean±SD, (n=4)

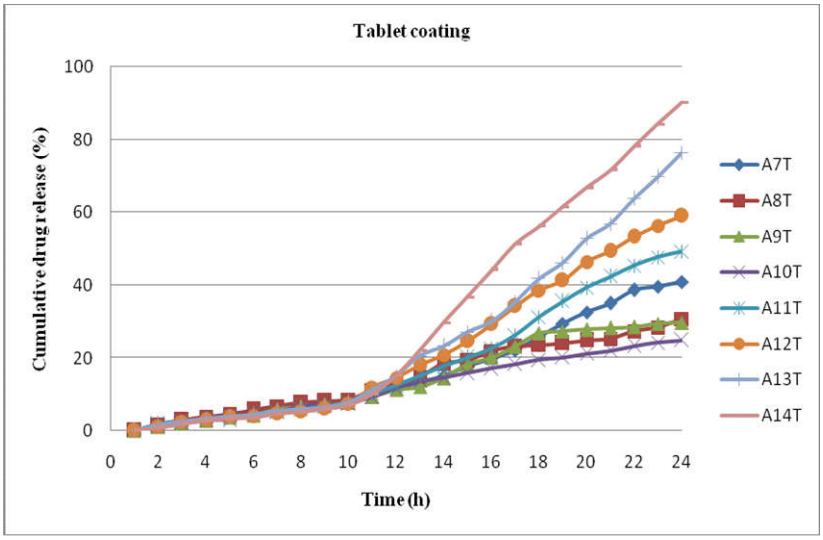

Fig. 4(d): Dissolution profile of A7T-A14T, *results are expressed as mean±SD, (n=4)

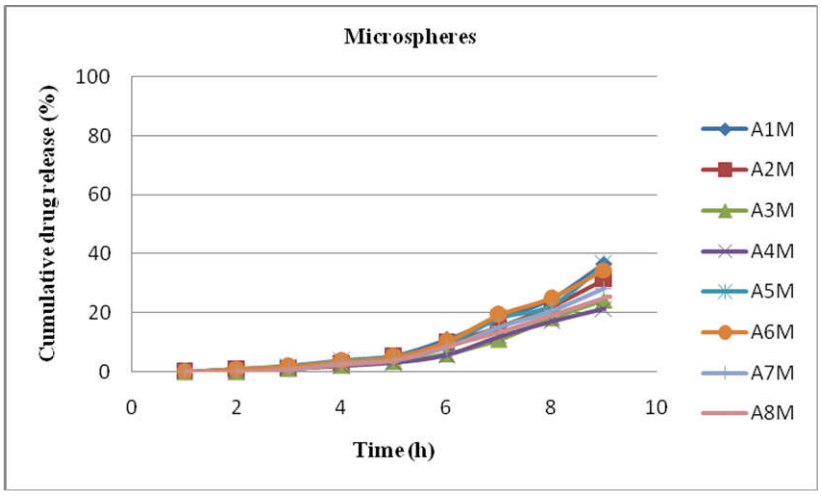

Fig. 5(a): Dissolution profile of A1M-A8M, *results are expressed as mean±SD, (n=4)

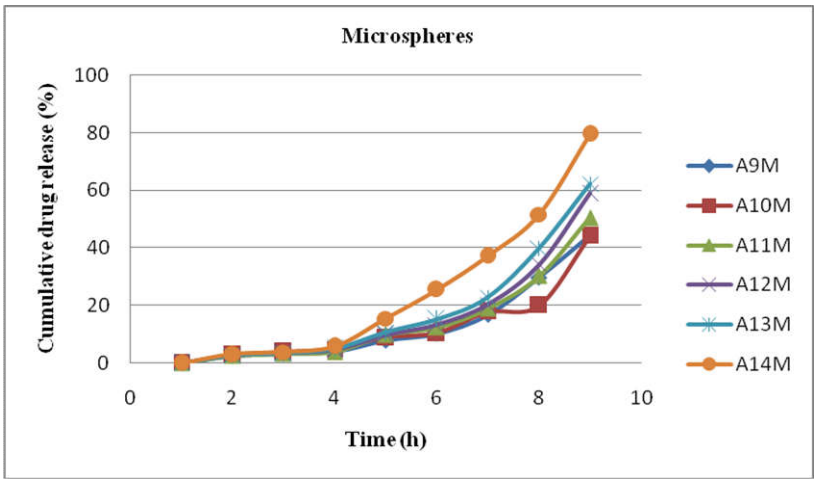

Fig. 5(b): Dissolution profile of A9M-A14M, *results are expressed as mean±SD, (n=4)

