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Original Article

FORMULATION AND DEVELOPMENT OF MICROSPHERES FOR THE TREATMENT OF FAMILIAL ADENOMATOUS POLYPOSIS

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ABSTRACT

Objective: Familial adenomatous polyposis (FAP) also known as familial polyposis coli, is a hereditary disease characterized by progressive appearance of numerous polyps mainly in the large intestine. Polyps are initially benign but can easily become cancerous and as such it is a life threatening condition. Celecoxib, a cyclooxygenase-2 (COX-2) inhibitor is thought to induce cell death, and thus prevent or delay the growth of polyps. So in the present study celecoxib loaded microspheres were prepared using control release Hydroxy propyl methyl cellulose (HPMC K4M) and pH dependent polymer eudragit L 100-55 in different ratios (1:1 to 1:4) respectively. The main objective of the study is to identify the polymer concentration required to prevent the drug release in stomach region and promotes in intestinal region.

Methods: Emulsification solvent evaporation method was selected for the preparation and all the optimized formulations were evaluated for drug-polymer interactions, percentage yield, micrometric properties, entrapment efficiency, particle size analysis, differential scanning calorimetry and *in vitro* dissolution study.

Results: Drug and polymer interactions were evaluated by using FTIR and DSC. The FTIR spectrum and DSC thermograms stated that drug and polymer are compatible to each other. The micrometric properties of drug loaded microspheres were carried out and they were found to be as the angle of repose (18.26 °-40.69 °), bulk density (0.2846-0.3875), tapped density (0.4111-0.5428), Carr's index (9.66-14.77), Hausner's ratio (1.112-1.2642) which were within the limits. *In vitro* dissolution, drug release was found to be from 4.5 to 6.5 h for the prepared four formulations (F1–F4). From the kinetic data modeling the order of drug release was found to be zero order and korsmeyer-peppas with n value above 0.5 for all the formulations indicating non-fickian diffusion.

Conclusion: All the result demonstrated that celecoxib microspheres can be effectively used in the treatment of familial adenomatous polyposis

Keywords: Microspheres, Polyposis, Eudragit, pH dependent

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INTRODUCTION

Familial Adenomatous Polyposis (FAP), also known as familial polyposis coli, is a hereditary disease characterized by the progressive appearance of numerous polyps mainly in the large intestine especially in children. FAP may lead to cancer of the large intestine, and as such is a life-threatening condition. The opted therapy for this disease is endoscopic surveillance with the removal of polyps when required and use of anti-inflammatory agent like celecoxib which is thought to induce cell death, and thus prevents or delay the growth of polyps [1]. The better therapy for FAP is use site specific release systems which offers advantages like prevention of side effects of drugs on healthy tissues and enhancement of the uptake of the drug by targeted cells. Enhancing of drug uptake by specific cells not only permits the internalization of substances with low cellular permeability but also allow for the maintenance of low blood to cell concentration ratio thereby reducing therapy limiting side effects. The successful delivery of drugs to the colon via the gastrointestinal tract requires the protection of drug from being released in the stomach and small intestine. This might be achieved by the use of special drug delivery system that can protect the drug during its transfer to required site. Targeting relies on exploiting a unique feature of the intended site and protecting the active agent until it reaches that site and this is achieved with the use of a combination of pH dependent and controlled release polymer [2].

Microspheres 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 and ideally having a particle size less than 200µm. They are prepared in such a way that the incorporated drug is delivered at the target site while reducing side effects by keeping systemic circulation low. The risk of dose dumping also seems to be considerably lower than with single unit dosage form. Microspheres allow the administration of smaller doses that are normally required and this reduces local irritation when compared to single unit dosage forms [3-6]. Considering an advantage of microspheres, the present investigation was undertaken to prepare and evaluate celecoxib loaded microspheres with enteric polymers like eudragit and a rate controlling polymer like HPMC K_4M in different ratios so that the release of drug at gastric region is prevented with the use of ph dependent eudragit polymer and release of drug at controlled level is achieved with HPMCK4M.

