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CELECOXIB LOADED MICROBEADS: A TARGETED DRUG DELIVERY FOR COLORECTAL CANCER

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ABSTRACT

Celecoxib is a nonsteroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and antipyretic activities. Recently, considerable interest has been focused on the use of biodegradable polymers for specialized applications such as targeted release of drug formulations; meanwhile, microbeads drug delivery systems using various kinds of biodegradable polymers have been studied extensively during the past two decades. In the present investigation, it was aimed to prepare microbead formulations of celecoxib inclusion complex using sodium alginate and eudragit FS 30-D as a carrier for colonic administration to extend the retention of the drug in order to treat colorectal cancer. Microbead formulations were evaluated for entrapment efficiency, FT-IR, DSC, SEM, *In vitro* drug release, *In vitro* cell line study, Cytotoxicity Screening. Formulation F5 showed 91.99 ± 1.45% entrapment, which was uniformly dispersed and having smooth surface texture in formulation, F5 shown 92.11±2.32% drug release up to 8 hr. Coated Celecoxib microbeads (1:1ratio) showed cytotoxicity against HT-29 cells. DNA Fragmentation study confirms the better anti cancer activity of celecoxib microbeads against human colorectal adenocarcinoma cell line HT-29. Hence the formulations can be effectively tested for its anticancer activity.

Key words: Celecoxib, Targeted Drug Delivery, *In Vitro* Dissolution, Microbeads, Entrapment Efficiency.

INTRODUCTION

Development of colon drug delivery system through oral route has achieved the most attention and success in the recent years. Celecoxib is an anti-inflammatory drug that exhibits anti-inflammatory, analgesic and antipyretic activities, especially in arthritis. The mechanism of action of celecoxib is believed to be due to the inhibition of prostaglandin synthesis, primarily via inhibition of cyclo-oxygenase-2 (COX-II). It has been shown that at therapeutic concentration in humans, celecoxib does not inhibit COX-1 isoenzyme,[1] which is important for homeostatic protection of the gastric mucosa and kidney (Davidson, 1999). Celecoxib is thus, associated with a lower incidence of gastro duodenal ulcers than other non-steroidal anti-inflammatory drugs,[2] which are non-specific inhibitors of cyclo-oxygenase. But since COX-II is constitutively present in some organs, like kidney and brain and can be induced in other tissues, COX-II specific inhibitors are not devoid of side effects. Thus, COX-II inhibitors can cause thrombotic cardiovascular disease as well as renal disease.[3] Achieving higher concentrations of the drug at the arthritic joint and minimizing its distribution to the other tissues would minimize the side effects of the drug.

Microbeads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects.[4] Additionally, the beads maintain functionality under physiological conditions, can incorporate drug to deliver locally at high concentration ensuring that therapeutic levels are reached at the target site while reducing the side effects by keeping systemic concentration low. The microbeads are produced from several polymers such as cationic polymers e.g. chitosan, anionic polymers e.g. sodium alginate, and binding components e.g. gelatin, chondroitin sulfate, avidin in predetermined ratio.[5] Considering the advantages of microbeads, the present investigation was undertaken to prepare and evaluate celecoxib loaded sodium alginate, eudragit and enteric coated microbeads with an objective to reduce solubility in gastric medium and prolong the drug release in small intestinal environment and to improve the patient compliance. [6]

There are several ways in which drugs can be targeted on the colon when they are given by mouth. In time-dependent formulations the drug concerned is released during the period of gastrointestinal transit time. Release from formulations that contain pH-dependent polymers takes place on the basis that pH is higher in the terminal ileum and colon than in the upper parts of the gastrointestinal tract. The colon is also home to large numbers of bacteria of many kinds. Prodrugs and dosage forms from which drug release is triggered by the action of colonic bacterial enzymes have therefore been devised.[7]

Eudragit products are pH-dependent methacrylic acid polymers containing carboxyl groups. The number of esterified carboxyl groups affects the pH level at which dissolution takes place. Eudragit S is soluble above pH 7 and Eudragit L above pH 6. Eudragit S coatings protect well against drug liberation in the upper parts of the gastrointestinal tract and have been used in preparing colon-specific formulations. When sites of disintegration of Eudragit S-coated single-unit tablets were investigated using a gamma camera they were found to lie between the ileum and splenic flexure. Site- specificity of Eudragit S formulations, both single- and multiple-unit, is usually poor.[8]

MATERIALS AND METHODS

Materials

Celecoxib was received as gift samples from Micro labs Pvt. Ltd. Bangalore, India. Sodium alginate was received as gift samples from Sreenivasa Marine Ltd. Madurai, India. Eudragit FS-30 D was received as gift samples from Vikram Thermonik Pvt. Ltd. Hyderabad, India. β- Cyclodextrin was received as gift samples from Universal Medicament Pvt. Ltd. Nagpur, India. Pectin (classic AF707)gift sample from Herbsteith & Fox K.G. Neuenburg.

