

Preparation and Development of Capecitabine Microspheres for Colorectal Cancer

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Abstract

The main aim of the present investigation was to formulate and evaluate Capecitabine microspheres for colon cancer and to reduce dosing frequency and improve patient compliance. Microspheres of Capecitabine were prepared by emulsion solvent evaporation method using hydroxy propyl methyl cellulose and ethyl cellulose and cellulose acetate phthalate are polymers. The microspheres obtained were evaluated for particle size, shape, flow properties, drug content, bulk density, entrapment efficiency, in vitro dissolution studies and surface thickness by scanning electron microscope (SEM). Drug excipient compatibility was determined by FTIR and DTA. Accelerated stability studies were also carried out following ICH guidelines. The formulated microspheres were discrete, spherical with relatively smooth surface, and with good flow properties. Capecitabine-loaded microspheres demonstrated good entrapment efficiency (73% to 83%). The release study was done simulated gastro intestinal fluid for two hours in SGF (PH 1.2), remaining hrs in SIF (PH 7.4) and have showed that the drug was protected from being release in the physiological environment of the stomach and small intestine and efficiently released in colon (89.78%). FITR and DSC results showed Capcitabine was compatible with excipients. The prepared formulation can be further evaluated in vivo for correlating in vitro study results

Key words: Bioavailability, Capecitabine, colon targeting, ethyl cellulose, Microspheres.

INTRODUCTION

Oral colon-specific drug delivery system (CDDS) is more advantageous over conventional cancer chemotherapy as it is ineffective in delivering drugs to the colon due to absorption or degradation of the active ingredient in the upper gastrointestinal tract [1]. CDDS as an effective and safe therapy for colon cancer provides therapeutic concentrations of anticancer agent at the site of action and spare the normal tissues, with reduced dose and reduced duration of therapy [2]. The successful targeted delivery of drug to the colon via the gastrointestinal tract (GIT) requires the protection of a drug from degradation and release in the stomach and small intestine and then ensures abrupt or controlled release in the proximal colon [3]. Colorectal cancer is the second most common cancer killer overall and third most common cause of cancer related death in the United States in both males and females [4].

The successful targeted delivery of drug to the colon via the gastrointestinal tract (GIT) requires the protection of a drug from degradation and release in the stomach and small intestine and then ensures abrupt or controlled release in the proximal colon [5]. Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of colorectal cancer and metastatic breast cancer. Capecitabine is a prodrug that is enzymatically converted to fluorouracil (antimetabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissues since it is readily absorbed from the gastrointestinal tract. The recommended daily dose is large, i.e., 2.5 g/m² and it has a short elimination half-life of 0.5–1 h [6].

It is metabolized in the liver by 60 K Da Carboxylesterase. By converting into controlled, site specific release dosage form first pass metabolism can be minimized, bioavailability can be increased, deactivation in gastric pH can be avoided, reduce the dose frequency and minimize side effects when compared with the conventional treatment [7]. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 µm. The range of techniques for the preparation of microspheres offers a variety of opportunities to control aspects of drug administration and enhance the therapeutic efficacy of a given drug. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs also known as microparticles. It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs. In future by combining various other strategies, microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective in vivo delivery.

The term microspheres describe a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles [8]. They can also be defined as a structure made up of continuous phase of one or more miscible polymers in which the particulate drug is dispersed at the macroscopic or

molecular level. Microspheres provide constant and prolonged therapeutic effect, reduced the GI toxic effects and dosing frequency and thereby improve the patient compliance. They could be injected in to the body due to the spherical shape and smaller size. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects. Microsphere morphology allows a controllable variability in degradation and drug release. Although number of polymers can be used, natural or semi-synthetic polysaccharides, such as cellulose derivatives, play an important role in microencapsulation processes. For example, Ethylcellulose (EC) a biocompatible and water insoluble polymer that has been used in the preparation of coated and matrix tablets [9], micro and nano capsules, beads [10] and other coated solid pharmaceutical dosage forms [11]. In present work ethyl cellulose is selected as the retardant material for CBN. EC, used as an encapsulating material is extensively studied by many researchers for the controlled release of CBN. The microspheres prepared using emulsion/solvent evaporation method shown only 78% entrapment efficiency. The purpose of the present work was to prepare and evaluate oral controlled release microparticulate drug delivery system of CBN using ethylcellulose with high entrapment capacity and sustained release.