MATERIALS AND METHODS

Materials

Celecoxib, HPMC K_4M, Eudragit L 100-55, Acetone, Alcohol, Dibutyl phthalate, Span 80, Liquid paraffin, Petroleum ether.

Method

For the preparation of celecoxib microspheres, emulsification solvent evaporation method opted. HPMC K_4M and Eudragit L 100-55 in different ratios were dissolved in ethanol (S_1) and acetone (S_2) in 1:2 ratio to form a homogeneous polymer solution. Celecoxib was added into the polymer solution and mixed thoroughly. Plasticizer (dibutyl phthalate 3% w/v) was added to above solution. The above organic phase was slowly poured at 30 °C into liquid paraffin (15 ml) containing varying concentrations of span 80 while stirring speed maintained at 2000 rpm to form a smooth emulsion and stirring was continued until residual acetone and ethanol were evaporated and smooth walled, rigid and discrete microspheres were formed. The microspheres were collected by decantation and the product was washed with petroleum ether three times and dried at room temperature for 3 h.

The microspheres were stored in a dessicator over fused calcium chloride for further use. Total twelve trial formulations were carried out [7. From that four main formulations were formulated with optimized span concentration. The detailed composition of the various formulations prepared are mentioned in below table 1 and table 2.

Table 1: Details of trial formulations prepared

Formulation code	Polymer ratio HPMC: eudragit	S_1 (ml)	S_2 (ml)	Span concentration (%)
TF1	1:1	5	10	1.2
TF2	1:2	5	10	1.2
TF3	1:3	5	10	1.2
TF4	1:4	5	10	1.2
TF5	1:1	5	10	1.0
TF6	1:2	5	10	1.0
TF7	1:3	5	10	1.0
TF8	1:4	5	10	1.0
TF9	1:1	5	10	0.8
TF10	1:2	5	10	0.8
TF11	1:3	5	10	0.8
TF12	1:4	5	10	0.8

Table 2: Design of main formulations

Formulation code	Drug (mg)	Polymer ratio HPMC: eudragit	Span concentration (%)
F1	100	1:1	1%
F2	100	1:2	1%
F3	100	1:3	1%
F4	100	1:4	1%

Evaluation

Drug-polymer interactions (FTIR study)

FTIR spectroscopy of drug, polymers and physical mixture was performed using fourier transform infrared spectrophotometer (BRUKER, ALPHA). The sample was taken and by using potassium bromide, pellets were prepared using hydraulic pellet press and the spectrum was taken.

Percentage yield

The dried microspheres were collected and weighed. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres gives percentage yield and it can be calculated using the following formula [8].

$$\% \text{ Yield} = \left(\frac{\text{actual weight of obtained product}}{\text{total weight of excipients and drug}}\right) \times 100$$

Micromeritic properties of microspheres

The flow properties of the microspheres were investigated by determining the angle of repose, bulk density, tapped density, carr's index, hausner's ratio. The angle of repose was determined by the fixed–based funnel method. Bulk and tapped density were measured in 10 ml of the graduated cylinder. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. The volume occupied by the sample after tapping was recorded and subsequently, bulk density, tapped density, carr's index and hausner's ratio was calculated [5].

The angle of repose was determined using the formula:

$$\theta = \tan^{-1}\frac{h}{r}$$

Entrapment efficiency

Microspheres containing the equivalent to 50 mg of drug was allowed to equilibrate in 100 ml of phosphate buffer pH 6.8 for 24 h. The solution was filtered using whatmann filter paper. The resulting solution was analysed using a UV spectrometric method at 255 nm in the presence of blank prepared from microspheres containing all materials except the drug.

$$\% \text{ drug entrapment} = \frac{\text{calculated drug concentration}}{\text{theoretical drug concentration}} \times 100$$

Particle size analysis

Particle size analysis of drug-loaded microspheres was by optical microscopy (Olympus Model Szx-12). A small amount of microspheres was suspended in purified water (10 ml). Mount the sample on a clean glass slide and placed it on mechanical stage of the microscope. The eye piece of a microscope fitted with a micrometre by which the size of the spheres could be determined. The process was repeated for each batch of prepared microspheres.