Experimental

Preparation of microbeads

Inclusion complex of celecoxib and - β-cyclodextrin (1:1 ratio),[9] due to poor solubility of the celecoxib, was prepared and used for microbeads preparation. Ionic gelation method was used to prepare calcium alginate microbeads.[10] A 2 % w/v of inclusion complex of celecoxib was added to a 1.75 % w/v aqueous solution of sodium alginate (F1). This solution was dropped manually through a needle size no. 26G from a hypodermic syringe in to a 2 % w/v solution of $CaCl_2$. The gel microbeads formed were allowed to harden in gelling bath for 30 min. After washing with bidistilled water, the beads were air dried at room temperature and stored in vacuum desiccator until constant weight was achieved. Calcium alginate-pectinate microbeads were prepared using method as described above, the 2 % w/v of drug was added to an aqueous solution comprising 1.75 % w/v sodium alginate and 0.3, 0.5 and 1.0 % w/v of pectin in F2, F3 and F4 respectively. Eudragit FS 30 DL -coated microbeads were prepared by the procedure as described earlier.[11] Selected batch of microbeads (F4) were transferred in to 0.5 %w/v and 1.0 %w/v solution of Eudragit FS 30 DL in acetone for F5 and F6 respectively and remained for 15 min under gentle magnetic stirring, filtered and dried in air at room temperature (Table 1).

Table 1: Composition of microbeads formulations

Batch code	Drug (%w/v)	Sodium Alginate (%w/v)	Pectin (%w/v)	Eudragit FS 30 DL solution (%w/v)
				-
F1	2	1.75	-	-
F2	2	1.75	0.3	-
F3	2	1.75	0.5	-
F4	2	1.75	1.0	-
F5	2	1.75	1.0	0.5
F6	2	1.75	1.0	1.0

Evaluation of Microbeads

FT-IR Study

The Celecoxib micro beads were subjected to FT-IR analysis by the following method, an approximately minimum quantity (about 1mg) of sample was thoroughly blended with adequate quantity of IR grade KBr (about 5mg) in mortar. The mix was then made into thin films on a sample plate using a hand operated compression lever. The samples were then analyzed in a double beam IR spectrometer using KBr film as negative control (blank). The scanning range was 400-4000 cm^{-1}, resolution was 4 cm^{-1}.[12]

Differential scanning colorimetric study

DSC thermogram of celecoxib inclusion complex, sodium alginate polymer and formulated micro beads were recorded using a differential scanning calorimeter (DSC60,Shimazu,Japan).Each sample 2-2.5 mg was accurately weighed in to a40- µl aluminum pan without an aluminum cover. The measurement was performed between30 and 350 ċ at a heating rate of 10°C/mins.[13]

Scanning electron microscopy study

SEM photographs were taken with JSM 5600 scanning Microscope (Japan) to examine the morphology and surface structure of the beads at the required magnification at room temperature. The beads were deposited on brass hold on sputtered with a thin coat of gold under vacuum. Acceleration voltage used was 20kV with the secondary electron as a detector.[14]

Micromeritic properties

Average particle size of beads was determined by sieving method using automatic sieve shaker (HICON, India). Flowability of beads was determined by dynamic angle of repose using lab fabricated rotating cylinder. The beads were subjected to bulk density determination using tap density tester (HICON, India).[15]

Determination of entrapment efficiency

10 mg of the crushed microbeads were dissolved in 10 ml of methanol, vortexed for 5 min and filtered though whatmann filter paper no. 4. The filtered samples were suitably diluted and analyzed spectrophotometrically at 252 nm against suitably constructed calibration curve.[16]

Dissolution studies

The dissolution studies were carried out using USP XII dissolution rate test apparatus type I at 75 rpm and 37±0.5°C. The beads equivalent to 250 mg of drug were filled in to colorless hard gelatin capsules and placed in basket separately. The dissolution medium (900 ml) was 0.1 N HCl pH 1.2 as simulated gastric fluid (SGF) for the first 2 h, followed by phosphate buffer pH 6.8 and pH 7.4 as simulated intestinal fluid (SIF) for the next 6 h. 5 ml samples were withdrawn at specified time intervals and was replaced immediately with an equal volume of fresh medium. Samples were suitably diluted and analyzed at 252 nm (Shimadzu 1700). All the tests were carried out in triplicate.

Kinetics of drug release

In order to understand the mechanism and kinetics of drug release, the drug release data of the in-vitro dissolution study was analyzed with various kinetic equations like zero-order, higuchi and peppas equation. Coefficient of correlation (r) values were calculated for the linear curves obtained by regression analysis of the above plots.

