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Design and Evaluation of Microcapsules of Flurbiprofen for Colon Specific Drug Delivery.

Mukesh Kumar Sharma[1]*, K Kavitha[1], S D Jagadeesh Singh[1], and M Rupesh kumar[2].

[1]East Point College of Pharmacy, Bangalore - 49, Karnataka, India.
[2]The Oxford College of Pharmacy, Bangalore -68, Karnataka, India.

ABSTRACT

The main objective of the present research work was to develop and evaluate Flurbiprofen (FP) loaded microcapsules using pH dependent polymers for colon specific drug delivery. Microcapsules of FP were prepared by emulsion solvent evaporation techniques by using Eudragit RS/RL-100 as polymers. The basic design of pulsatile device consists of an insoluble capsule body, filled with FP microcapsules and sealed with hydrogel polymer plug. The entire device was enteric coated with 5% Cellulose Acetate phthalate (CAP) solution, The prepared FP microcapsules were subjected to evaluate surface morphology by scanning electron microscopy (SEM), particle size and size distribution, %yield, drug content, entrapment efficiency, *in vitro* dissolution studies, and release kinetics. Three optimized formulation were selected and further used into fabrication of pulsatile capsule. The FP microcapsules was spherical in nature, which was confirmed by SEM. FP microcapsules with normal frequency distribution were obtained. A maximum of 89.50% drug entrapment efficiency was obtained. The *In-vitro* performance showed that sustained release was dependent upon the polymer concentration. The regression co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model were found to be Non-Fickian. The present study conclusively demonstrates the programmable pulsatile, colon specific release has been achieved from a capsule device over a 24 hr period, consistent with the demands of chronotherapeutic drug delivery.

Keywords: Pulsatile, colon specific device, Chronotherapeutics, Circadian rhythm, Flurbiprofen, Arthritis.

Corresponding author

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INTRODUCTION

The drug delivery systems have drawn an increasing interest over the last few decades. Colon targeted drug delivery is used to deliver the substances that are degraded by the digestive enzymes in the stomach such as proteins and peptides. Colon targeted drug delivery of drugs reduces the systemic side effects. Colon targeted drug delivery system increases the absorption of poorly absorbable drugs due to the high retention time of the colon. Colon-specific formulation could also be used to prolong the drug delivery. [1-3]

Microencapsulation is a process in which tiny particles or a coating to give small capsules with many useful properties surrounds droplets. One of the advantages of microencapsulation is that the administered dose of a drug is subdivided into small units that are spread over a large area of the gastrointestinal tract, which may enhance absorption by diminishing localized drug concentration. [4-9]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are an important class of drug commonly administered for both acute relief of pain, inflammation and fever and a variety of chronic conditions from arthritis to cardiovascular disease, the latter primarily due to association of aspirin consumption and a lowered incidence of stroke and cardiac events. NSAIDs are commonly used, but also have risks associated with their use, including significant upper gastrointestinal tract bleeding. [10]

Flurbiprofen, a, 2-(2-flurobiphenyl-4yl) propionic acid belongs to a group of non-steroidal anti-inflammatory drugs used for the treatment of mild to moderate pain. [11]They impart their action by inhibiting the synthesis of prostaglandins involved in pain and inflammation. [12]

There are many methods for microencapsulation and selection of method depends on hydrophilicity or hydrophobicity of the drug. A well designed modified drug delivery system can overcome many of the problems of conventional dosage forms and enhance the therapeutic efficacy of the administered drug.

The concept of this formulation can also be utilized to minimize the irritant effect of weakly acidic drugs on the stomach by avoiding direct contact with the mucosa. The present work was carried out for the development and evaluation of microencapsulated flurbiprofen for colon specific drug delivery. [13-15]

EXPERIMENTAL

MATERIALS

Flurbiprofenpure sample obtained from Sun Pharmaceuticals Industries Ltd, Mumbai. Eudragit **R**L-100 and Eudragit **R**S-100 (Evonik Industries, Mumbai).Cellulose Acetate Phthalate (Spectrochem Pvt Ltd., Mumbai). Ethyl cellulose and HPMC 15K (Colorcon Asia Pvt. Ltd., Goa), All other reagents and solvents used are of analytical grade.