Differential scanning calorimetry

DSC studies were performed using a universal V4.5A TA instruments. Accurately weighed samples (about 3 mg) were placed in a sealed aluminium pan, before heating under nitrogen flow (20 ml/min) at a scanning rate 20 °C/min from 40 to 300 °C. An empty aluminium pan was used as reference. DSC thermograms of pure substances, their physical mixtures and drug loaded microspheres were recorded.

***In vitro* drug release study**

In vitro drug release study of microspheres was performed in pH progression medium at 37 °C±0.5 °C. The drug dissolution test of microspheres was performed by the basket method (USP dissolution apparatus type-I, LABINDIA DISSO 8000). Microspheres that are equivalent to 50 mg were weighed accurately and placed in the basket setup which is in 900 ml of dissolution medium and test was carried out in pH 1.2 buffer for 2 h using 0.1N HCl. After 2 h, the dissolution medium was replaced with phosphate buffer pH 6.8 and maintained up to 6 h. The samples were withdrawn from the dissolution medium at various time intervals using a syringe the rate of drug release was analyzed using UV-Visible spectrophotometer.

RESULTS AND DISCUSSION

Out of the twelve trial formulations with varying span concentrations (1.2%, 1%, 0.8 %), an optimum span concentration of 1% was chosen and four main formulations were formulated by incorporation of the drug. The resultant formulations with varying sizes were obtained as shown in fig. 1.

Standard calibration

Calibration of pure celecoxib was carried out in pH 1.2 buffer and phosphate buffer pH 6.8. The wavelength maxima was found to be 255 nm for the drug. The calibration curves for celecoxib in 1.2 and 6.8 pH buffers were given in fig. 2 and fig. 3 respectively.

Drug-polymer interactions (FTIR study)

The compatibility between the drug and the selected polymers were evaluated using FT–IR peak matching method. The IR spectra of pure drug, individual polymers and final formulation were shown in the fig. 4, 5, 6 and 7 respectively. The standard peaks 1159 cm-1is due to S=O stretching, 3338 cm-1by NH2 stretching and 1563 cm-1by N-H stretching. There was no appearance or disappearance of peaks in the formulation, which confirmed the absence of chemical interaction between drug and polymers.