In vitro cell line Studies

From the *In vitro* release data for uncoated and enteric coated celecoxib micro beads (F4 and F5), F5 showed better results, hence F5 was taken further for cytotoxicity studies against human colorectal adenocarcinoma cell line HT-29 cell line. [17-18]

Cytotoxicity against cell lines *in vitro*

Celecoxib micro beads uncoated and enteric coated (F4 and F5) along with the pure sample was checked for its cytotoxicity against human colorectal adenocarcinoma cell line HT-29 cell line using Microculture Tetrazolium (MTT) assay. All the above three samples were incubated at pH 1.5 for 2 hours, subsequently transferred and incubated at pH 6.8 for three hours and finally transferred and incubated at pH 7.4 for an additional three hours in a rotating agitator. Sampling was done at one hour interval starting from zero time. The samples collected at different time intervals were centrifuged and the drug concentration was measured using spectrophotometer. Human colorectal adenocarcinoma cell line HT-29 cell line expressing high levels of Cox-2 was cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum, penicillin (100 U) and streptomycin (100 µg). The HT-29 cells were plated and treated with samples collected at different time intervals from the rotating agitator. Cell viability was measured using MTT assay. Method for passaging the cells, cytotoxicity screening by MTT assay were given below, followed by the results.

Cytotoxicity Screening

Determination of Mitochondrial Synthesis by Microculture Tetrazolium (MTT) Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100µl of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTT in MEM was added to each well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 50µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm.

DNA Fragmentation Analysis

HT-29 cells were cultured in the presence or absence of uncoated, coated and pure sample Celecoxib for 24 hours. Floating and adherent cells were collected, rinsed twice with phosphate-buffered saline, and resuspended in lysis buffer containing 1% Nonidet P-40, 2 mM EDTA, and 50 mM Tris (pH 7.5) for 1 h. After centrifugation, the supernatants were treated with 5 µg/ml of ribonuclease A at 37 °C for 1 h, and then proteinase K was added at 2.5 µg/ml for 2 h at 37 °C. DNA was precipitated by 75% ethanol and 3 M sodium acetate at -80 °C for 2 h, and pellet was resuspended in TE buffer. Each sample was electrophoresed on a 1.5% agarose gel and visualized by ethidium bromide staining.[19]

RESULTS

The compatibility between the drug and the selected polymers were evaluated using FT-IR peak matching method. The IR Spectra of pure drug, polymers and the physical mixtures are shown in fig. 1,2 and 3 respectively. There was no appearance or disappearance of peaks in polymer and mixture, which confirmed the absence of chemical interaction between drug and polymers.

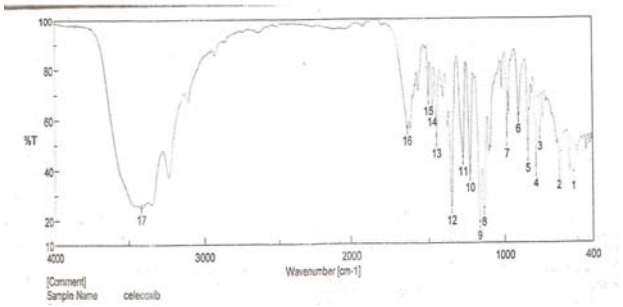


Fig. 1: FTIR of celecoxib

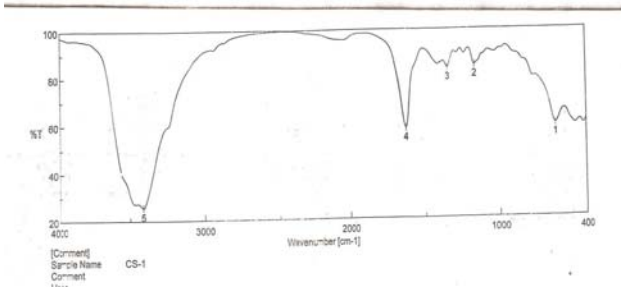


Fig. 2: FTIR of celecoxib, sodium alginate and pectin

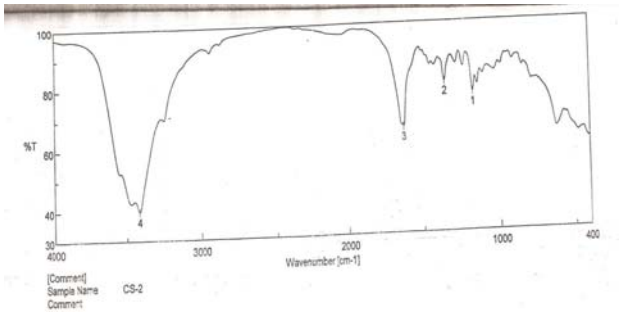


Fig. 3: FTIR of celecoxib, cyclodextrin and sodium alginate

The DSC thermograms of pure drug Celecoxib, sodium alginate, and formulated micro beads ratio 1:1 showed on Fig. 4, 5 and 6 celecoxib exhibited a sharp endothermic peak at 158°C. The peak of the drug did not appear in the thermogram of any type of the prepared micro bead containing drug .This may indicate that the maximum drug was uniformly dispersed at the molecular level in the beads.