METHODS

PREPARATION OF MICROCAPSULES OF FLURBIPROFEN (FP)

Emulsification-solvent evaporation method.

Table 1: Formulation Design of FP Microcapsules.

Sl No	Ingredients	Formulation code					
		FP1	FP2	FP3	FP4	FP5	FP6
1	Drug(mg)	500	500	500	500	500	500
2	Eudragit **R**L-100 (mg)	170	250	330	170	-	-
3	Eudragit **R**S -100 (mg)	330	500	-	-	170	330
4	Span-80 (ml)	0.5	0.5	0.5	0.5	0.5	0.5
5	Acetone (ml)	25	25	25	25	25	25

Accurately weighted Eudragit **R**L-100 and **R**S-100 in 1:2 ratios were dissolved in acetone to form a homogenous polymer solution. Core material, i.e. FP was added to the polymer solution and mixed thoroughly.

This organic phase was slowly poured at 15oC into liquid paraffin (150 ml) containing 1% w/w of Span-80 with stirring at 1200 rpm to form a smooth emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone evaporated and smooth-walled, rigid and discrete microcapsules were formed. The microcapsules were collected by decantation and the product was washed with petroleum ether (40-60oC), four times and dried at room temperature for 3 hrs. The microcapsules were then stored in a desiccators over fused calcium chloride. Six batches from FP1 to FP6 were prepared with different proportions of core to coat materials. Where FP1 and FP2 contain the drug with polymers Eudragit RL/RS 100, FP3 and FP4 contains the drug with Eudragit RL100 and FP5 and FP6 contain the drug with Eudragit RS100 (Table 1).

EVALUATION OF MICROCAPSULES

Particle size and Surface morphology

Determination of average particle size of FP microcapsules was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microcapsules was spread on a clean glass slide and average size of 300 microcapsules was determined in each batch.

Scanning Electron Microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. The studies were carried out by using JEOL JSM T-330 a Scanning microscope (Japan). Dry microcapsules were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microcapsules were taken by random scanning of the stub.

Frequency distribution analysis

Determination of average particle size of FP microcapsules was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microcapsules was spread on a clean glass slide and average size of 300 FP microcapsules was determined in each batch. In order to be able to define a frequency distribution or compare the characteristics of particles with many different diameters, the frequency distribution can be broken down into different size ranges, which can be presented in the form of a histogram. Histogram presents an interpretation of the frequency distribution and enables the percentage of particles having a given equivalent diameter to be determined.

% Yield and Drug content

The percent yield of each of the sample was calculated from the expression:

$$\% \text{ Yield} = \frac{weight\,of\,microparticles}{weight\,of\,solid\,starting\,materials} \text{ X } 100$$

Determination of percentage drug entrapment efficiency (PDE)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula

$$\text{PDE} = \frac{Practical\ drug\ content}{theoretical\ drug\ content} \text{ X } 100$$

Drug content

In a 100 ml volumetric flask, 25 mg of crushed microcapsules were taken, and volume was made up to mark with pH 7.4. The flask was shaken for 12 hours using an orbital shaker incubator. Then the solution was filtered and from the filtrate appropriate dilutions were made and absorbance was measured at 247nm.

***In-vitro* dissolution studies**

In-vitro dissolution profile of each formulation was determined by employing USP XXIII rotating basket method (900 ml pH 1.2, pH 6.8 and pH 7.4 phosphate buffer, 100 rpm, 37o±0.5oC). Microcapsules equivalent to 150 mg of FP was loaded into the basket of the dissolution apparatus. 5 ml of the sample was withdrawn from the dissolution media at suitable time intervals and the same amount was replaced with fresh buffer. The absorbance of the filtrate was determined at wavelength of 247nm against pH 7.4 blank. The amount of drug present in the filtrate was then determined from the calibration curve and cumulative percent of drug release was calculated Data obtained was also subjected to kinetic treatment to obtain the order of release and release mechanism.

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q0-Q) v/s t], Higuchi's square root of time (Q v/s t1/2) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0-Q) is the cumulative percentage of drug remaining after time t.

