RESEARCH ARTICLE

FORMULATION DEVELOPMENT OF CONTROLLED RELEASE MUCOADHESIVE BEADS OF CAPECITABINE

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ABSTRACT

The aim of the present investigation was to formulate and evaluate controlled release beads containing capecitabine in order to decrease the dosing frequency. The beads were prepared using sodium alginate and chitosan by ionotropic gelation method. The concentrations of the polymers were optimized to obtain the spherical beads with sufficient integrity. The drug loaded beads were characterized for entrapment efficiency, size, shape, morphology, swelling index, mucoadhesion and in-vitro drug release and in-vitro cytotoxicity study. The prepared beads had spherical shape with smooth surface and improved micromeritic properties. The entrapment efficiency was found to be 58%, swelling index was 60% and they exhibited mucoadhesion to the intestinal tissue for more than 6 hours. The in-vitro drug release studies indicated that the beads were able to give zero order controlled release for a period of 6 hours.

Key Words: Capecitabine, Chitosan, Sodium alginate, Ionotropic gelation

INTRODUCTION

Cancer, a most dreadful disease and a major cause of deaths worldwide occurs due to malignant growth of body cells that start growing beyond their usual boundaries because of some biochemical or physical damage to the normal cells. As per the data of World Health Organization, cancer is the second leading cause of death after Heart diseases. Out of the various types of cancers, Breast cancer is widely prevalent amongst the elderly females and is the third leading cause of cancer deaths in the United States. In 2014, more than 2 lakh breast cancer cases were reported which accounted for 14% of all new cancer cases while around 40,000 deaths accounting for 6.8% of all cancer deaths. Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both. Various chemotherapeutic agents such as Docetaxel, Paclitaxel, Cisplatin, Carboplatin, Vinorelbine, Capecitabine, Gemcitabine etc are used alone or in combination for treatment of breast cancer. Most of these are administered as intravenous injections needing hospitalization leading to poor patient compliance, discomfort and possibility of infections. The advent of orally effective chemotherapeutic agents such as Capacitabine has led to improved patient compliance because of possibility of self administration and no requirement of hospitalization. This leads to significant cost savings, both in terms of treatment costs and lost wages incurred by patient and family during physician visits[1]. Capecitabine, an orally-administered chemotherapeutic agent is a prodrug, that is enzymatically converted to active 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows down growth of tumor tissue[2] Capecitabine is completely absorbed from the gastrointestinal tract having almost 100% bioavailability, but it's very short plasma half life (0.85 hours) leads to its rapid elimination from the body necessitating its frequent administration. In order to achieve the Cmax of 3-5 mg/L, it needs to be administered at a very high dose of 1250 mg/m^{2}. Commercially, it is available as a film coated tablet dosage form at two different strengths viz.150 mg and 500mg. The dosage frequency is 3-6 tablets twice a day depending on the body surface area. Such a high frequency and dose leads to serious side effects like hand and foot syndrome, vomiting, pain in stomach, fever or infection leading to poor patient compliance. Capacitabine is absorbed throughout the gastrointestinal tract. However, it gets degraded in the acidic environment of the stomach necessitating the avoidance of its exposure to the gastric environment. Design of a formulation which could protect capecitabine from degradation in stomach and release the drug in controlled manner in the intestine would thus lead to reduction in the dose and dosing frequency resulting in decreased side effects and dose related toxicity.

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Different strategies such as incorporation of matrix forming polymers[3], coating[4], microsphere[5] and microcapsule[6] based formulations have been used for controlling the release rate of the entrapped drug. Controlled release microspheres with use of polymers have been reported for capecitabine[7]. Manufacturing of microspheres is a complicated process requiring high energy stirrers. Beads, on the other hand, are much simpler to manufacture. Natural polymers such as sodium alginate and chitosan are highly effective in controlling the drug release. The use of bioadhesive polymers results in a prolonged intestinal retention and the swelling causes a formation of a matrix and a diffusional path for the drug to be released resulting in controlled release.

Beads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form. Numerous anticancer drugs have been incorporated into beads like doxorubicin[8], irinotecan[9], 5-flourouracil[10], paclitaxel[11].

Thus, the aim of the present investigation was to prepare controlled release mucoadhesive beads for capecitabine in order to protect it from stomach environment and release the drug in controlled manner in the intestine so that the frequency of administration is decreased resulting in increased patient compliance and reduced side effects.

MATERIALS AND METHODS

Capecitabine was kindly gifted by Dishman Pvt ltd. (Ahmedabad, India). Sodium alginate and calcium chloride were purchased from SD Fine chemicals (India). Pepsin (1:1000), pancreatin and pectinase enzymes were procured from Himedia. Chitosan was kindly gifted by Mahtani Chitosan Pvt ltd (India). All other materials and solvents were of analytical reagent grade.