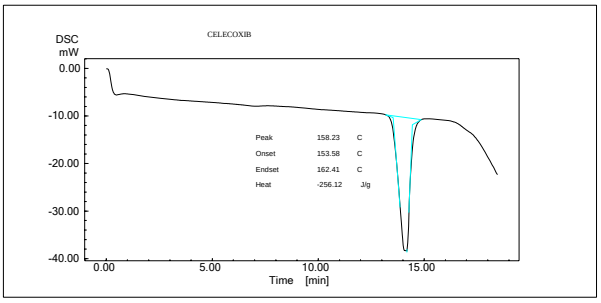


Fig. 4: Differntial scanning colorimetric spectra of celecoxib

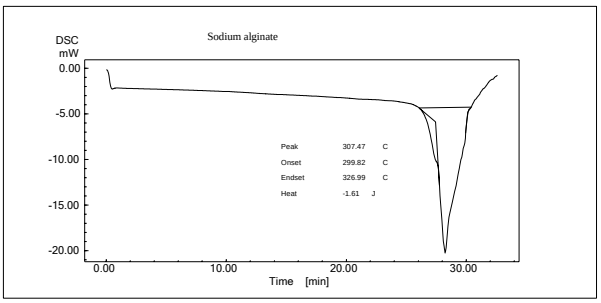


Fig. 5: Differential scanning colorimetic spectra of sodium alginate polymer

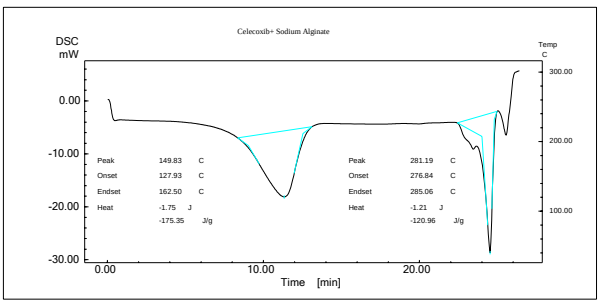
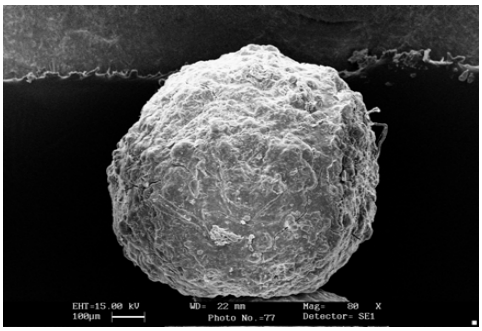
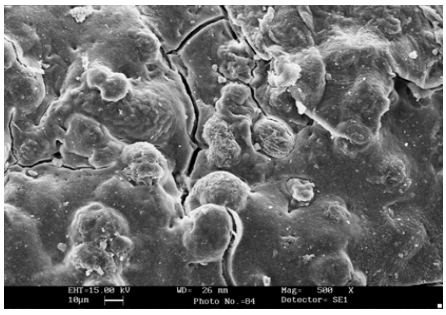


Fig. 6: differential scanning colorimetric spectra of celecoxib micro beads

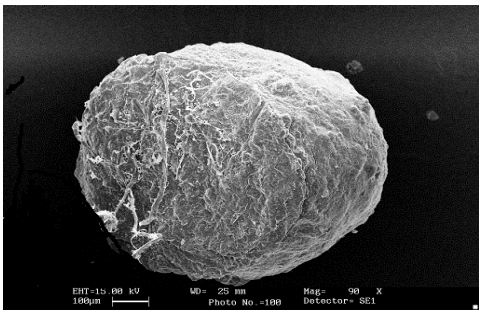
The SEM micro graph the shape of micro beads shown in Fig.7 was found to pearl, which was clearly evident and the surface texture of the micro beads was found to be smooth from the SEM analysis.