MATERIALS AND METHODS

Materials

Capecitabine were procured from Dr Reddy's Labs, Medak Dist.,Telangana., India (gift sample), Ethylcellulose (25 cps viscosity), DCM, polyethylene glycol, polyvinyl alcohol, hydroxyl ethyl cellulose and ethyl cellulose (s.d. fine chemicals, Mumbai) were obtained from commercial sources. All the other reagents used were of analytical grade.

Methods

Preformulation Studies:

Infrared (IR) absorption spectroscopy: To investigate any possible interaction between the drug and the polymer, the IR spectra of pure drug capecitabine, its physical mixture with polymers were carried out using Infrared spectrophotometer FTIR- 8400S Shimadzu (TOKYO JAPAN). The sample were prepared as KBr disks compressed under a pressure 8 ton/cm² and the wave length selected ranged between 400-4000cm⁻¹. The IR spectrum of the physical mixture was compared with those of pure drug and polymer to detect any appearance or disappearance of peaks.

Preparation of Standard Graph Of Capecitabine:

An accurately weighed quantity of 100mg Capecitabine was transferred into a 100ml standard flask and volume was made up to the mark using Phosphate buffer of pH 7.4. From the primary stock solution 2ml was transferred to 100 ml volumetric flask and diluted upto the mark using Phosphate

buffer of Ph 7.6. The concentration of the solution will be 20µg/ml. Various concentrations 4µg, 8µg, 12 µg, 16µg, 20µg, 24µg, 28µg 32µg, were prepared by diluting 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml of the stock solution to 10ml using buffer of pH 7.4 respectively. The absorbance was noted at 239nm using UV/Vis Spectrophotometer. Similarly the various standard graphs of capecitabine were prepared in 0.1 N HCl pH 1.2 with phosphate buffer of pH 6.8, (Fig.No.1).

Table No.1. Calibration curve data

S.No	Conc (µg/ml)	Absorbance (nm)
1	0	0
2	4	0.545
3	8	1.051
4	12	1.473
5	16	1.906
6	20	2.294
7	24	2.662
8	28	3.062
9	32	3.34

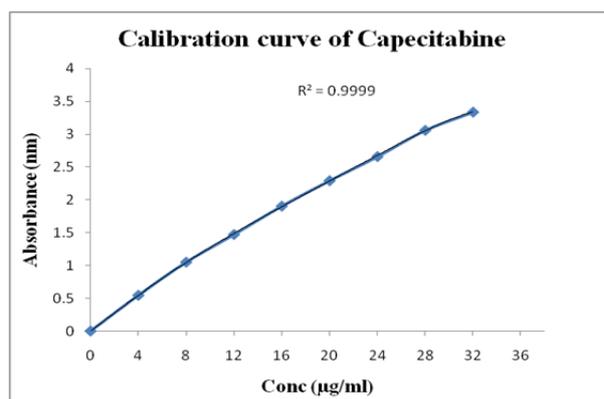


Fig.No.1: Calibration curve of Capaectabine

Preparation of Microspheres:

The microspheres were prepared by (o/w) solvent evaporation method, since capecitabine is a slightly water-soluble drug. Polymers ethyl cellulose and HPMC were dissolved in 20ml of DCM. These polymers and drug are mixed vigorously to form a clear solution. Then 0.1% of polyethylene glycol was added which acts as a surfactant. Then the above solution was emulsified by adding drop by drop into the aqueous solution containing 160 ml of 0.46% w/v of PVA as an emulsifier. Dichloromethane was removed at 35°C by evaporation. As the solvent was being removed, the emulsifier continued to maintain the oil droplets in their spherical configuration and prevented from aggregating until the solvent was completely removed, and the microspheres were hardened as discrete particles. Finally, the hardened microspheres were washed with distilled water for 5 times and dried (Tabel 2).