PREPARATION OF CROSS-LINKED GELATIN CAPSULES

Formaldehyde treatment

Formalin treatment has been employed to modify the solubility of the gelatin capsules. Hard gelatin capsules of size 0 and 100 in number were taken. Their bodies were separated from the caps. 25 ml of 15% v/v formaldehyde was taken into dessicator and a pinch of potassium permanganate was added to it, to generate formalin vapors. The wire mesh containing the bodies of the capsule was then exposed to formaldehyde vapors. The dessicator was tightly closed. The reaction was carried out for 12 hrs after which the bodies were removed and dried at 50oC for 30 minutes to ensure completion of reaction between gelatin and formaldehyde vapors. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated caps and stored in a polythene bag.

Test for formaldehyde treated empty capsules

Various physical and chemical tests such as Identification attributes, visual defects, dimensions, Solubility studies of the treated capsules and qualitative test for free formaldehyde were carried out simultaneously for formaldehyde treated and untreated capsules.

FORMULATION OF PULSATILE (MODIFIED PULSINCAP) DRUG DELIVERY SYSTEM

Formaldehyde treated hard gelatin capsules of 'size 0' were chosen for the formulation. The bodies and caps were separated manually. Microcapsules equivalent to 150 mg of FP were accurately weighed and filled into the treated bodies by hand filling. The capsules containing the microcapsules were then plugged with HPMC hydrogel at different concentration. The joint of the capsule body and cap was sealed with a small amount of the 5% Ethyl Cellulose ethanolic solution. The sealed capsules were completely coated with 5% Cellulose Acetate Phthalate (CAP) to prevent variable gastric emptying. Coating was repeated until an 8-12% increase in weight is obtained. % Weight gain of the capsules before and after coating was determined. The whole system thus produced is modified pulsincap.

Coating of pulsincap

5% w/w solution of CAP was prepared by using acetone: ethanol (8:2) as solvent and dibutyl phthalate as plasticizer (0.75%). Dip coating method was followed to develop the pulsincap. The capsules were alternatively dipped in 5 % CAP solution and dried. Coating was repeated until an expected weight gain of 8-12 % was obtained and the capsule resists disintegration in 0.1 N HCl for a minimum period of 2 hrs.

EVALUATION OF MODIFIED FP PULSINCAP

Thickness of cellulose acetate phthalate coating

ISSN: 0975-8585

The thickness of the cellulose acetate phthalate coating was measured using screw gauge. It was expressed in mm.

Weight variation

10 capsules were selected randomly from each batch and weighed individually for weight variation. The test requirements are met if none of the individual weights are less than 90% or more than 110% of the average.

***In- vitro* release profile**

Dissolution studies were carried out by using USP XXIII dissolution test apparatus (paddle type) with rotation speed of 100 rpm. 900 ml of the dissolution medium was used and the temperature of the medium was maintained at 37oC± 0.5oC. The pH 1.2 buffer for 2 hours (since the average gastric emptying time is 2 hrs), pH 7.4 buffer for 3 hours (average small intestinal transit time is 3 hours) and pH 6.8 buffer for subsequent hours were used. Capsules were tied to paddle with a cotton thread in each dissolution vessel to prevent floating. 5 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed and the amount of FP released was determined by UV absorption spectroscopy.

STABILITY STUDIES

The stability study was carried out for selected formulation as per ICH guidelines. Various ICH storage conditions are available which are as 250C ± 20C (60 % ± 5% RH), 300C ± 20C (65 % ± 5% RH) and 400C ± 20C (75 % ± 5% RH). The microcapsules of the best formulation (F2) were placed in screw capped glass container and stored at various ICH storage condition for a period of 60 days. The samples were analyzed for physical appearance and for the drug content at regular interval of 15 days.

STATISTICAL ANALYSIS

The data were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by using Graph Pad (Instat) prism software version 5.0.