PREPARATION OF BEADS

The concentrations of sodium alginate and calcium chloride were optimized by preliminary experimentation in which blank beads were prepared using varying concentrations. The concentrations which yielded spherical beads having sufficient integrity were selected for preparing drug loaded beads. The composition of the various batches along with the observation is shown in Table 1. Drug loaded chitosan-alginate beads were prepared by ionotropic gelation method[12]. The following solutions were made separately: Solution A-Accurately weighed quantities of sodium alginate (3%w/v-450 mg) and

capecitabine were dissolved in 15 ml de-ionized water by continuous stirring for 30 minutes using a magnetic stirrer. Solution B: Accurately weighed quantity of calcium chloride (5%w/v) was dissolved in 35 ml de-ionized water. To this solution, 10ml of solution containing chitosan (dissolved in 2% glacial acetic acid) with pH adjusted to 4.5±1 was added. Solution A was then extruded dropwise into solution B through hypodermic syringe with needle (20G) and stirred for 15 minutes at 50rpm using magnetic stirrer. This led to ionotropic gelation of sodium alginate in the form of beads. Stirring was continued for 30 minutes for completion of the gelation. The beads were then filtered using a whatman filter paper and air dried overnight. Various batches where prepared by increasing concentrations of drug and chitosan as described in table 2. The optimized batch was selected and crosslinked by glutaraldehyde vapors. The beads were spread uniformly in a petridish and kept in a desiccators containing glutaraldehyde at the bottom. The beads were exposed to the glutaraldehyde vapours for 24 hours.

Table 1: Optimization of sodium alginate and calcium chloride concentrations

Batch code	Calcium chloride (%w/v)	Sodium alginate (%w/v)	Observation
T1	1%	1%	Beads not formed
T2		3%	Fragile beads
T3		5%	Difficult to inject
T4	2%	1%	Fragile beads
T5		3%	Fragile beads
T6		4%	No spherical shape
T7	3%	1%	Fragile beads
T8		3%	Fragile beads
T9		4%	No spherical shape
T10	4%	1%	No spherical shape
T11		3%	No spherical shape
T12	5%	1%	Nearly spherical shape
T13		2%	Nearly spherical shape
T14		3%	Spherical shape
T15		4%	Spherical shape

Table 2: Optimization of capecitabine and Chitosan concentration

Batch	Amount of Drug (mg)	Chitosan(%)	Yield (%)	Drug loading (%w/w)	%Entrapment Efficiency*
C1	500	1%	31.38	65	38.97±1.27
C2	700	1%	43.24	64.35	45.81±0.66
C3	700	2%	45.92	77.26	58.34±1.49
C4	800	2%	47.88	69.53	52.02±1.37

Values are expressed as mean±S.D(n=3)

EVALUATION OF BEADS

%Yield

The prepared beads were weighed using a calibrated weighing balance and the % yield was calculated using the following equation:

$$\% \text{ yield} = W/T, \text{ where}$$

W= weight of the beads

T= Total weight of sodium alginate and capecitabine taken

Drug loading and entrapment efficiency

Accurately weighed beads (10 mg) were transferred to a beaker containing 10 ml Phosphate buffer pH 6.8 and the mixture was allowed to stand for 24 hours. The contents of the beaker were stirred for 1-2 hours using a magnetic stirrer for complete breakage of the beads, followed by filtration using whatman filter paper. The amount of capecitabine(x) in 10 mg beads was then estimated in the filtrate by measuring the absorbance at 239.5 nm using U.V.-visible spectrophotometer. Drug loading and Entrapment efficiency was then calculated by following formulae:

Drug loading (%w/w) = $10x$

% Entrapment efficiency=$(x /T)*100$

Where, x = Actual quantity of drug present in beads

T = Theoretical quantity of drug added during preparation

Micromeritic properties

The size of beads was determined using optical microscopy technique. The beads were spread uniformly on a glass slide and observed under microscope. The size of 50 beads from each batch was determined using a calibrated eye piece micrometer and average was calculated. Angle of repose was measured by fixed base cone method. Bulk and tapped densities as measures of pack-ability of beads were measured using a 5 ml graduated cylinder. 1 gm of sample was added in the cylinder and volume occupied by sample was noted and bulk density was calculated. The cylinder was tapped 100 times and volume occupied was again measured to calculate tapped density.

Bulk density = (weight of sample / volume occupied by sample before tapping)

Tapped density= (weight of sample/ sample occupied by sample after tapping)

Carr's index and Hausner's ratio were also calculated as measures of powder flow properties using the following equations:

Carr's index= (Tapped density – Bulk density) x 100 / Tapped density

Hausner's ratio= (Tapped density / Bulk density)

SIZE, SHAPE AND SURFACE MORPHOLOGY

The beads were analyzed microscopically under digital microscope (Nikon digital sight, DS-Fi20) to study their size and shape. The size of the beads was determined by using a calibrated eye piece micrometer. The surface morphology of beads was studied using scanning electron microscopy (JSM-5610LV, JEOL, Japan). For this, samples were attached to sample stubs, silver coating was done and then viewed using an accelerating voltage at the magnification of 15000X.