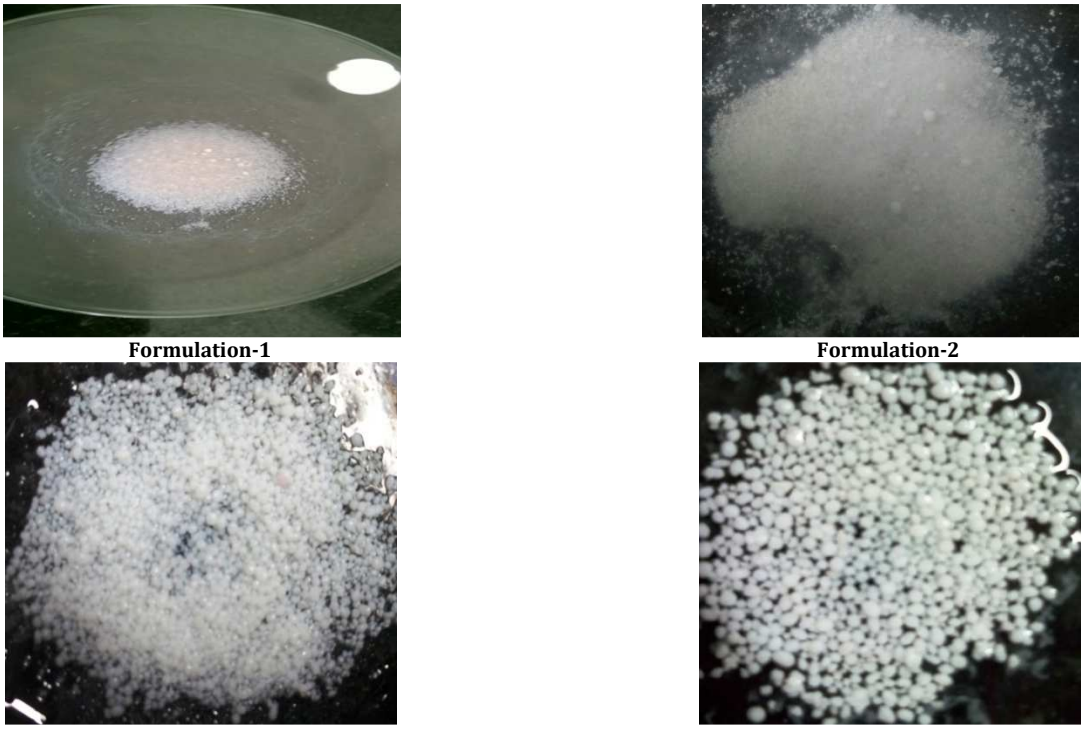

Fig. 1: Images of different prepared formulations F1-F4

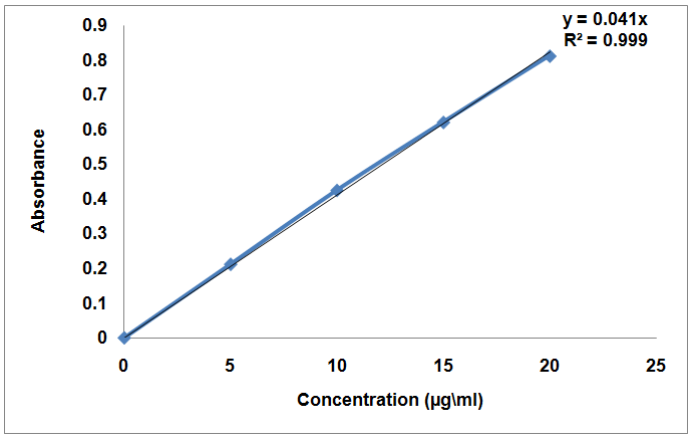

Fig. 2: Standard calibration curve of celecoxib in pH 1.2 buffer

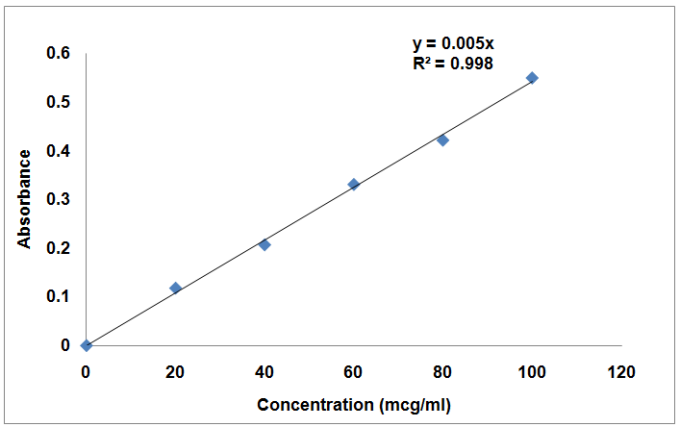

Fig. 3: Standard calibration curve of celecoxib in phosphate buffer pH 6.8

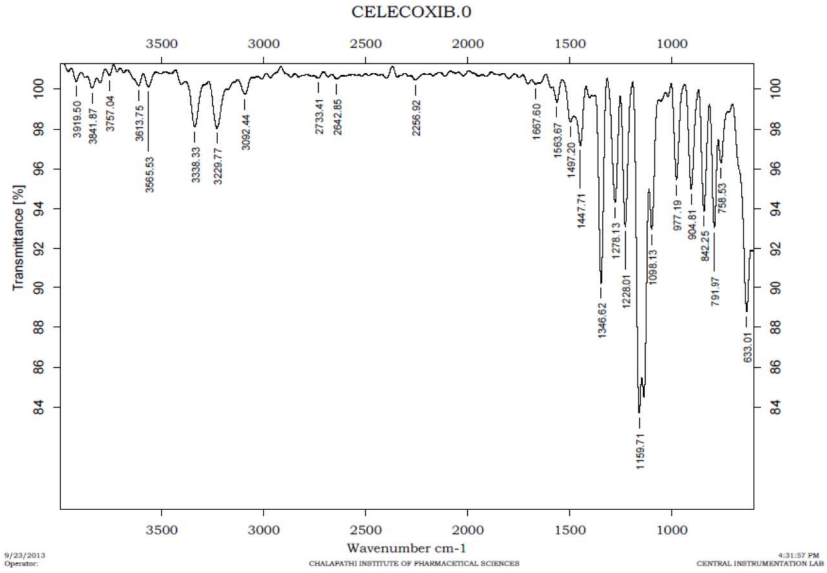

Fig. 4: FTIR spectrum of celecoxib

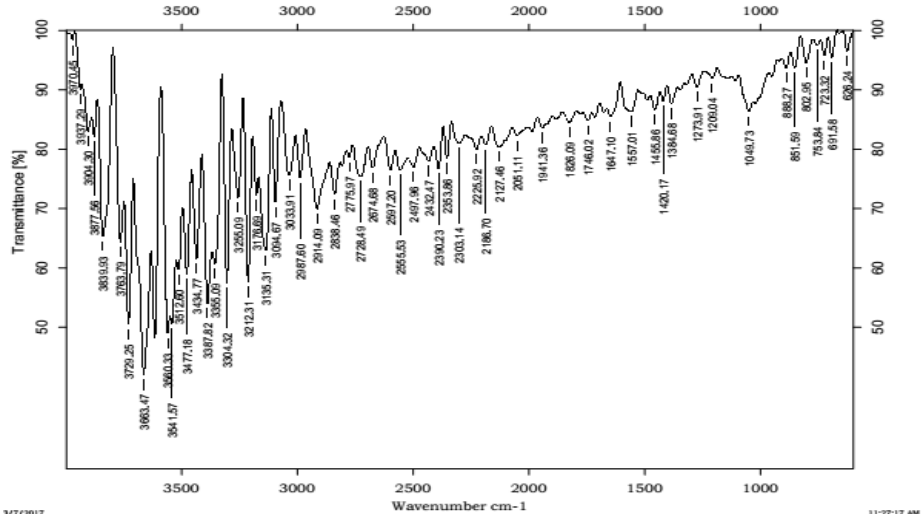

Fig. 5: FTIR spectrum of HPMC K_4M

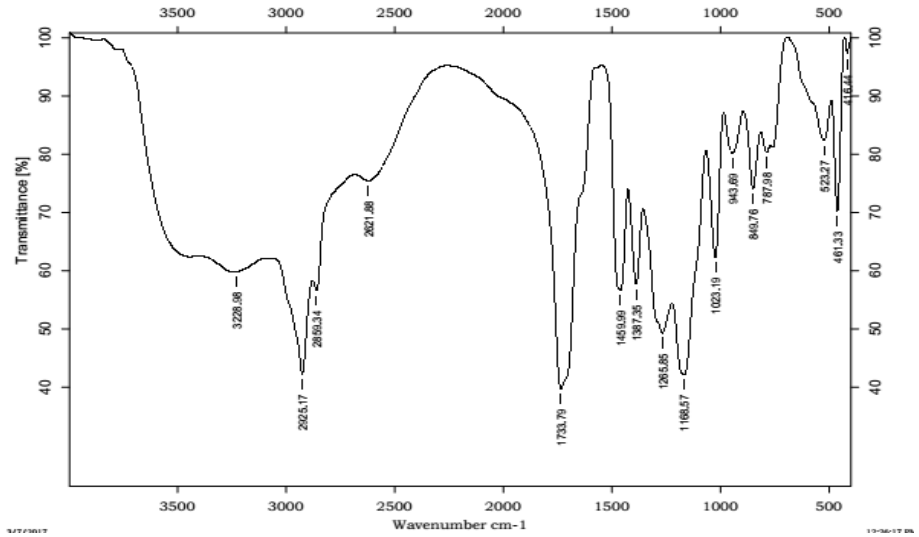

Fig. 6: FTIR spectrum of eudragit L 100-55

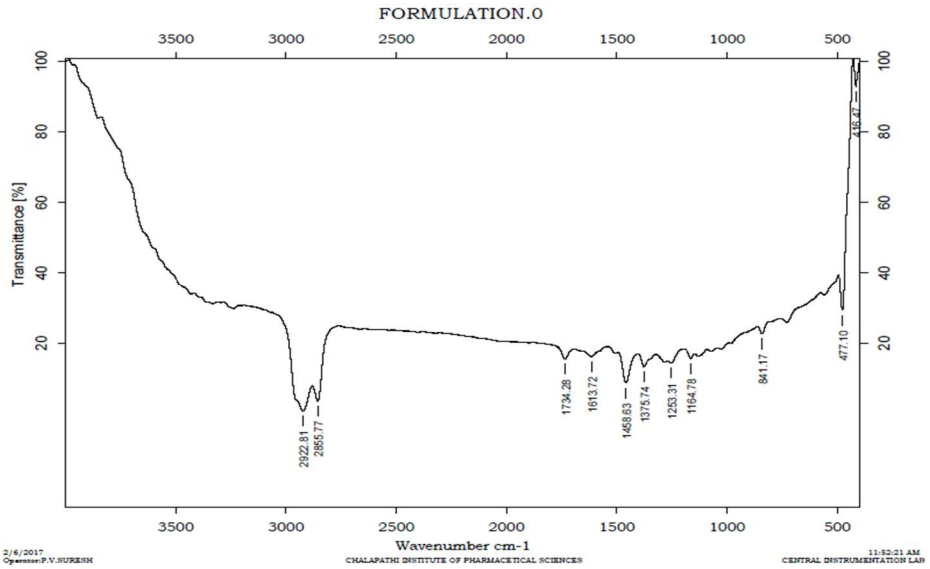

Fig. 7: FTIR spectrum of formulation

Micromeritic properties of prepared microspheres

Micromeritic studies like the determination of the angle of repose, bulk and tapped densities, compressibility index and hausner's ratio were performed for all the four main formulations and the results were reported in table 3**.**