The dose of OX is on the lower end compared to that of 5-FU and with that small dose, microspheres were comfortably prepared. Based on the solubility profile of oxaliplatin, the ionotropic gelation method was adopted as the method of choice for the formulation of oxaliplatin microspheres and finally prepared microspheres were filled into a capsule. At the end of the 5th hour, not more than 5% of 5-FU was released. Again alginate slows down the drug release noticeably from OX microspheres. Since OX microspheres had to release the drug completely in 10 h, the weight of alginate was reduced in the microspheres. Here in microspheres, the alginate concentration reduction isn't that easy as compared to a 5-FU tablet. A1M and A7M were selected to proceed further by reducing the alginate concentration, here the amount of alginate was reduced concerning the other polymers. Zinc sulfate helped in sustaining the drug release but it was not used in other formulations. Eudragit S-100 coat on capsules was reduced to 8% and carried out further formulations concerning A1M and A7M. The reason behind choosing these two formulations is their high encapsulation efficiencies at 73.2% and 74.4% respectively. The absence of zinc sulfate didn't show any effect on the encapsulation efficiencies and by carefully controlling the amount of alginate and coating on to the capsules the cumulative release for A13M and A14M were recorded as 62.31% and 79.63% respectively. The detailed percentage drug release for OX microspheres were shown in fig. 5.

Ex vivo drug release study using rat caecal content

The ex vivo studies were carried out in an everted colon technique in the presence of caecal content. Since the A14M and A14T formulations exhibited a higher rate of drug release we are taking them as optimized formulations to carryout *ex vivo* studies. The pH-sensitive polymer-coated capsule was not used in this study along with microspheres and tablets due to the test directly carried out in the simulated colonic condition. The results were found to be 76% and 78% of drug released for OX microspheres (A14M) and 5-FU tablets (A14T) respectively at specific time points as shown in fig. 6.

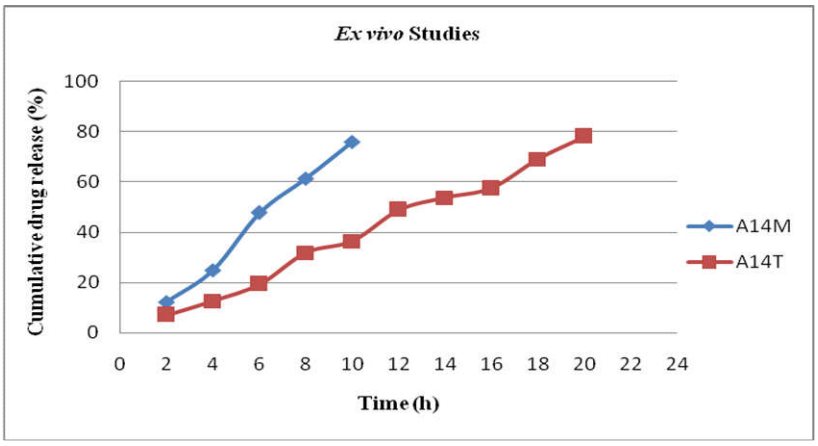

Fig. 6: *Ex vivo* drug release study of OX microspheres (A14M) and 5-FU tablet (A14T), *results are expressed as mean±SD, (n=4)

CONCLUSION

The two different formulations were designed selectively to deliver 5-FU and OX to the colon region in synchronization with circadian timing system (Chronotherapy). Both the tablet and microsphere formulations were found to be successful in case of controlled drug release. Among all the other formulations, A14T and A14M were showed maximum drug release at 24 h and 9 h respectively. This demonstrated that optimization of polymer ratio and concentrations of coating solution are very important to achieve delayed drug release. Wherefore the current study believed that 5-FU tablets and OX microspheres prepared by using varied concentration of polymers that were further filled into capsules and coated with pH-sensitive polymer eudragit S-100 can be efficiently used to control the rate of drug release to the colon in synchronization with circadian timing system same as chronomodulated chemotherapy treatment given through intravenous route in the belief of improved therapeutic efficacy, tolerability and overall survival rate of cancer

patients. Thus it can be a very good alternative for intravenous route based chronomodulated chemotherapy.

ACKNOWLEDGEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. WHO Cancer Fact Sheet. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer. [Last accessed on 11 Sep 2018]
2. Basini J, Rayadurgam S, Dakshinamurthy S. An overview of colorectal cancer: implication of two medicinal plants in their treatment. Asian J Pharm Clin Res 2019;12:47-52.
3. Chena Y, Li N, Xu B, Wu M, Yan X, Zhong L, *et al*. Polymer-based nanoparticles for chemo/gene-therapy: evaluation its therapeutic efficacy and toxicity against colorectal carcinoma. Biomed Pharmacother 2019;118:109-257.
4. Sunoqrot S, Abujamous L. pH-sensitive polymeric nanoparticles of quercetin as a potential colon cancer-targeted nanomedicine. J Drug Delivery Sci Tech 2019;52:670-6.
5. Neugut AI, Lin A, Raab GT, Hillyer GC, Keller D, Neil DSO, *et al*. FOLFOX and FOLFIRI use in stage IV colon cancer: analysis of SEER medicare data. Clin Colorectal Cancer 2019;18:133-40.
6. You X, Kang Y, Hollett G, Chen X, Zhao W, Gu Z, *et al*. Polymeric nanoparticles for colon cancer therapy: overview and perspectives. J Mater Chem B 2016;4:7779-92.
7. Shena MY, Liu T, Yu TW, Kv R, Chiang WH, Tsai YC, *et al*. Hierarchically targetable polysaccharide-coated solid lipid nanoparticles as an oral chemo/thermotherapy delivery system for local treatment of colon cancer. Biomaterials 2019;197:86-100.
8. Gupta SK, Huneza A, Patra S. Formulation, development and *in vitro* evaluation of tramadol extended-release tablets. Int J Pharm Pharm Sci 2019;11:63-73.
9. Li W, Liu D, Zhang H, Correia A, Makila E, Salonen J, *et al*. Microfluidic assembly of a nano-in-micro dual drug delivery platform composed of halloysite nanotubes and a pH-responsive polymer for colon cancer therapy. Acta Biomater 2017;48:238-46.
10. Lijun S, Liping L, Xiaoying S, Honglang C, Shumin Z, Wenfeng C, *et al*. Optimizing pH-sensitive and time-dependent polymer formula of colonic pH-responsive pellets to achieve precise drug release. Asian J Pharm Sci 2019;14:413-22.
11. Mahto A, Mishra S. Design, development and validation of guar gum based pH sensitive drug delivery carrier via graft copolymerization reaction using microwave irradiations. Int J Biol Macromol 2019;138:278-91.
12. Vemula SK. Formulation and pharmacokinetics of colon-specific double-compression coated mini-tablets: chronopharmaceutical delivery of ketorolac tromethamine. Int J Pharm 2015;491:35-41.
13. Sen SO, Devbhuti P, Sen KK, Ghosh A. Development and evaluation of sustain release microparticles of metoprolol succinate. Int J Appl Pharm 2019;11:166-72.
14. Cerciello A, Auriemma G, Gaudio PD, Sansone F, Aquino RP, Russo P. A novel coreeshell chronotherapeutic system for the oral administration of ketoprofen. J Drug Delivery Sci Tech 2016;32:126-31.
15. Jose S, Prema MT, Chacko AJ, Thomas AC, Souto EB. Colon specific chitosan microspheres for chronotherapy of chronic stable angina. Colloids Surf B 2011;83:277-83.
16. Levi F. Circadian chronotherapy for human cancers. Lancet Oncol 2001;2:307-15.
17. Youan BBC. Chronopharmaceutics: gimmick or clinically relevant approach to drug delivery? J Controlled Release 2004;98:337-53.
18. Oishi K, Koyanagi S, Matsunaga N, Kadota K, Ikeda E, Hayashida S, *et al*. Bezafibrate induces plasminogen activator inhibitor-1 gene expression in a CLOCK dependent circadian manner. Mol Pharmacol 2010;78:135-41.