Table 2. Formulation for Capecitabine microspheres

S.No	Drug (g)	Ethyl Cellulose (g)	HPMC (g)	DCM (ml)	PVA (0.47% w/v)	SPEED (rpm)
1	0.5	2	1	20	160 ml	800
2	0.5	1	2	20	160 ml	800

*Dichloromethane (DCM), *Polyvinyl alcohol (PVA)

Table 3 Micromeritic properties of microspheres

	Bulk density	Tapped density	Angle Of repose	Carr's index	Hausners ratio
F1	0.426±0.015	0.512±0.025	25.92±0.30	11.8±0.42	1.12±0.016
F2	0.456±0.015	0.54±0.026	26.06±0.39	13.6±0.53	1.13±0.031

Characterization of the Microspheres:

Particle size of microspheres:

The particle size of the microspheres was determined by using optical microscopy method [13]. A small amount of dry microspheres was suspended in distilled water. A small drop of suspension was placed on a clean glass slide. The slide containing suspended microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The process was repeated three times for each batch prepared.

Morphology:

Shape and surface morphology was studied with projection microscope and photographs were taken and the selected formulations were further investigated using Environmental Scanning Electron Microscopy (ESEM, XL30, Philips, Netherlands). The samples were randomly scanned and photomicrographs were taken with ESEM (Fig.2).

Flow Properties [13]

The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr's and Hausner's ratio. Each parameter was calculated three times for each batch prepared and results were averaged.

(i) Angle of Repose

Angle of repose (θ) was measured according to the fixed funnel of Banker and Anderson. A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height of 1cm (H), above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel. Thus, the R being the radius of the base of the microspheres conical pile:

$$\tan \theta = H/R$$

$$\theta = \tan^{-1}(H/R)$$

Where, θ = Angle of repose

H = Height of pile

R = Radius of pile.

(ii) Carr's Index and Hausner's Ratio

Poured density was determined by placing exact quantity 'M' of microsphere into a graduated cylinder and measuring the volume 'V' occupied by the

microspheres.

$$\text{Poured Density} = M/V$$

Tapped density was determined by placing a graduated cylinder containing a known quantity (M) of the prepared microspheres on a mechanical tapping apparatus, which was operated for a fixed number of taps until the bed volume reached to a minimum.

$$\text{Tapped Density} = M/V$$

The Carr's Index and Hausner's ratio were calculated using formula:

$$\text{Carr's index (\%)} = \frac{\text{Tapped} - \text{Poured density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Poured density}}$$

Percentage yield:

The microspheres were evaluated for percentage yield. The yield was calculated by

$$\text{Percentage yield} = \frac{\text{weight of microsphere recovered}}{\text{Weight of drug} + \text{weight of polymer}} \times 100$$

Percent Entrapment Efficiency:

25 mg of drug loaded core microspheres was weighed and washed with 10ml of phosphate buffer of pH 6.8 to remove the surface – associated drug. Then microspheres were kept in phosphate buffer of pH 7.4 for digestion for 24 hrs and sonicated for 1 hr at room temperature. From that 1ml of sample is withdrawn and diluted 1000 times using phosphate buffer pH 7.4 and quantified spectrophotometrically at 239nm entrapment efficiency is determined by using the formula.

$$\% \text{ Drug Entrapment} = \left(\frac{\text{Practical yield from graph}}{\text{Initial amount loaded}} \right) \times 100$$