SWELLING INDEX

Accurately weighed 50mg of beads were taken in a beaker containing 10 ml of phosphate buffer pH 6.8 and allowed to stand at room temperature for 6 hr. The excess liquid adhered to the surface of the beads was removed by blotting with filter paper and the swollen beads were weighed. Each experiment was carried out in triplicate. The swelling index of the beads was calculated by using the formula[13]:

$$\text{Swelling Index} = \frac{Wt - W1}{W} \times 100$$

Where, Wi= Weight of the beads after swelling

Wt= Initial weight of dried beads

MUCOADHESIVITY TEST[14]

The mucoadhesivity test was carried out using rat intestinal mucosa. All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of The Maharaja Sayajirao University of Baroda and were in accordance with the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. One rat was sacrificed and the mucosa was removed and cut into pieces (2 x 2 cm) and rinsed with phosphate buffer pH 6.8. Pieces of wet rat intestinal mucosa were mounted onto glass slides with acrylate glue. 50 beads were counted and spread uniformly over the surface of wet mucosa. A glass slide was connected with a support and was hung on the arm of a USP tablet disintegration test

apparatus (Figure 1). The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the beaker containing alkaline phosphate buffer pH 6.8. The temperature was maintained at 37±5°C throughout the study. The numbers of beads still adhering to the tissue were counted at the end of 6 hours . % Mucoadhesion was calculated by following formula:

$$\% \text{ Mucoadhesion} = \frac{\text{Number of adhered beads}}{\text{Total number of applied beads}} \times 100$$
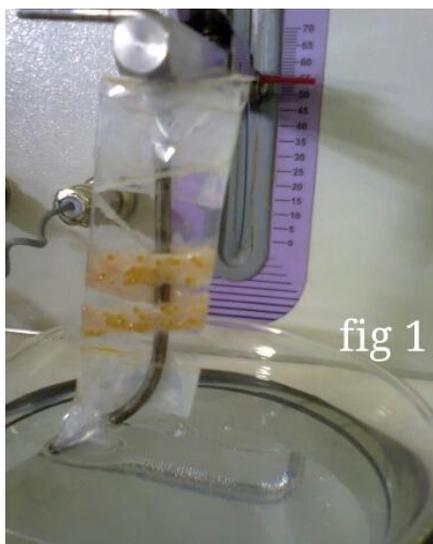

Figure 1: Experimental setup for mucoadhesivity test

IN-VITRO DISSOLUTION STUDIES

The dissolution studies were carried out using USP XII dissolution apparatus type I(basket type) at 100 rpm and 37±0.5°C. The beads equivalent to 200 mg of drug were filled in to enteric coated hard gelatin capsules and placed in basket separately. The dissolution medium (500 ml) was simulated gastric fluid (SGF pH 1.2) for the first 2 h, followed by simulated intestinal fluid (SIF pH 6.8) for the next 5 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 239.5 nm UV-Spectrophotometer (Shimadzu 1700). All the tests were carried out in triplicate. The dissolution study of marketed formulation Xeloda-500mg tablet was carried out using USP dissolution apparatus II (paddle) at 100 rpm and 37±0.5°C.

DRUG-EXCIPIENT COMPATIBILITY STUDIES

The compatibility of Capecitabine with the excipients was confirmed by DSC and FTIR studies. The DSC thermograms of Capecitabine, Chitosan, Sodium alginate, Calcium chloride and the optimized Beads formulation were recorded using Differential Scanning Calorimeter DSC-70, Shimadzu; between 30-300°C at a heating rate of 10.00 °C/min with constant nitrogen flow of 50.0 mL/min. The FTIR spectra of moisture free powdered samples of drug, excipients, drug containing beads and blank beads(without drug) were recorded

using Bruker-αt, FTIR spectrophotometer, Japan; The samples were mixed with potassium bromide and compressed into a pellet before recording the spectra.

IN-VITRO CYTOTOXICITY STUDIES [15]

The anticancer potential of the Capecitabine beads was evaluated and compared with its marketed tablet (Xeloda®, Roche Inc., Philippines) using the MTT based cytotoxic assay on the HT29 colorectal cancer cells. The MTT assay involves colorimetric estimation of purple colored formazan developed via reduction of yellow colored MTT dye by mitochondrial dehydrogenase within living cells and thereby, gives a direct estimate of number of viable cells. Briefly, 5×10^3 cells/ml in their exponential growth phase were plated in 96-well flat-bottom tissue culture plates and incubated at 37°C, 5% CO_2 in an incubator for 24 hr to allow the cells to adhere and grow as a monolayer. Concurrently, optimized formulation of Capecitabine beads and marketed Xeloda® tablet (amount equivalent to 400 mg drug) were incubated in simulated body fluids in the same way as adapted for in vitro drug release study and 5ml samples were withdrawn at 1 hr interval starting from zero time for the estimation of Capecitabine concentration and its cytotoxicity. In the well plates, when a monolayer was formed the supernatant was removed, the monolayer washed once with phosphate buffer saline at pH 7.4 and 100μL of above mentioned samples were added to cells in well plate. The plate was then incubated at 37°C and 5 % CO_2 for 4 hr. At the end of the exposure period, the medium was removed from all the wells, and the wells were washed with phosphate buffer saline at pH 7.4. Then 50 μL of MTT dye solution (1 mg/mL) was added to all of wells and incubated for 4 hr. After 4 hr, the plate was taken out and the medium along with the MTT was removed from the wells without disturbing the formazan crystals. Cell lysis and solubilization of formazan crystals was done by adding 200 μL of DMSO to all of the wells and their absorbance were measured using a microplate reader at a wavelength of 540 nm.