Percentage yield and entrapment efficiency

Percentage yield and entrapment efficiency for all the four main formulations containing drug were determined and reported in the table 4**.**

Particle size analysis

All the main formulations were observed under Olympus imaging binocular microscope and particle size was determined. The average size and standard deviations were given in table 5 and the images were shown in fig. 8.

***In vitro* drug release studies**

In vitro drug release was studied by *in vitro* dissolution test carried out using basket apparatus.

Prior to dissolution, in order to know about the stability of the microspheres at gastric pH, a study was conducted to determine the enteric capacity of the polymer, where the formulation was equally weighed and placed in 100 ml of each pH 1.2 buffer and phosphate buffer pH 6.8 for 2 h and it was observed that the microspheres in the beaker with pH 1.2 buffer as the medium remained as such where as those in beaker with phosphate buffer pH 6.8 were dissolved gradually as the time prolonged. They are shown in fig. 11. As the concentration of eudragit was increased the drug release in acidic pH was found to be reduced and the the presence of HPMC made the prolonged release up to 6 h.

Table 3: Micromeritic properties of drug loaded microspheres

Batch	Angle of repose (°)	Bulk density (gm/cm³)	Tapped density (gm/cm³)	Carr's index (%)	Hausner's ratio
F1	14.26 °	0.3875	0.4428	9.66	1.112
F2	16.69 °	0.3208	0.4277	11.99	1.1632
F3	19.25 °	0.38	0.5428	12.99	1.2184
F4	22.0 °	0.2846	0.4111	14.77	1.2642

Table 4: Percentage yield and entrapment efficiency

Formulation code	Percentage yield	Entrapment efficiency
F1	62.5%	68.52 %
F2	69.0 %	76.25 %
F3	74.28 %	81.46 %
F4	65.0 %	84.36%