Table 4 Yield of Microspheres

Formulation code	% yield
F1	83.05±1.29
F2	79.68±2.01

Table 5 Entrapment Efficiency

Formulation code	Drug entrapment
F1	83.04±1.94
F2	79.68±2.1

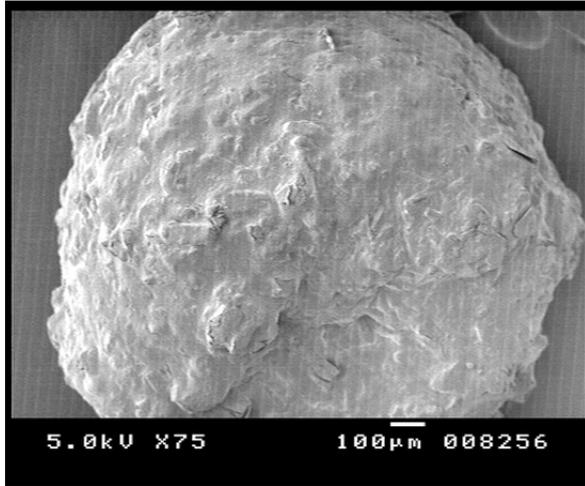


Fig. 2 SEM analysis

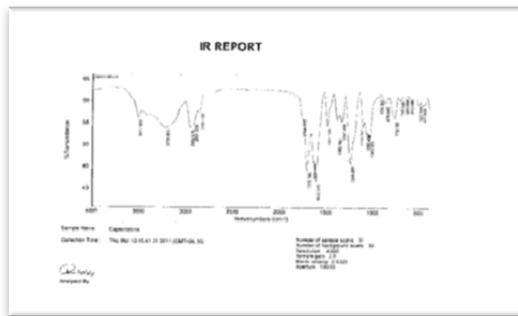


Fig.3 FTIR of pure Capecitabine

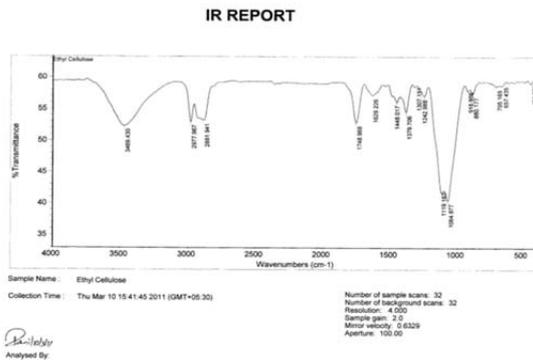
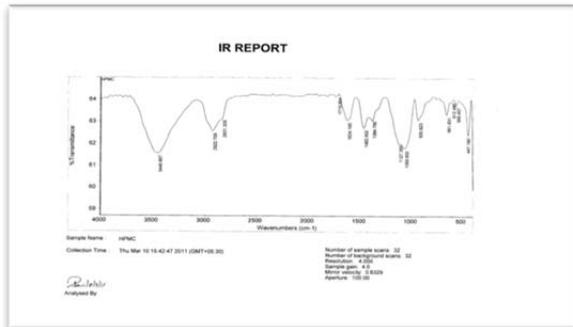