(a)



(b)



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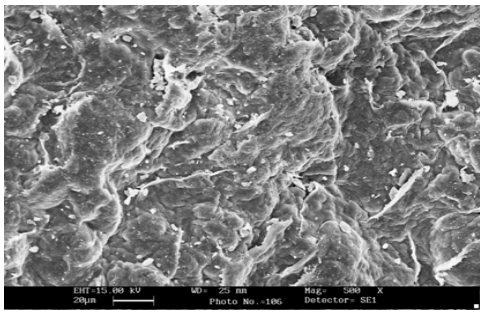
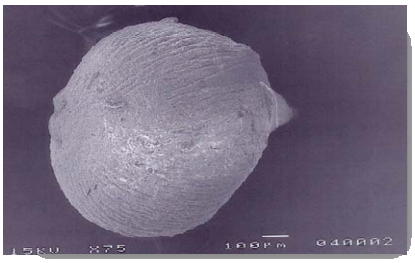


(d)



(e)

Fig. 7: Sem images of formulations. (a): f1 at 80 x magnification (b): f1 at 500 x magnification, (c): f4 at 90 x magnification (d): f4 at 500 x magnification. (e) f 5 at 75 x magnification.

The numerical values of compressibility index in the range of 11.17% to 14.99%, Hausner's ratio less than 1.2 and dynamic angle of repose (20° to 25°) suggested excellent flow properties of all microbeads formulations unlike cohesive pure drug (Table 2). The average particle size of pure drug was 16.61 µm and that of microbeads were ranged between 629.80 to 688.52 µm.

Formulation F5 (calcium alginate-pectinate) exhibited highest drug loading and % entrapment efficiency values of 39.99 ± 0.55 % and 91.99 ± 1.45% respectively, whereas F1 microbeads (calcium alginate) showed the least value of drug loading and % entrapment efficiency as 30.94 ± 0.21 % and 63.74 ± 0.88% respectively (Table 3).

Table 2: Comparative micromeritic studies of pure drug and formulations

Parameters	Drug	F1	F2	F3	F4	F5	F6
Poured density (g/cm3)	0.33± 0.008	0.61± 0.004	0.64± 0.01	0.72± 0.009	0.75± 0.011	0.77± 0.008	0.78± 0.012
Tapped density (g/cm3)	0.58± 0.007	0.69± 0.005	0.75± 0.009	0.83± 0.011	0.85± 0.008	0.89± 0.009	0.94± 0.013
Compressibility index (%)	39.62± 0.12	11.17± 0.15	12.80± 0.31	12.76± 0.44	13.98± 0.22	14.24± 0.41	14.99± 0.37
Hausner's ratio	1.67± 0.012	1.11± 0.015	1.14± 0.031	1.15± 0.040	1.15± 0.021	1.18± 0.027	1.19± 0.031
Dynamic angle of repose	71°± 3.4	24°± 1.11	25°± 1.14	23°± 0.99	24°± 1.45	22°± 0.98	22°± 1.17
Average particle size (µm)	16.61 ±0.67	629.80 ±5.98	667.65 ±4.51	674.80 ±6.00	669.77 ±5.92	674.37 ±4.99	688.52 ±6.21

Table 3: Drug content and percentage entrapment efficiency of microbeads

Formulation	Drug content (%)*	Entrapment efficiency (%)
F1	30.94 ± 0.21	63.74 ± 0.88
F2	32.45 ± 0.46	65.66 ± 1.22
F3	34.88 ± 0.68	78.61 ± 1.34
F4	36.76 ± 0.62	84.33 ± 1.67
F5	39.99 ± 0.55	91.99 ± 1.45
F6	38.93 ± 0.47	90.11 ± 1.78

* n = 3

The dissolution studies showed that the release of drug from calcium alginate microbeads (F1) was found to be 10.26±0.077% in pH 1.2 within 2 h. After 2 h, the calcium alginate microbeads (F1) disintegrated and lost remaining drug within 3 h in the dissolution medium SIF (pH 6.8). With the addition of pectin with sodium alginate i.e. calcium alginate-pectinate microbeads (F2, F3 and F4), the release of entrapped drug during first 2 h in SGF was significantly reduced. Three different pectin concentrations with sodium alginate were used in order to study the effect of pectin concentration on drug release from microbeads. The F2, F3, and F4 released 4.21±0.053, 3.15±0.038 and 2.07±0.03 % of celecoxib respectively at pH 1.2 within 2 h but after 2 h, only F4 followed the drug release pattern extending up to 7 h (Fig. 8). This suggested that at least 1.0 % w/v conc. is required to delay the release up to 7 h. Selected formulation F4 was coated with enteric polymer Eudragit (Table 4, 5 and 6).