Table 5: Particle size analysis data

S. No.	Formulation code	Average minimum radius(µ)	Average maximum radius (µ)	Average circle diameter (µ)
01	F1	1.0618±1.6051	1.712±2.0072	5.5312±7.4666
02	F2	3.7245±3.7752	4.6474±3.9243	14.322±10.536
03	F3	1.7612±1.3127	3.2710±2.5049	8.5150±5.9215
04	F4	2.7088±2.9990	2.8715±2.9026	10.613±11.114

Values shown in the table indicates mean±standard devoation for n=3

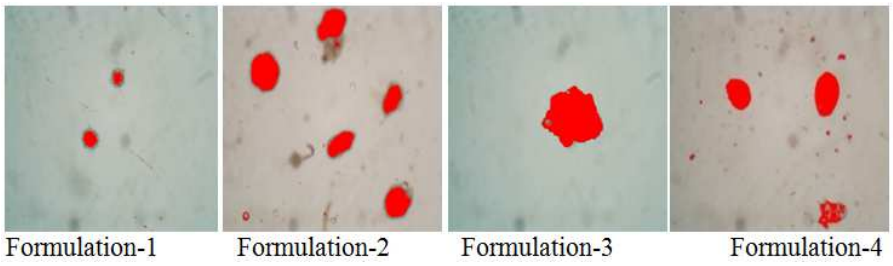

Fig. 8: Images from olympus binocular microscope of all four main formulations

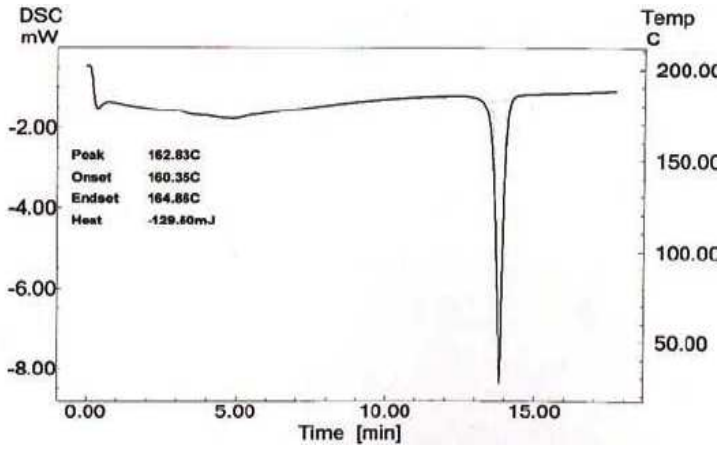

Fig. 9: Thermogram of celecoxib

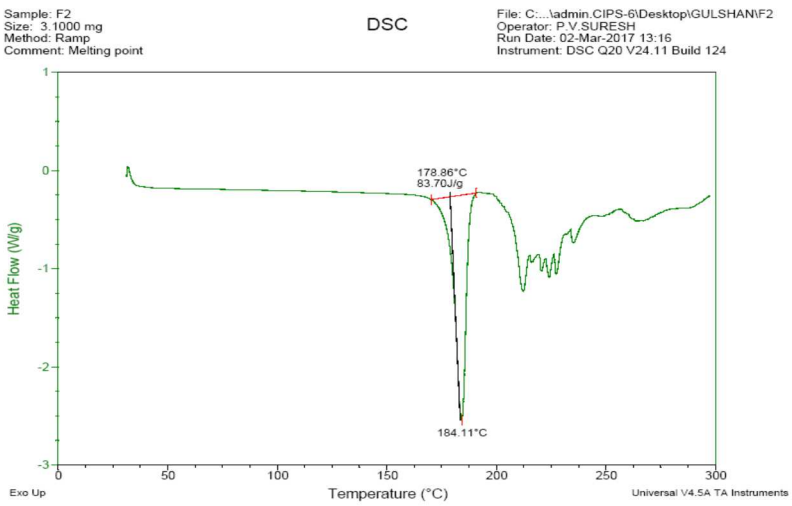

Fig. 10: Thermogram of celecoxib final formulation

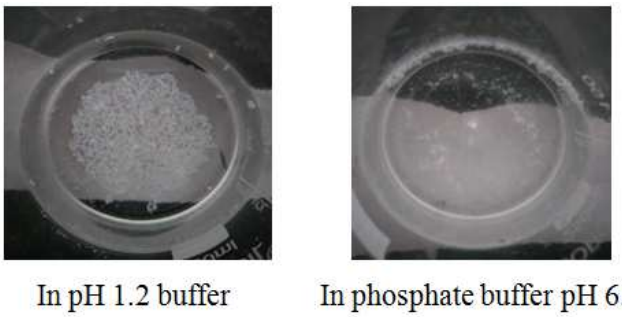

Fig. 11: Fate of micro spheres in two buffers

Kinetic treatment of data

The prepared formulations were subjected to dissolution study and the obtained data were fitted into different kinetic modelling to identify the drug release model and mechanism. The comparative dissolution data and kinetic data was given in the 7. Graphs were plotted for drug release and it was shown in the fig. 12.

Table 7: Kinetic modeling data

Formul-ation	Zero order plot			First order plot			Higuchi plot			Korsemeyer peppas plot		
	K_0	R^2	r	K_1	R^2	r	K_H	R^2	r	n	R^2	r
F_1	18.21	0.931	0.972	0.4928	0.5081	0.9648	34.61	0.7265	0.866	0.8664	0.9833	1.737
F_2	10.42	0.996	0.998	0.4157	0.9659	0.9652	21.66	0.8807	0.9508	0.9847	0.9895	1.0531
F_3	12.09	0.952	0.9800	0.1870	0.9023	0.9980	24.94	0.789	0.9097	0.6868	0.8411	1.6108