Fig. 4 FTIR of pure HPMC and EC

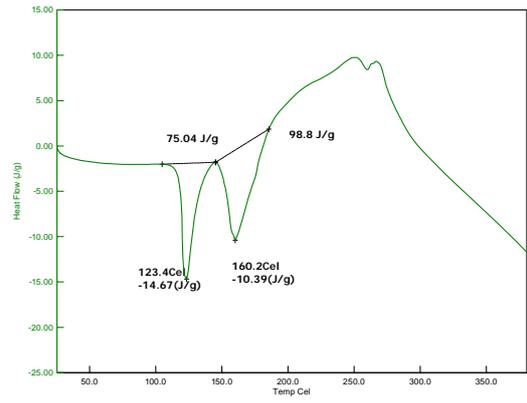


Fig.5 DSC of pure Capecitabine

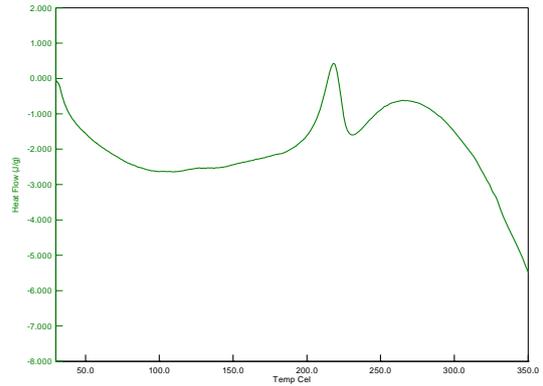


Fig.6 DSC of Ethyl Cellulose

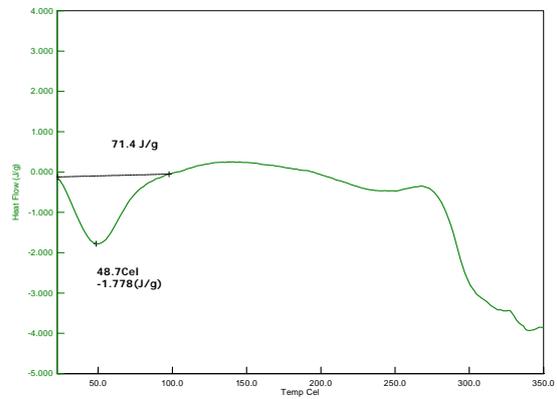


Fig.7 DSC of HPMC

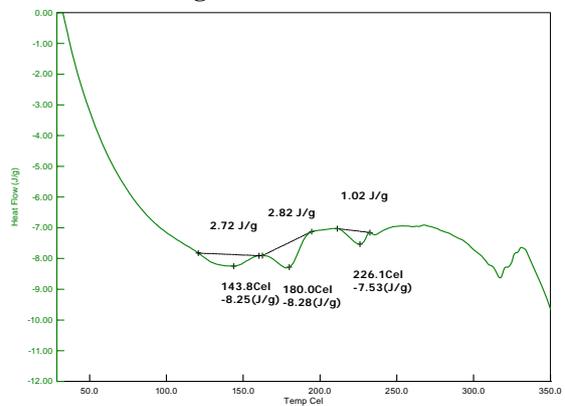


Fig. 8 DSC of physical mixture of Capecitabine, Ethyl Cellulose, and HPMC

In Vitro Release Studies:

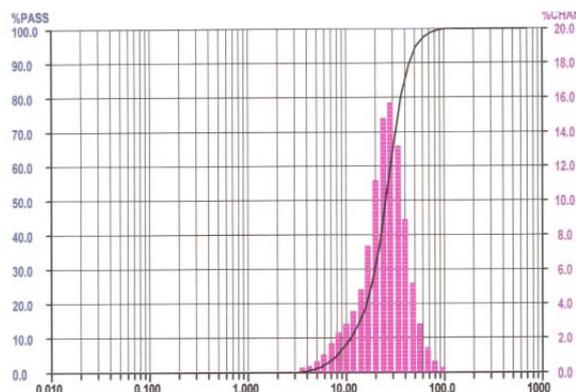
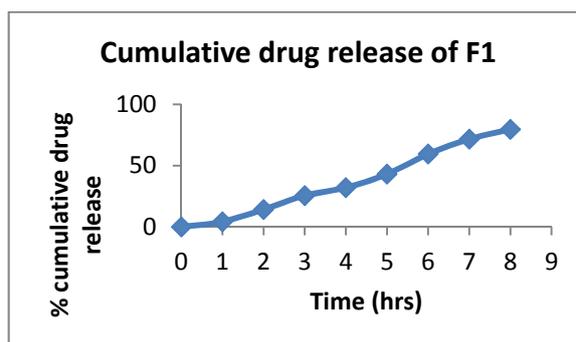
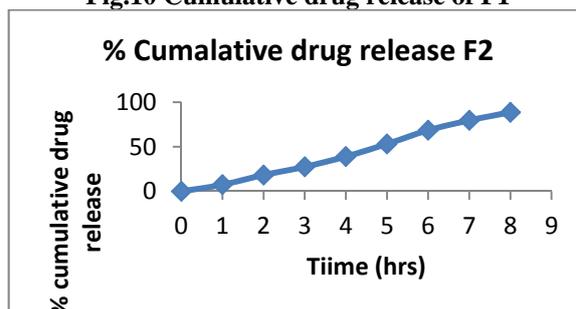
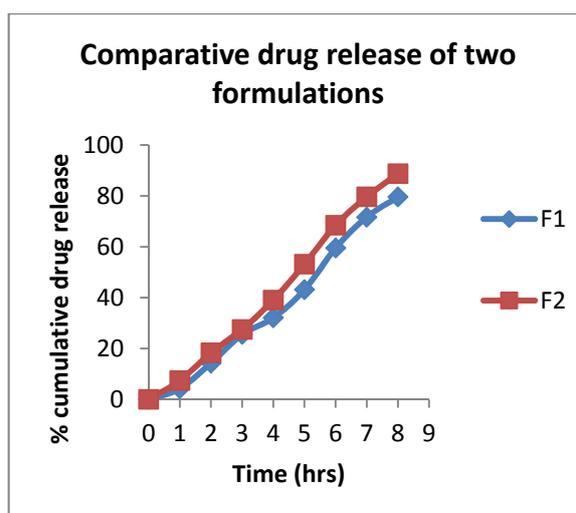
In vitro drug release was investigated in SGF (0.1N HCl, pH 1.2) for the first 2 h, followed by the SIF (0.05M potassium dihydrogen phosphate, pH 7.4 phosphate buffer), until complete dissolution. The drug dissolution test of microspheres was performed by the paddle method using USP XXIII paddle type dissolution apparatus (TDT-08L, Electro lab India, Mumbai) at 100 rpm and $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Microspheres (100 mg) were weighed accurately and filled in tea bags. The tea bags were tied using thread with paddle and loaded into the basket of dissolution apparatus containing 900mL of dissolution medium. The samples (5mL) were withdrawn from the dissolution medium at time interval of 1 hr using a pipette fitted with a microfilter at its tips and analyzed for drug by UV spectrophotometer against a standard curve ($R^2 > 0.99$) obtained at $\lambda = 239$ nm. Perfect sink condition was maintained during the drug dissolution study period with the addition of an equal volume of fresh release medium at the same temperature.

Table 6: In vitro Drug release studies of F1

S.No	Time (hrs)	% cumulative drug release
1	0	0
2	1	4.23±0.36
3	2	14.26± 1.63
4	3	25.52±1.21
5	4	32.06±0.7
6	5	43.12±1.29
7	6	59.51±0.87
8	7	71.65±1.58
9	8	79.58±0.94

Table 7: In vitro Drug release studies of F2

S.No	Time (hrs)	% cumulative drug release
1	0	0
2	1	7.43±0.26
3	2	18.31± 1.03
4	3	27.52±0.21
5	4	39.06±0.63
6	5	53.18±1.57
7	6	68.51±1.08
8	7	79.65±0.94
9	8	88.74±1.80

**Fig: 9 Particle size analysis****Fig.10 Cumulative drug release of F1****Fig. 11 Cumulative drug release of F2****Fig.12 Comparative drug release of F1 and F2**