STABILITY STUDY

The optimized batch was filled into hard gelatin enteric coated capsule and kept in the stability chamber under conditions of 25 ±2°C, 60% RH ±5% and at accelerated conditions of 40 ±2°C, 75% RH ±5%. At intervals of 0, 1, 2 and 3 months, formulations were characterized for % drug content and in-vitro drug dissolution using the procedure mentioned earlier.

RESULT AND DISCUSSION

The present investigation aimed to prepare mucoadhesive beads for controlled release of Capecitabine. The selection of the polymers was done based on the preliminary experimentation and literature survey. The main characteristics required were the bioadhesive nature and ability of control the release rate along with biodegradability, non-toxicity and economic viability. In recent years, the use of natural polymers as carriers has been increased as they are biocompatible, biodegradable and easily available at reasonable cost. Ionotropic gelation is a simple technique used for

preparing sodium alginate beads, which have the property of swelling in aqueous media and controlling the release rate of entrapped drug[16].When an aqueous solution of sodium alginate is dropped into counter-ions solution (calcium), the metallic calcium ions rapidly diffuse into alginate solution droplets because of their smaller size than polymeric ions, and bind to unoccupied binding sites on these polymers to form calcium alginate beads by ion-exchange. The results of the experiments for optimization of the sodium alginate and calcium chloride concentration are shown in table 1. It is evident that at lower concentration of Calcium chloride, the beads are not formed. This might be because of the insufficient gelation due to availability of lower amounts of counter ions. The optimum calcium chloride concentration was found to be 5%. In case of sodium alginate concentration, lower concentrations resulted in fragile and irregular shaped beads whereas too high concentration of 5% was difficult to inject because of high viscosity. Thus, 3% sodium alginate concentration was considered to be optimum which resulted in spherical shaped beads with sufficient integrity. When capecitabine loaded beads were prepared using 3% sodium alginate and 5% calcium chloride, very low entrapment efficiency was obtained. This was because of the tendency of capecitabine, a highly water soluble drug to leach out in the aqueous calcium chloride solution. This observation was confirmed by estimating the drug in the external phase. Additional use of chitosan in chitosan-alginate beads has already been recognized as stabilizing the "egg-box" structure and thereby reducing the problem of drug leaching during bead preparation[17]. Thus, varying concentrations of chitosan were added to the calcium chloride solution in order to increase the entrapment efficiency. It is evident from the table 2 that on increasing the chitosan concentration, the drug loading and %EE increased significantly. This may be due to the fact that higher chitosan concentration results in the formation of a denser matrix structure that probably decreases the loss of drug to the curing medium. In addition, electrostatic attraction between the negatively charged capecitabine and the positively charged chitosan also becomes stronger, promoting the drug entrapment. Thus, the optimized chitosan concentration and the amount of capecitabine to be added during the preparation were 2% and 700 mg respectively. Further increase in capecitabine amount to 800 mg led to a decrease in entrapment efficiency maybe because of availability of higher amount of drug to be leached out in the external aqueous medium. As the capecitabine loading concentration increased, more capecitabine molecules were just electrostatically adsorbed onto the surface of chitosan and were easily separated from beads resulting in a decrease in the entrapment efficiency. The results of the micromeritic studies are shown in table 3.

Table 3: Micromeritic properties of pure drug and beads

Batch	Angle of repose (θ)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Hausner's ratio	Carr's index (%)	Average particle size (µm)
Pure drug	49.68 ±0.02	0.389 ±0.006	0.591 ±0.005	1.52 ±0.03	34.08 ±1.14	-
C1	22.85 ±0.03	0.611 ±0.002	0.723 ±0.003	1.18 ±0.01	15.57 ±0.50	1121.35
C2	23.25 ±0.03	0.638 ±0.003	0.752 ±0.006	1.18 ±0.02	15.07 ±1.12	1126.27
C3	27.57 ±0.05	0.656 ±0.004	0.785 ±0.003	1.20 ±0.01	16.47 ±0.47	1388.36
C4	27.97 ±0.04	0.675 ±0.004	0.835 ±0.004	1.24 ±0.01	19.17 ±0.84	1381.65

The values of the different micromeritic parameters indicate the poor flow properties of pure drug while all the bead formulations showed acceptable flow properties and compression characteristics. The improved flow properties might be due to the spherical shape and smooth surface of the beads which show minimum resistance to flow. An increase in chitosan concentration led to an increase in particle size. This result is expected since capecitabine carried negative charge and electro statically interacted with chitosan, which would promote formation of beads through ionic cross-linking. Thus, as the chitosan concentration was increased; the particle size was also increased. The optimized batch had a mean size of around 1.3 mm. The SEM images are shown in figures 2.