RESULTS AND DISCUSSION

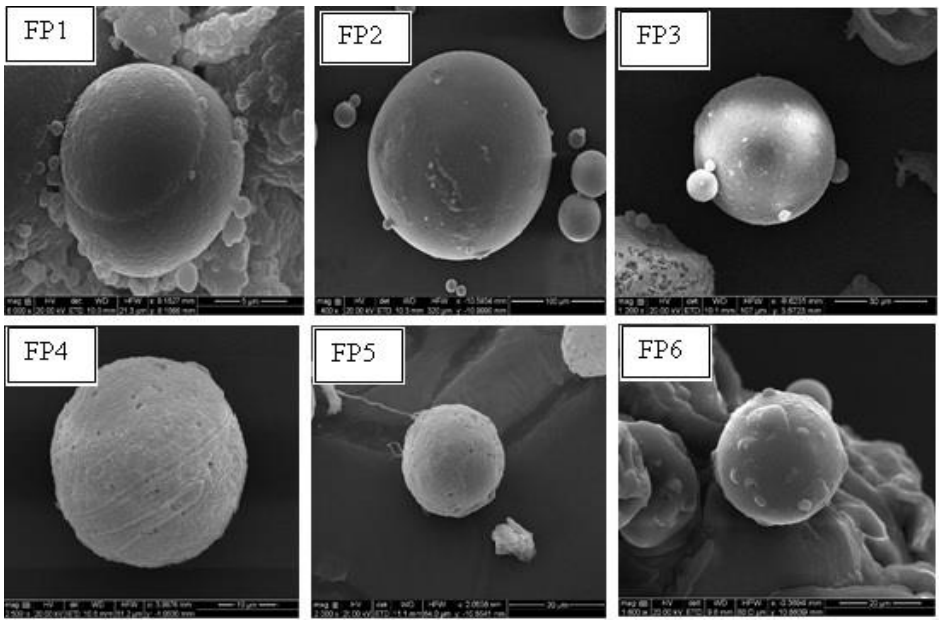

Figure1: SEM Photography of FP microcapsules

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In the present study, an attempt was made to develop and evaluate pulsatile drug delivery system containing Eudragit microcapsules of FP for colon specific delivery and for better treatment of Arthritis. Colonic delivery of FP could prevent unwanted systemic side effects and subsequently a lower dose of the drug may be sufficient to prevent the early morning symptoms in the Arthritis.

Scanning electron microscopy was performed to characterize the surface of the formed microcapsules. Surface smoothness of FP microcapsules was increased by increasing the polymer concentration. FP microcapsules with smooth surface were obtained and are shown in Figure 1.

The mean particle size of the microcapsules significantly increased with increase in polymer concentration and was ranged in between 564 to 810µm as shown in Figure 2. The reason must be, the viscosity of medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This may be resulting in the formation of larger particles. FP microcapsules showed the normal frequency distribution of particles (Figure3)