RESULTS AND DISCUSSION

Microspheres of capecitabine were successfully prepared by o/w emulsification-solvent evaporation technique. This method is used for microsphere preparation because of its simplicity, reproducibility, and fast processing with minimum controllable process variables that can be easily implemented at the industrial level. The percentage yield of different formulations was calculated and the results were shown in (Table 2) the yield was found in the range of 88.05% to 79.68% for all the formulations (F1 to F2). The results indicated that the method o/w emulsification-solvent evaporation yields better percentage of capecitabine microspheres. As shown in figure the average particle size of microspheres increased with increasing polymer concentration, as higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres were formed (Fig.9). It can be clearly observed from the photographs of the microspheres prepared by solvent evaporation technique, that the microspheres are small, spherical and discrete. The shape and surface morphology was further confirmed with the SEM photograph (Fig.2). The microspheres were spherical and have almost smooth surface. The values of angles of repose were in the range of $25.92^\circ \pm 0.30$ to $26.0^\circ \pm 0.39$, the values of Carr's index were in the range of 11.8% to 13.6 % and the values of Hausner ratio were ranged from 1.12 to 1.14 for all the formulations (Table 3). Comparison of calculated results with standard values indicates an overall good free flowing nature of microspheres of all batches. Values of angle of repose $\leq 30^\circ$ usually indicate a free flowing material, while values of compressibility index below 20 % give rise to good flow characteristics. Percent entrapment efficiency of the formulations was found in the range of 73.28% to 83.76 in both the formulations given in (Table 5). All the formulations show good entrapment efficiency. It was reported in the literature that the encapsulation efficiency depends on the solubility of the drug in the solvent and continuous phase. An increase in the concentration of polymer in a fixed volume of organic solvent resulted in an increase in encapsulation efficiency [14]. Hence capecitabine being aqueous soluble drug required high concentration of polymer in dosage form for better formulation development.

In vitro drug release study of pH dependent CPB microspheres was performed in pH of medium and the in vitro drug release data of CPB in simulated gastrointestinal fluids (SGF, SIF) for all the formulations are given in figure. The F1 formulation showed a longer duration 8hrs of 79.59% of drug release (Fig.10) and F2 showed 89.78% of drug release in 8hrs (Fig.11). The comparison of the two formulations was presented in (Fig.12). It was noticed that the increase in the amount of ethyl cellulose showed slow release of the drug for a longer period of time. The result shows that the cumulative drug

release decreased as the polymer concentration increased. It may be due to the fact that the increase in polymer concentration increases the density of polymer matrix.

Fourier transform infrared (FTIR) spectral studies:

FTIR spectra of the Ethyl Cellulose, HPMC, Capecitabine and Capecitabine-loaded microspheres were obtained. In order to investigate the possible reaction between Ethyl cellulose and Capecitabine. The ratio (mL/mg) and concentration of Ethyl Cellulose as well as capecitabine was kept identical to that used in formulations. The time of exposure was also kept identical to that of microsphere preparation, i.e., 2 h. Then, capecitabine was washed with double distilled water. After drying, FTIR spectrum was recorded. The samples were crushed with KBr to get pellets by applying a pressure of 600 kg/cm^2 . Spectral scans were taken in the range between 4000 and 500 cm^{-1} on a Nicolet (Model Impact 410, Milwaukee, WI, USA) instrument.

Differential scanning calorimetry (DSC) studies:

Differential scanning calorimetry (DSC) was performed on Ethyl Cellulose, HPMC, Capecitabine and Capecitabine-loaded microspheres. DSC measurements were done on a Rheometric Scientific (DSC-SP, Surrey, UK) by heating the samples from ambient to 400°C at the heating rate of $10^\circ\text{C}/\text{min}$ in a nitrogen atmosphere (flow rate, 20 mL/min).

CONCLUSION:

In this study, colon targeted microspheres of anticancer drug Capecitabine were formulated successfully using polymers HPMC, EC separately and in combination for colonic delivery of drug. Spherical and free-flowing microspheres were prepared by emulsion solvent evaporation method. The good flowability and packability of microspheres, indicates that they can be successfully handled and either filled into a capsule or compressed to tablet dosage form. All the formulations were found to be efficient with good recovery yield and percent drug entrapment. The study revealed that the release profile of microspheres was affected by polymer concentration and microspheres were capable to retard the release of CPB until it reaches the colon. The prepared formulation can be further evaluated in vivo for correlating in vitro study results. The prepared microspheres is a promising colon targeted delivery device for achieving better kinetic profile with improved bioavailability.

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