These images confirmed that the formulated beads were spherical in shape with a relatively smooth surface texture. Moreover, in the SEM images, no drug crystals were found on bead surface, which indicate that the drug particles were present in the form of a finely dispersed state in the polymeric matrix of beads. Moreover the size obtained through SEM images is in concurrence with that obtained by optical microscopy. The glutaraldehyde crosslinking does not significantly affect the size of the beads. The in-vitro drug release studies were done for all the batches as well as glutaraldehyde crosslinked batch C3. Crosslinking was done using vapors of glutaraldehyde in order to minimize its residue and thereby toxicity in the final product. Sustained and controlled drug release may be achieved by selecting a polymer with the proper molecular weight and swelling properties.
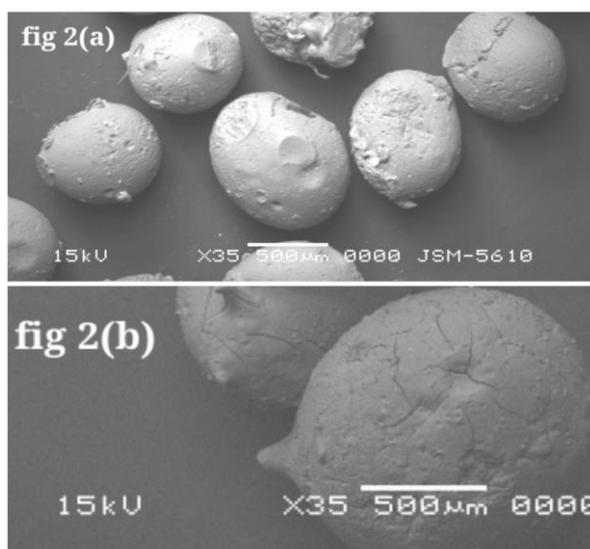
Figure 2: a) SEM of sodium alginate-chitosan beads of capecitabine (Without crosslinked)

b) SEM of sodium alginate-chitosan beads of capecitabine (crosslinked)

Upon coming in contact with gastric fluid, the polymer imbibes water and swells. The extensive swelling of these polymers is a result of the presence of physical–chemical crosslinks in the hydrophilic polymer network. These cross-links prevent the dissolution of the polymer and thus maintain the physical integrity of the dosage form. A balance between the extent and duration of swelling is maintained by the degree of crosslinking between the polymeric chains. A high degree of crosslinking retards the swelling ability of the system and maintains its physical integrity for a prolonged period. On the other hand, a low degree of cross-linking results in extensive swelling followed by the rapid dissolution of the polymer. An optimum amount of cross-linking is required to maintain a balance between swelling and dissolution[18]. Swelling index was determined by measuring the extent of swelling of beads in the given buffer. Here, swelling index was evaluated in terms of weight. All the batches exhibited very high values of swelling index as can be seen from table 4.

Table 4: Swelling Index and % Mucoadhesion of beads

Batch	Swelling Index	% Mucoadhesion
C1	2015.17±1.97	34
C2	2022.50±2.71	32
C3	1203.70±2.19	60
C4	1209.60±1.57	60

It was found that an increase in chitosan concentration in beads resulted in decreased swelling. The reason may be that in swelling process, the Ca^{2+} ions present in the polymannuronate units of alginate, are exchanged with Na^+ ions present in the buffer solution, which ultimately causes chain relaxation and enhances gel formation or swelling[19]. The adhesive property of the carrier towards the mucus membrane lining the intestine is reported to increase the residence time of the delivery system in the intestine ultimately leading to controlled release. Mucoadhesivity test was done and the results are shown in table 4. It can be seen from the results that the highest mucoadhesion of 60% was found in batch C3 and C4 containing 2% of chitosan at the end of 6 hours. Mucoadhesion of 32 % was observed with beads containing 1% of Chitosan. Thus, mucoadhesivity increased when chitosan concentration was increased. The basis of mucoadhesion could be described in terms of electronic theory: electron transfer occurred between the positively charged chitosan and sodium alginate polymers of the formulation and the negatively charged mucus glycoprotein network. This led to the formation of an electrical double layer that resulted in adherence to beads for a longer time.
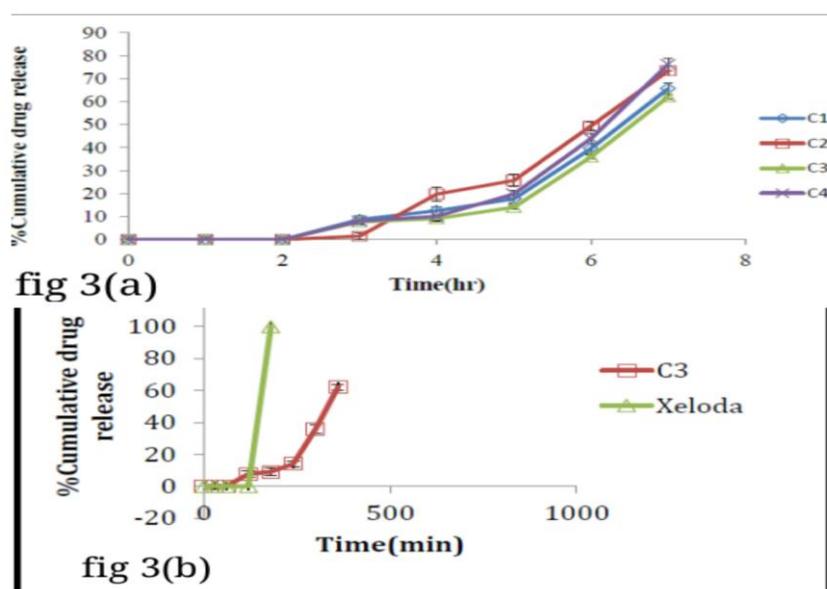

Figure 3: a) In-vitro release of capecitabine beads
b) Comparative release profile with marketed preparation

Moreover, increased polymer concentrations resulted in increased viscosity of gel that was formed and ultimately led to higher adhesion. This helps in the release of drug in a sustained manner before the beads were eroded away. There was no significant effect of drug loading on the mucoadhesivity.