Table 4: *In vitro* % cumulative release of celecoxib-alginate micro beads in ph 1.2

Time (min)	Medium (pH)	Pure sample	Drug carrier ratio % Cumulative release					
			F1	F2	F3	F4	F5	F6
15	1.2	9± 0.34	1.98± 0.03	2.02± 0.045	1.45± 0.036	1.18± 0.052	-	-
30	1.2	9.73± 0.97	2.02± 0.041	2.27± 0.037	1.57± 0.043	1.25± 0.028	-	-
45	1.2	10.55± 0.56	4.25± 0.046	2.39± 0.38	1.65± 0.031	1.39± 0.028	-	-
60	1.2	10.92± 0.72	7.11± 0.054	3.12± 0.046	1.98± 0.041	1.56± 0.039	-	-
90	1.2	11.29± 1.01	8.26± 0.038	3.48± 0.041	2.65± 0.035	1.79± 0.029	-	-
120	1.2	12.38± 0.85	10.26± 0.077	4.21± 0.053	3.15± 0.038	2.07± 0.033	-	-

Table 5: *In vitro* % cumulative release of celecoxib-alginate micro beads in ph 6.8

Time (min)	Medium (pH)	Pure sample	Drug carrier ratio % Cumulative release					
			F1	F2	F3	F4	F5	F6
150	6.8	12.41± 0.56	22.12± 0.092	11.47± 0.062	9.67± 0.045	8.61± 0.051	8.41± 0.035	5.85± 0.42
180	6.8	14.94± 0.71	43.95± 0.56	24.25± 0.92	19.56± 0.21	14.62± 0.069	8.57± 0.075	6.23± 0.08
210	6.8	15.67± 1.21	53.2± 1.02	25.81± 0.74	24.71± 0.48	21.11± 0.18	10.03± 0.093	6.35± 0.11
240	6.8	17.41± 1.11	64.29± 1.52	49.55± 1.16	33.41± 0.92	32.74± 0.67	14.23± 0.09	6.59± 0.32
270	6.8	19.96± 0.91	79.74± 1.73	54.37± 1.08	42.12± 0.83	39.92± 0.88	15.91± 0.76	6.78± 0.09
300	6.8	21.7± 1.73	97.83± 2.03	66.46± 1.74	52.89± 1.21	47.34± 0.79	16.59± 0.43	7.14± 0.32

Table 6: *In vitro* % cumulative release of celecoxib-alginate micro beads in ph 7.4

Time (min)	Medium (pH)	Pure sample	Drug carrier ratio % Cumulative release					
			F1	F2	F3	F4	F5	F6
330	7.4	22.7± 0.93	-	88.98± 2.17	69.45± 1.54	60.11± 1.21	38.26± 2.01	35.45± 1.03
360	7.4	26.63± 0.81	-	97.34± 1.84	89.91± 1.32	75.11± 2.12	45.01± 1.44	39.73± 0.84
390	7.4	28.34± 1.32	-	-	99.33± 2.38	87.88± 1.943	59.86± 1.24	42.28± 0.823
420	7.4	32.45± 1.14	-	-	-	98.87± 2.43	67.88± 0.932	44.03± 1.13
450	7.4	38.58± 2.01	-	-	-	-	85.68± 1.67	57.88± 1.52
480	7.4	45.78± 2.45	-	-	-	-	92.11± 2.32	76.11± 1.55

Celline cytotoxicity study

All the three samples, viz pure sample (Celecoxib), Uncoated Celecoxib microbeads (F4) and Coated Celecoxib microbeads (F5) showed cytotoxicity against HT-29 cells (Table 7 and Fig. 9). Hence the formulations can be effectively tested for its anticancer activity. Pure sample Celecoxib showed drug release from the first hour itself at pH 1.5, followed at each hour. Maximum percentage cell viability it reduced up to 36 percentage. Meaning that maximum of 64 percentage cell death occurred in HT-29 cells.

Uncoated Celecoxib micro beads showed very significant results compared with the pure drug. The uncoated microbeads showed maximum release at 5th hour in pH 6.8. Maximum percentage cell viability is reduced up to 25 percentages at fifth hour meaning that 75 percentage cell death occurred in HT-29 cells after treatment with uncoated Celecoxib- Alginate

microbeads. Drug release pattern was little slow compared to the pure drug. Most of the drug release is observed at pH 6.8, after that the release reduced at pH 7.4, but still showed good cytotoxicity.

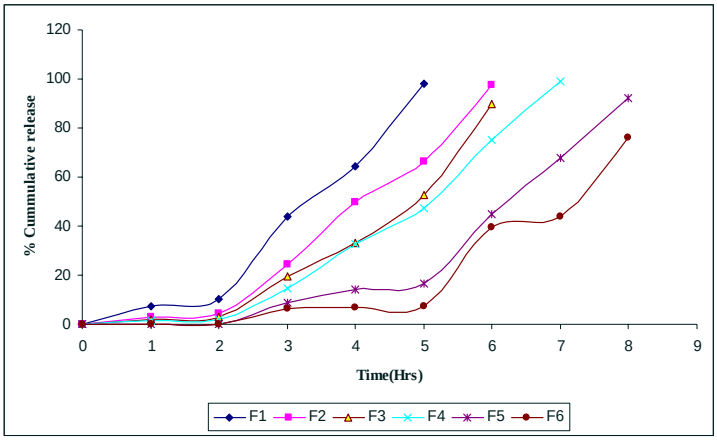


Fig. 8: *In vitro* % cumulative release of celecoxib-alginate micro beads in ph 1.2, ph 6.8 and ph 7.4

Eudragit FS 30D Coated Celecoxib micro beads showed the best results compared the other two samples. Coated Celecoxib didn't show much drug release for the first 3 hours. But at pH 6.8 it started better release and followed by pH 7.4, coated celecoxib micro beads showed the best release and maximum cytotoxicity. It reduced cell viability up to 17 percentages, meaning that 83 percentage cell deaths occurred at HT-cells after treatment with coated Celecoxib micro beads.