19. Marra G, Anti M, Percesepe A, Armelao F, Ficarelli R, Coco C, *et al*. Circadian variations of epithelial cell proliferation in human rectal crypts. Gastroenterology 1994;106:982-7.
20. Levi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol 2007;47:593-628.
21. Innominato PF, Levi FA, Bjarnason GA. Chronotherapy and the molecular clock: clinical implications in oncology. Adv Drug Delivery Rev 2010;62:979-1001.
22. Levi F, Focan C, Karaboue A, De la Valette V, Henrard DF, Baron B, *et al*. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. Adv Drug Delivery Rev 2007;59:1015-35.
23. Takimoto CH. Chronomodulated chemotherapy for colorectal cancer: failing the test of time? Eur J Cancer 2006;42:574-81.
24. Kiran HC, Venkatesh DN, Kumar RR. Formulation and characterisation of sustained release microbeads loaded with zaltoprofen. Int J Appl Pharm 2019;11:173-80.
25. Venkatesh DN, Kumar SS, Rajeshkumar R. Sustained release microbeads of ritonavir: *in vitro* and *in vivo* evaluation. Int J Appl Pharm 2019;11:189-98.
26. Venkatesh DN, Rao P, Rajeshkumar R. Enhanced oral bioavailability of tenofovir from ionotropically gelled microbeads. Int J Appl Pharm 2019;11:242-50.
27. Rodriguez MLG, Holgado MA, Lafuente CS, Rabasco AM, Fini A. Alginate/chitosan particulate systems for sodium diclofenac release. Int J Pharm 2002;232:225-34.
28. Shankar SJ, Gowda JBH, Nimitha AG, Metikurki B. Formulation and evaluation of hydroxyapatite microspheres of an antimicrobial drug for the treatment of osteomyelitis. Indo Am J Pharm Res 2019;7:3145-56.
29. Indian Pharmacopoeia, Government of India, Ministry of health and family welfare. 8th ed. New Delhi: The Controller of Publications; 2018.
30. Karthika C, Sureshkumar R, Suhail A. Formulation development and *in vitro* evaluation of curcumin-loaded solid self-nanoemulsifying drug delivery system for colon carcinoma. Asian J Pharm Clin Res 2019;12:243-7.
31. Wang QS, Wang GF, Zhou J, Gao LN, Cui YL. Colon targeted oral drug delivery system based on chitosan/alginate microspheres loaded with icariin in the treatment of ulcerative colitis. Int J Pharm 2016;515:176-85.
32. Sharma S, Seth N. Exploring the potential of eudragit for development of microparticles of water-soluble drug using quality by design approach. Int J Appl Pharm 2019;11:110-6.
33. Al-tahami K. Preparation, characterization, and *in vitro* release of ketoprofen loaded alginate microspheres. Int J Appl Pharm 2014;6:9-12.
34. Alange VV, Birajdar RP, Kulkarni RV. Functionally modified polyacrylamide-graft-gum karaya pH-sensitive spray dried microspheres for colon targeting of an anti-cancer drug. Int J Biol Macromol 2017;102:829-39.
35. Dodov MG, Calis S, Crcarevska MS, Geskovski N, Petrovska V, Goracinova K. Wheat germ agglutinin-conjugated chitosan–Ca–alginate microparticles for local colon delivery of 5-FU: development and *in vitro* characterization. Int J Pharm 2009;381:166-75.
36. Jain A, Jain SK, Ganesh N, Barve J, Beg AM. Design and development of ligand-appended polysaccharidic nanoparticles for the delivery of oxaliplatin in colorectal cancer. Nanomed: Nanotechnol Biol Med Food Hydrocolloids 2010;6:179-90.
37. Aulton ME. Pharmaceutics the science of dosage form design. Bangalore, India: Sapna Book House; 2002. p. 247.
38. Lachman L, Liberman HA, Kanig JL. The theory and practice of industrial pharmacy. Bangalore, India: Sapna Book House; 1987. p. 293-345.