It can be seen from and figure 3 that there was almost no drug release in first two hours because the beads were filled in enteric capsules and the release medium used was simulated gastric fluid. There was no significant effect of chitosan concentration on the drug release as evidenced by the results for batch C2 and C3. However, crosslinking with glutaraldehyde led to a significant decrease in the drug release. Glutaraldehyde crosslinking leads to a formation of dense matrix and hence retardation of drug release. The comparison of the release profile of the developed formulation C3 and the marketed tablet formulation is shown in figure 3. In the case of the marketed tablet preparation, almost entire amount of the drug released within an hour. The rapid release necessitates frequent administration of the marketed tablet preparation. The bead formulation, on the other hand is able to control the release rate for more than 7 hours leading to a possibility of reduction of the dosing frequency. Model fitting of the drug release data is shown in table 5.

Table 5: Model fitting of in-vitro release studies

Formulation code	First order	Zero order	Higuchi	Hixon-Crowel	Korsmeyer-Peppas	
			r^2			N
C3	0.9396	0.8024	0.7055	0.7485	0.8913	2.2124

The drug is release by first order as indicated by the high r^2 value. The chemical compatibility between capecitabine and other formulation components (excipients) was ensured using DSC and FTIR studies. Thermal analysis of pure capecitabine and capecitabine-loaded beads were conducted using DSC. These experiments measure the heat gain or loss from chemical or physical changes within a sample as a function of temperature. The DSC thermograms of the various samples are shown in figure 4.
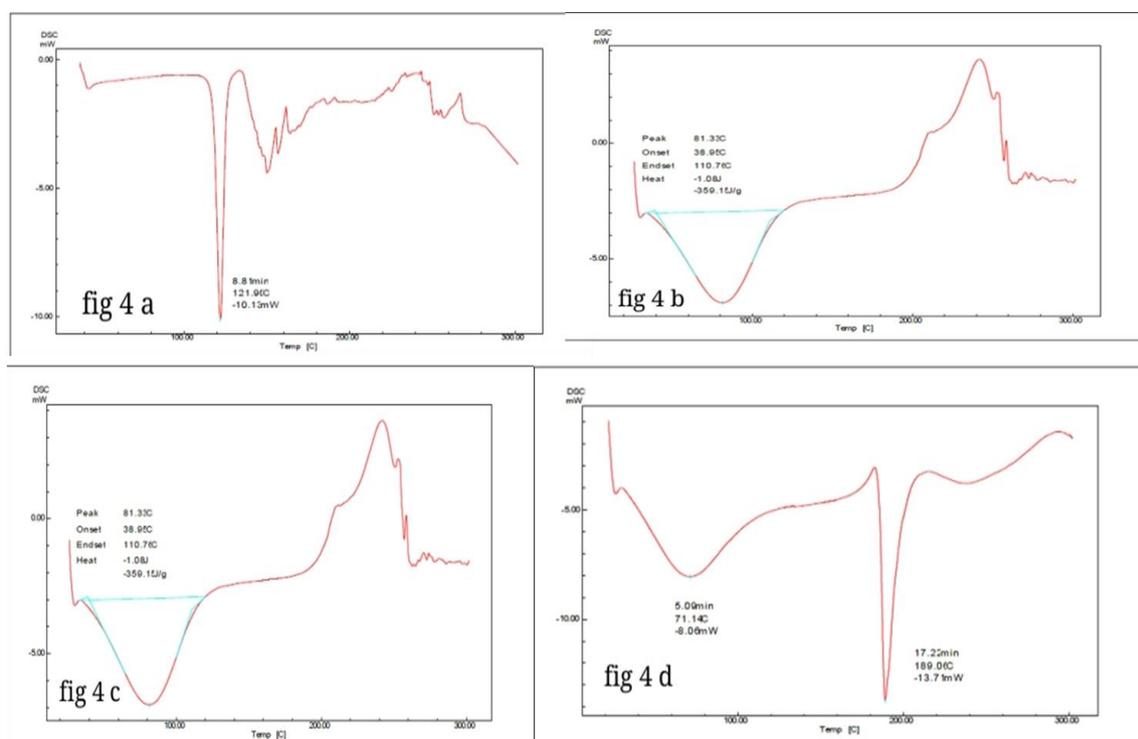

Figure 4: DSC thermograms of a) capecitabine b) Chitosan c) Sodium alginate d) optimized batch C3