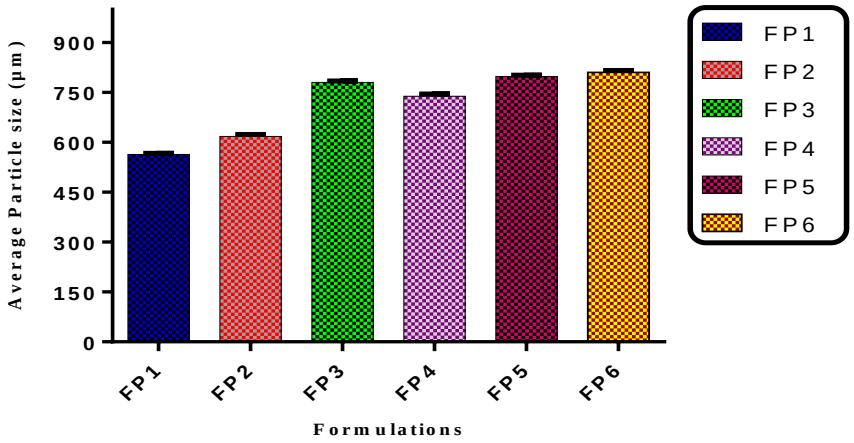

Figure2: Average Diameter of FP microcapsules

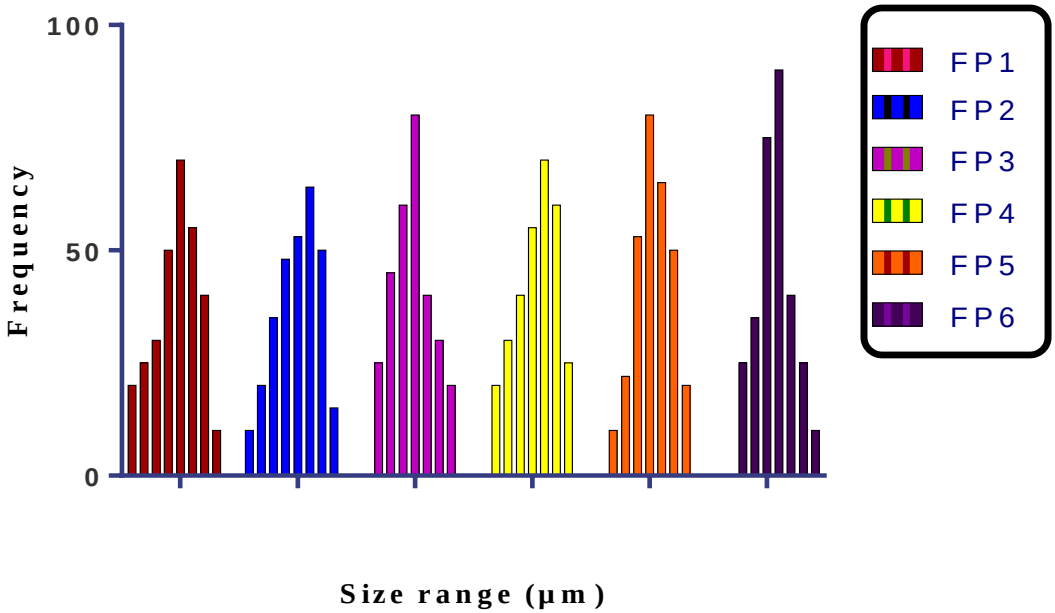

Figure 3: Frequency distribution of FP microcapsules

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The mean particle yield for all the formulations was more as shown Table 2. A positive correlation between solid content and percentage yield was observed. This may be explained by the fact that though a constant amount of material is always lost in processing, this loss is proportionately less significant when the solid content is more (e.g. If the loss in processing is 10 mg then it is more significant for a 100 mg sample, but much less significant for a 500 mg sample).

The drug content was found to be very high in all the cases probably due to polymer loss by adherence to the container as a result of viscous nature of slurry. Amount of microcapsules to be taken for *in vitro* release studies and further development of pulsincap was calculated based on content of drug present in each formulation.

Entrapment efficiency increase with increase in the polymer concentration from the results it can be inferred that there is a proper distribution FP in the microcapsules and the deviation is within the acceptable limits. The percentage of the drug content found to be in the range of 20% to 85%. The percentage entrapment efficiency was found to be 60% to 90%. The result obtained was given in the Table 2 and Figure 4.

Table 2: Drug entrapment efficiency of FP microcapsules

Formulation	% Yield	% Drug Content	Entrapment Efficiency (%)±SD
FP1	66.49	70.82	60.60 ± 2.50
FP2	86.99	80.32	89.50 ± 1.29
FP3	69.85	50.59	70.00 ± 3.50
FP4	89.18	69.82	86.20 ± 4.07
FP5	59.61	30.22	66.00 ± 3.30
FP6	82.66	70.68	80.00 ± 1.85

SD= Standard Deviation (n=3)