Table 7: Cytotoxicity against cell line ht-29 using mtt assay

Time hr.	Pure sample		Uncoated Celecoxib Micro beads(1:1)		Coated Celecoxib Micro beads(1:1)	
	Concentration (µg)	% cell Viability	Concentration (µg)	% cell Viability	Concentration (µg)	% cell Viability
	0	100 %	0	100 %	0	100 %
1	110	72 %	40	78 %	3	100 %
2	140	70 %	46	72 %	3	98 %
3	160	66 %	280	44 %	6	96 %
4	224	60 %	340	30 %	84	70 %
5	254	58 %	400	25 %	120	54 %
6	269	52 %	270	34 %	380	40 %
7	290	44 %	100	38 %	440	28 %
8	300	36 %	80	40 %	490	17 %

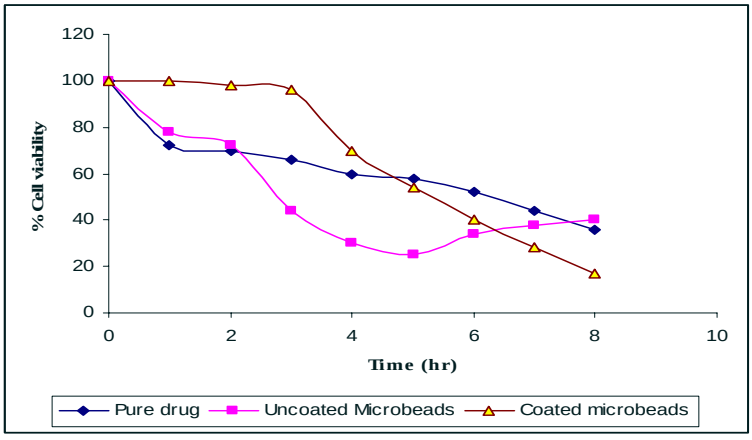


Fig. 9: Cytotoxicity against cell line ht-29 using mtt assay profile

Table 8: Correlation coefficient (r^2) values for the fit of different kinetic models

Formulations	Zero order plots	Higuchi's plots	Peppa's plots	
	r^2	r^2	r^2	n
F1	0.9261	0.9727	0.7886	0.8774
F2	0.8937	0.9369	0.7026	0.8602
F3	0.8871	0.9302	0.6748	0.8945
F4	0.9043	0.9131	0.6484	0.9284
F5	0.8377	0.8849	0.6019	0.9059
F6	0.7884	0.8510	0.5608	0.8039

DNA Fragmentation study

DNA was isolated from HT-29 cells with and without different formulations of Celecoxib micro beads was shown in Fig 10. The results indicate, there is clear DNA damage to the HT-29 cells after treatment with Celecoxib and its formulated micro beads. This confirms the better anti cancer activity of Celecoxib micro beads against human colorectal adenocarcinoma cell line HT-29.

Samples from left

Well number 01& 06: Normal DNA of HT-29.

Well number 02 :Uncoated Celecoxib (5 hour sample 400 µg/mL) treated.

Well number 03 & 04: Pure Celecoxib (8 hour sample, 300 µg / mL) treated.

Well number 05 :Coated Celecoxib (8 hour sample 490 µg/ mL) treated.