The thermogram of capecitabine showed a sharp endothermic peak at 121.9°C, which nearly corresponded to the melting point of capecitabine (116–118°C). This peak was absent in both the thermograms of drug loaded beads formulation, confirming complete entrapment of the drug in polymer matrix. The FTIR spectra of the different samples are shown in figure 5.
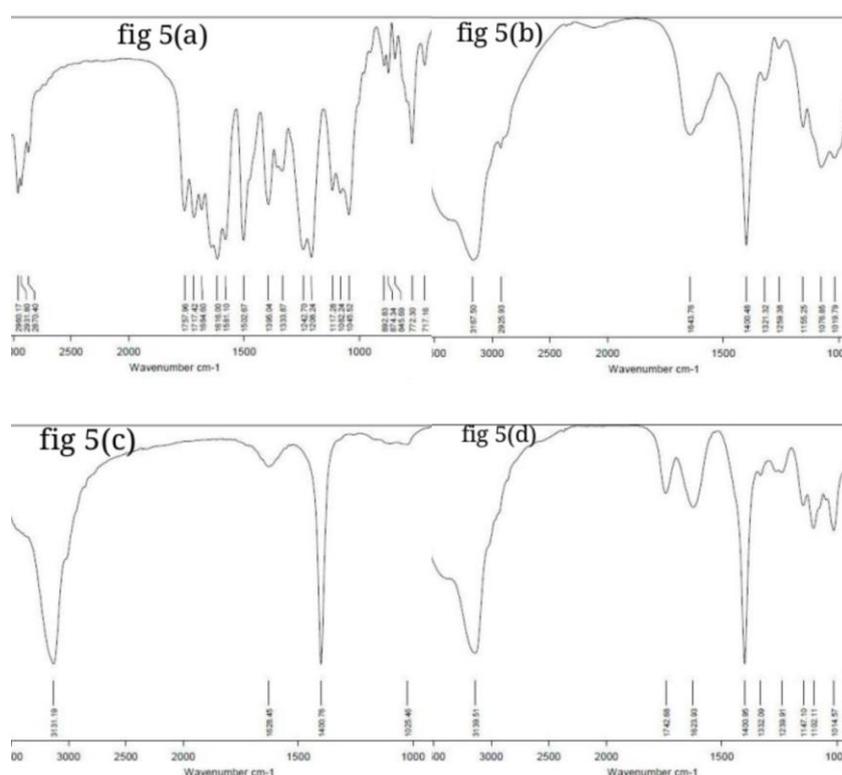
Figure 5: FTIR spectra of   a) Capecitabine   b) Chitosan   c) Sodium alginate d) optimized batch C3

In the spectrum of capecitabine, there are sharp peaks at 1757, 1333, 1242, and 1117 cm^{-1} corresponding to C=O stretching vibrations (pyrimidine carbonyl), C-N bending vibrations, N-H bending vibrations (tetrahydro furan), and C-F stretching vibrations respectively. These peaks were found in drug-loaded chitosan-sodium alginate beads. This confirms that no chemical interaction was found between the drug and polymers, thus confirming drug compatibility with these excipients. The result of the in-vitro cytotoxicity studies is shown in table 6.

Table 6: Invitro cytotoxicity studies

Time(h)	Batch C3		Xeloda tablet	
	Conc. (µg/mL)	%cell viability	Conc. (µg/mL)	%cell viability
0	0.00	0.00	0.00	0.00
1	0.00	100.12±1.26	0.00	0.00
2	0.00	101.23±1.48	0.00	0.00
3	34.96	97.07±0.98	444	5.32±0.91
4	40.028	95.34±2.58	996	6.76±2.44
5	62.922	94.45±2.27	-	-
6	158.24	76.88±2.76	-	-
7	274.788	56.65±1.24	-	-
8	-		-	-
9	-		-	-

Blank beads did not show any cytotoxicity against HT-29 cells indicating its safe use as a drug delivery system. The beads formulation showed cytotoxicity against HT-29 cells. Hence, the formulation can be effectively tested for its anticancer property. Xeloda tablet showed total drug release within first hour itself at SIF pH 6.8. The percentage cell viability was reduced up to 2% meaning 98% cell death occurred in HT-29 cells. Chitosan-Sodium alginate beads showed slower controlled drug release with 56% cell viability at the end of 7 hr meaning that 44 % cell death occurred at this time. Hence, in spite of slower drug release, the formulation showed good cytotoxicity. The results of stability studies at 25°C, 60% RH and 40°C, 75% RH of the optimized formulation (batch C3) are shown in figure 6.
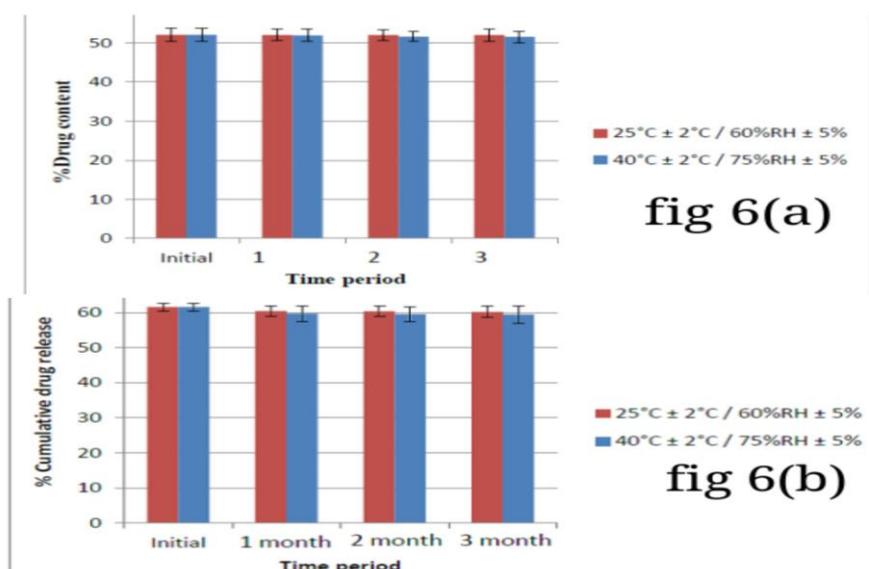
Figure 6:   Stability Study- Effect of temperature and relative humidity on
a) % drug content of chitosan-sodium alginate beads batch C3
b) % drug release of chitosan-sodium alginate beads batch C3