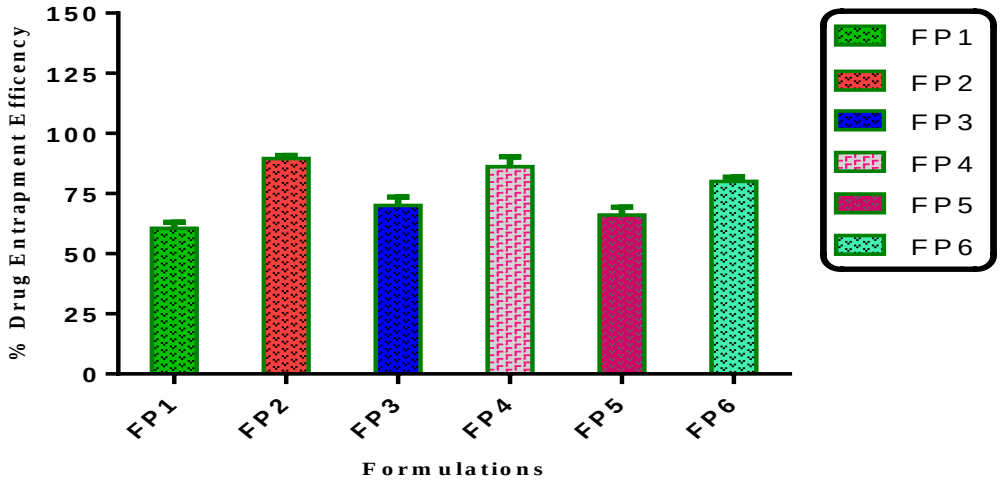

Figure4: Drug entrapment efficiency of FP microcapsules

It was observed that the drug release from the formulations decreased with increase in the amount of polymer added in each formulation. The release showed a bi-phasic release with an initial burst effect. In the first hour drug release was 7.03%, 8.25%, 15.32%, 12.46%, 17.09% and 14.14% for FP1 to FP6 respectively. The mechanism for the burst release can be attributed to the drug loaded on the microcapsules or imperfect entrapment of drug. The overall cumulative % release for FP1, FP2, FP3, FP4, FP5 and FP6 were found to be 93.92%, 82.84%, 91.34%, 85.43%, 89.85% and 87.34% at the end of 12th hour. The release data obtained for all the six formulations were shown in Figure 5.

ISSN: 0975-8585

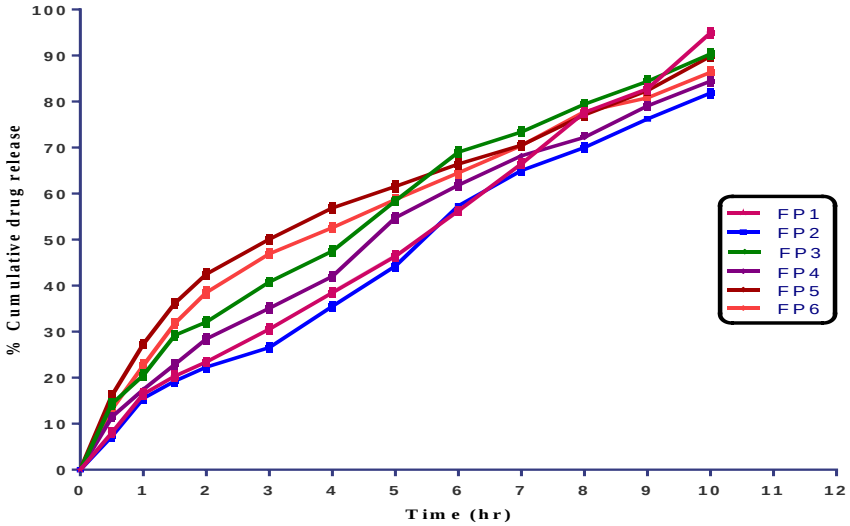

Figure 5: *In-vitro*release profile of FP microcapsules

In-vitro release kinetics Plots of zero order, first order, Higuchi matrix, Peppas depicted in Fig 6. Regression co-efficient (r2) values of different kinetic models and diffusion exponent (n) of Peppas model for FP microcapsules are indicated in Table 3. From the regression co-efficient (r2) values it was observed that the FP microcapsules followed the zero order release kinetics. Higuchi matrix kinetics indicated that the diffusion release of drug from FP microcapsules. Diffusion exponent (n) of Peppas model for FP microcapsules confirmed the Non Fickian diffusion mechanism of drug from FP microcapsules.