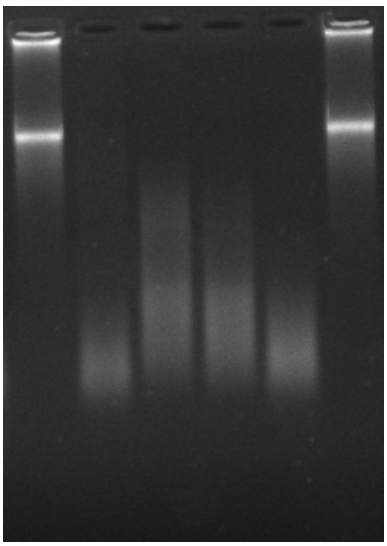


Fig. 10: DNA fragmentation assay on HT-29 cells

DISCUSSION

The gelled microbeads were formed by ionic interaction between the negatively charged carboxyl groups of sodium alginate and the positively charged counter ion such as Ca++. The addition of the divalent ions such as Ca++ produced a partial neutralization of carboxylate groups present on the alginate chain, forming insoluble gelatineous microbeads. Pectin with low degree of esterification (35%) along with sodium alginate formed gel microbeads by ionic gelation with divalent calcium ions. Gelation occurred due to intermolecular cross linking between the divalent calcium ions and the negatively charged carboxyl groups of pectin and sodium alginate molecules. Pectin was used in conc. of 0.3, 0.5 and 1.0% W/V, higher conc. of pectin resulted in much higher viscosity of solution which was difficult to process for preparing the microbeads. The SEM images showed surface of calcium alginate micro beads (F1) with cracks, while the calcium alginate-pectinate microbeads (F4), though exhibited rough surface, were devoid of cracks. Absence of cracks may be due to pectin as additional component present in the microbeads. Moreover, the dispersion of celecoxib as fine crystalline particles on the surface of microbeads was also observed. The celecoxib microbeads were evaluated for flow properties using percentage compressibility index, Hausner's ratio as well as dynamic angle of repose suggesting excellent flow properties of all microbeads formulations unlike cohesive pure drug. This might be explained as spherical microbeads exhibited low interparticle friction resulting in good flow property. Microbeads were assayed for drug content and percentage entrapment efficiency. The F5 (calcium alginate-pectinate) exhibited highest drug loading and % entrapment efficiency values whereas F1 microbeads (calcium alginate) showed the least value of drug loading and % entrapment efficiency which might be attributed to the possible leakage of water soluble drug from calcium alginate microbeads having large gel porosity (Philip, 2004). The % entrapment efficiency was also proportional to concentration of pectin used. An increase in pectin concentration from 0.3 % w/v to 1.0 % w/v led to an increase in % entrapment efficiency, this effect might have occurred due to the increased viscosity of the preparative mixtures which hindered drug migration towards the external phase during microbeads preparation.

The dissolution studies showed that release of drug from calcium alginate microbeads (F1) was found to be 12.38±0.85 % in pH 1.2 within 2 h. After 2 h, the calcium alginate microbeads (F1) disintegrated and lost remaining drug within 3 h in the dissolution medium SIF (pH 7.4). According to literature, at pH 7.4, the water of environment penetrates in to the chains of alginate to form hydrogen bridges through their available –OH and COO- groups (Rodriguez *et al.*, 2002). As a consequence, the microbeads turn in to a

hydrogel and have their diameter increased, favoring the drug diffusion. This suggested that alginate microbeads had probably insufficient cross-linking density to prevent drug molecules to diffuse out. With the addition of pectin with sodium alginate i.e. calcium alginate-pectinate microbeads (F2, F3 and F4), the release of entrapped drug during first 2 h in SGF was significantly reduced. Further, the amount of drug release decreased with increase in pectin concentration. This was expected, since on increasing pectin amount with sodium alginate, interaction between two polymers had increased, forming a closer network, which decreased the diffusion of the drug outwards from the interiors of the microbeads. Therefore, in order to delay the drug release in pH 1.2, selected formulation F4 was coated with enteric polymer eudragit. Various release kinetic models[20] were applied to determine the mechanism of drug release from microbeads and observed that the highest correlation coefficient (r^2) found for Higuchi square root of time profile indicated that the drug release from the microbeads formulations occurred via diffusion mechanism suggesting uniform dispersion of water soluble drug in swellable polymer matrix. Drug release from coated microbeads were retarded till 8 h as compared to uncoated microbeads. This was obvious because of acid insolubility of enteric eudragit. The release data appeared to fit the zero order model better for the coated microbeads as compared to Higuchi matrix model for the uncoated microbeads. This suggested that water soluble drug from polymer matrices is released in a way which is proportional to the amount of drug remaining in its interior, in such a way that the amount of drug released by unit of time diminishes. The exponent (n), indicative of the mechanism of release from pharmaceutical formulation, was calculated from the well-known Peppas equation. The values of n were obtained by linear regression analysis. n values for the formulations F1, F2 and F6 were in between $0.45<n<0.89$ indicating anomalous (non-Fickian) diffusion. While formulations F3, F4 and F5 showed $n> 0.89$ indicating Super Case II transport.

CONCLUSION

In conclusion, the use of pectin with sodium alginate permits the retardant drug release behavior in gastric conditions and higher drug release at intestinal pH conditions which are of great interest for the delivery of water soluble drugs in to the intestine. The release of drug from microbeads in SGF could also be retarded by coating with polymer such as eudragit. Based on these findings, it has been concluded that the eudragit -coated microbeads are suitable for targeted release of drug in colon when administered orally. Further more, since these microbeads possessed excellent flow properties, they show sufficient promise for the formulation development into suitable oral solid dosage forms such as tablets or capsules.

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