There was no significant change in drug content and drug release for a period of three months at both the storage conditions indicating the stability of the formulation, which is expected because of the solid dosage form.

CONCLUSION

Controlled release mucoadhesive beads of Capecitabine were successfully prepared by ionic gelation which is a very simple technique. The beads were spherical in shape and had a smooth surface with very good flow properties and compressibility. The in-vitro dissolution studies indicated sustained release from the beads for more than 7 hours. The beads had good swelling property and mucoadhesivity and were found to have excellent stability, both at 40°C and 25°C. Extended research involving pharmacokinetic and pharmacodynamic studies in suitable animal models is expected to prove this formulation a superior to the presently available marketed formulation.

REFERENCES

1. DeMario MD, Ratain MJ; Oral chemotherapy: rationale and future directions. J Clin Oncol 1998; 16:2557-2567.
2. BC Cancer Agency Cancer Drug Manual; 2001; 1-7
3. Rowe C., Sheskey P., Owen S., Handbook of Pharmaceutical Excipients, 5th ed; London, The pharmaceutical press, 2005, 507-509.
4. Hemalatha K et al; International Journal of Pharmaceutical & Biological Archives 2011. 2(3):921-925
5. Agnihotri S., Aminabhavi TM. International Journal of Pharmaceutics. 2006; 324:103– 15.
6. Narasimharao R, Sampathkumar M, Dileepkumar K, Vineeth P. International Journal of Pharmacy & Technology. 2011;3(2):2599-632.
7. http://www.drugbank.ca/drugs/DB01101
8. Olukman M., Şanlı O., Solak E., "Release of Anticancer Drug 5-Fluorouracil from Different Ionically Crosslinked Alginate Beads," Journal of Biomaterials and Nanobiotechnology, 2012;3(4): 469-479. doi: 10.4236/jbnb.2012.34048
9. Olukman M., Şanlı O., Solak E., "Release of Anticancer Drug 5-Fluorouracil from Different Ionically Crosslinked Alginate Beads," Journal of Biomaterials and Nanobiotechnology. 2012;3(4):469-79
10. Sanderson K. Stealth Nanoparticles Sneak Past Immune System's Defenses; 2013; February 21.
11. Neoj V.et.al.International Journal of Current Pharmaceutical Research; 2010;2(1).
12. Sriamornsak P. Nunthanid J. Calcium pectinate gel beads for controlled release drug delivery I. Preparation and in-vitro release studies. *Int J Pharm*, 160, 1998, 207-301.
13. Fatemeh Atyabi, Sayeh Majzoob, Maryam Iman, Masoud Salehi, Dorkoosh F. In vitro evaluation and modification of pectinate gel beads containing trimethyl chitosan, as a multi-particulate system for delivery of water-soluble macromolecules to colon. Elsevier. 2005;61(1):39-51.
14. K.P.R. Chowdary, Deepthi KS, Rao YS. Mucoadhesive Microcapsules of Indomethacin: Evaluation for Controlled Release and Ulcerogenic Activity. International Journal of Pharmaceutical Sciences and Drug Research. 2009;1(2):74-9.
15. Giuseppe S. A. Longo-Sorbello GSm, Debabrata Banerjee, and Joseph R. Bertino. Cytotoxicity and Cell Growth Assays. Cell biology: A laboratory handbook. 3 ed. p. 315-24.
16. Poncelet D, Babak V, Dulieu C, A PA. Chemical approach to production of alginate beads by emulsification-internal ionotropic gelation. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 1999; 155:171-6.
17. Girhepunje K.M.et al /International Journal of Current Pharmaceutical Research 2010. 2(1): 53-55.
18. Chawla G GP, Koradia V, Bansal A. Gastroretention a Means to Address Regional Variability in Intestinal Drug Absorption. Pharmaceutical Technology. 2003:50-68.
19. Hemanta Kumar Sharma, Siba Prasad Pradhan, Sarangi B. Preparation and in vitro evaluation of Enteric Controlled release Pantoprazole loaded Microbeads using Natural Mucoadhesive Substance from Dillenia indica L. International Journal of PharmTech Research. 2010;2(1):542-51.