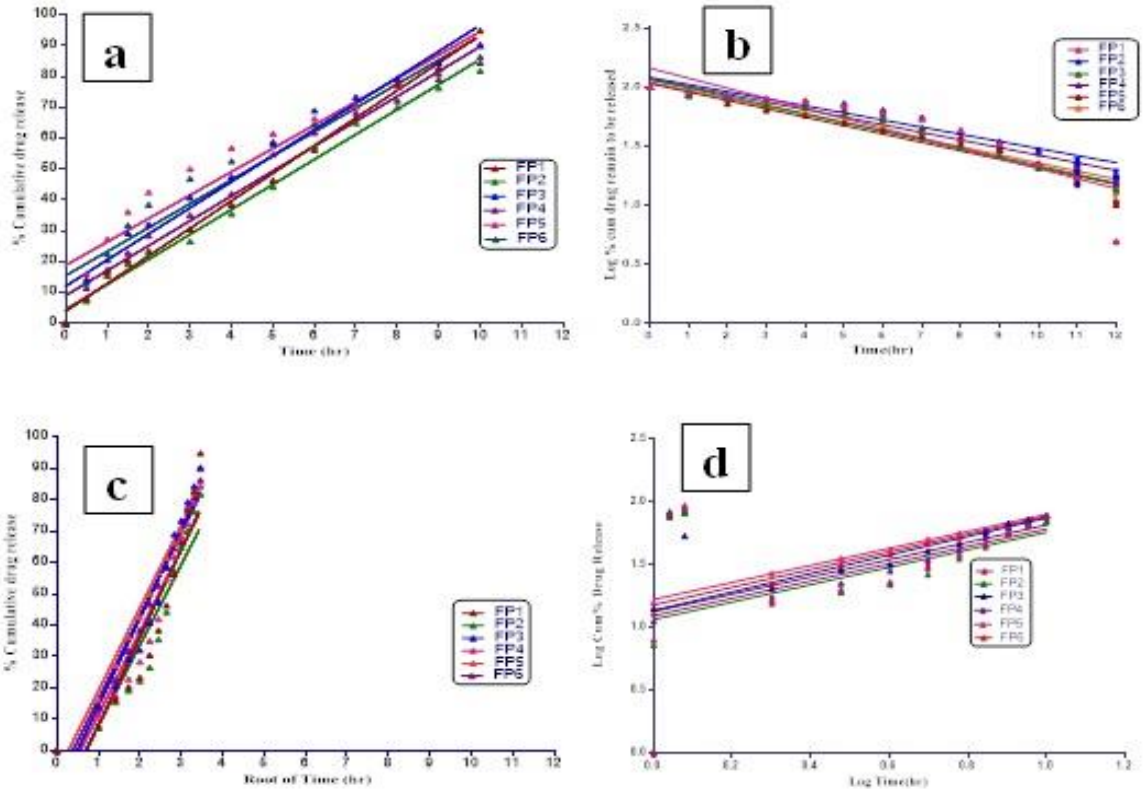

Figure 6: *In-vitro* release kinetics profile of FP microcapsules. (a) Zero order (b) first order (c) Higuchi matrix (d) Peppas

ISSN: 0975-8585

Table 3: Regression co-efficient (r2) values of different kinetic models and diffusion exponent (n) of Peppas model for FP microcapsules.

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				r2 value	n value
FP1	0.9860	0.9720	0.9867	0.9897	0.7708
FP2	0.9950	0.8414	0.8667	0.9364	0.8996
FP3	0.9954	0.9728	0.9514	0.9917	0.7826
FP4	0.9964	0.9643	0.9380	0.9897	0.8236
FP5	0.9798	0.9597	0.9911	0.9880	0.6415
FP6	0.9899	0.9787	0.9788	0.9969	0.7562

The bodies of hard gelatin capsules were made insoluble by formaldehyde treatment. This was done by exposing the bodies of the capsules to vapors offormaldehyde; the caps were not exposed leaving them water-soluble. The capsules were tested for physical and chemical changes caused by exposure to vapors of formaldehyde. On formaldehyde treatment, the '0' size capsule bodies showed a significant decrease in length and diameter. When the capsules were subjected to studies in different buffers, the untreated caps disintegrated within 10 minutes in all the media whereas the treated bodies remained intact for about 24 hours.

Limit test for the presence of residual formaldehyde, indicated that the amount of formaldehyde present in the treated capsules was well within limits (the sample solution was not more intensely colored than the standard inferring that less than 20 µg/ml of free formaldehyde is present in 25 capsules bodies) as per the literature.