F_4	13.09.	0.922	0.9642	0.2358	0.8994	0.9800	28.31	0.777	0.9062	0.4723	0.6781	1.954

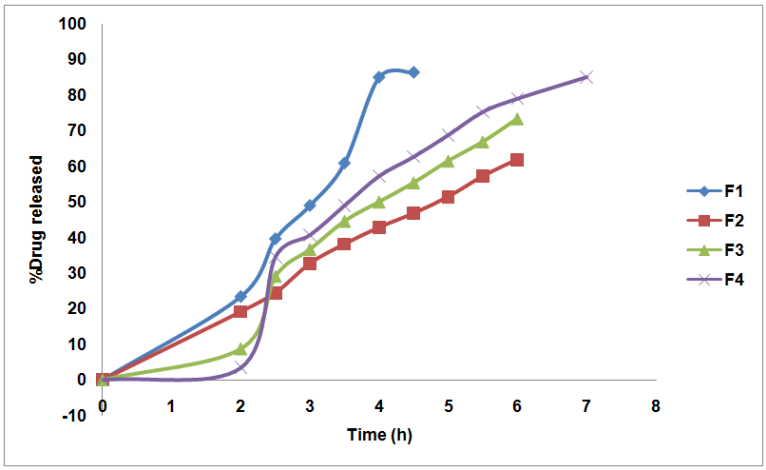

Fig. 12: Zero order plot for the formulations F1–F4

CONCLUSION

Documentation of adverse side effects of systemically delivered celecoxib envisaged the development of a formulation that could deliver the drug to the defined site. Studies have proven that local application of celcoxib is postulated to be advantageous over systemic circulation in the context of FAP for chemoprevention of cancer onset [9]. The current study data implies that microspheres containing celecoxib can be formulated by emulsification solvent evaporation technique by using HPMC K_4M and eudragit L 100-55 as polymers, alcohol and acetone as solvents, span 80 as a surfactant and dibutyl phthalate as a plasticizer. The I. R. spectra and DSC thermograms revealed that there was no interaction between polymers and drug. All the polymers used were compatible with the drug. The prepared microspheres were strong spherical with narrow size distributions could be prepared with high yields and good entrapment efficiencies. By increase in the percentage of polymer concentrations significantly affected on the size of spheres i.e. a gradual increase in the size of spheres and the optimum release of celecoxib in the intestinal region was achieved.

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CONFLICT OF INTERESTS

Declare none

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