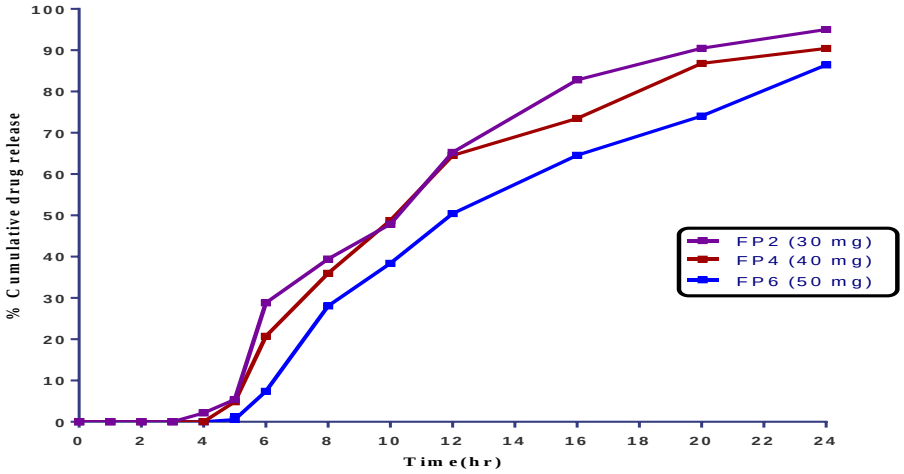

Figure 7: Comparative *Invitro* release profile of formulation containing HPMC as hydrogel plug in different concentration.

Microcapsules equivalent to 150 mg of FP were filled into the formaldehyde treated bodies and plugged with HPMC at different concentrations. The filled capsules were completely coated with 5% CAP cast solution. These pulsatile drug delivery systems were then evaluated for thickness of the CAP coating and *in-vitro* release. The thickness of the cap coating was measured by using screw gauge. The values ranged from 0.057-0.069 mm.

In-vitro release profiles of pulsatile device during 24 hrs studies were found to have very good sustaining efficacy (Fig 7). During dissolution studies, it was observed that, the enteric coat of the cellulose acetate phthalate was intact for 2 hours in pH 1.2, but dissolved in intestinal pH, leaving the soluble cap of capsule, which also dissolved in pH 7.4 then the exposed polymer plug absorbed the surrounding fluid, swelled and released the drug through the swollen matrix. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body; releasing the Eudragit microcapsules into simulated colonic fluid (pH 6.8 phosphate buffer). With all the formulations, there was absolutely no drug

release in pH 1.2, thus indicating the efficiency of 5% CAP for enteric coating. Very slight release was observed in pH 7.4.

With formulation FP2 (30 mg), FP4 (40 mg), at the end of 5th hour 6.38% and 7.40 % of drug was released respectively and at the end of 20th hour FP2 formulation had released 91.47 % of drug, whereas FP6 formulation released 87.82% of drug up to 24 hours in controlled manner.

In case of FP6 (50 mg), hydrogel plug ejected out in between 6th to 8th hour, indicating decrease in expelling power of plug, due to inadequate wetting of polymer at higher concentration. At the end of 24th hr 85.42% of drug was released.

The selected formulation (FP2) subjected to stability test the studies were carried out in view of the potential utility of the pulsatile device for targeting the FP to colon. The results indicated that the selected formulations showed no change in physical appearance and drug content (Table 4).

Statistical significance of all the data was analyzed by using Graph Pad (Instat) prism software version 5.0. The statistical values (< 0.05) were statistically significant.

Table 4: Data of Stability Studies

NO. of Days	250C ± 20C/60% RH ±5% RH		300C ± 20C/65% RH ±5% RH		400C ± 20C/75% ± 5% RH	
	Physical Appearance	% Drug Entrapment	Physical Appearance	%Drug Entrapment	Physical Appearance	%Drug Entrapment
0	No Change	100	No Change	100	No Change	100
15	No Change	99.58	No Change	98.44	No Change	98.12
30	No Change	99.15	No Change	98.21	No Change	97.44
45	No Change	98.71	No Change	97.30	No Change	96.10
60	No Change	98.04	No Change	96.97	No Change	95.16

CONCLUSION

Pulsatile drug release over a period of 2-24 hours, consistent with the requirements for chronopharmaceutical drug delivery was achieved from insoluble gelatin capsules, in which microencapsulated FP was sealed by means of a suitable hydrogel plug. Thus pulsatile drug delivery system can be considered as one of the promising formulation technique for preparing colon specific drug delivery systems and hence in chronotherapeutic management of Arthritis.

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