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FORMULATION AND EVALUATION OF PULSATILE DRUG DELIVERY CAPSULE OF KETOROLAC TROMETAMOL

Chauhan Amitsingh[*1] and Dr. L. D. Patel[2]

[1]503 B, Cosmos Orchid Blossom, Kasarvadavali, Thane, Maharashtra, India-400615.

[2]19, Devchhaya Society, Ghatlodia, Ahmedabad, Gujarat, India-380061.

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***Corresponding Author**
Chauhan Amitsingh
503 B, Cosmos Orchid Blossom, Kasarvadavali, Thane, Maharashtra, India-400615.

ABSTRACT

A time and pH dependent pulsatile drug delivery system of ketorolac trometamol (KT) was proposed for the chronotherapeutic treatment of rheumatoid arthritis. It was fabricated in three steps. First, KT was entrapped with cellulose acetate. Second, these core microspheres were coated with eudragit L-100 and S-100. Finally, the coated microspheres were filled in capsule with a soluble cap, sealed with hydrogel-plug (HPMC K100M/guar gum/xanthan gum) and entire capsule was enteric coated with Hydroxypropyl methylcellulose Phthalate (HPMC Phthalate). SEM revealed that the microspheres were discrete and uniform. The size distribution of the microspheres was narrow with an arithmetic mean size of 368.81µm. During dissolution, pulsatile capsules remained intact in pH 1.2 for 2 hours indicating the enteric coating efficiency of HPMC Phthalate. The enteric coat dissolved when pH was changed to 7.4, leaving the soluble cap apart. Then, the exposed polymeric plug absorbed the surrounding fluid, swelled and released the drug through solvent matrix. When polymeric plug was completely wetted by dissolution fluid, it was easily ejected out of the capsule and liberated the microspheres in to the simulated colonic fluid (pH 6.8 phosphate buffer). No drug release was observed in first three hours from all pulsatile capsules which was required for chronotherapeutic delivery of KT. The rank order of polymer plug for sustaining drug release was HPMC K100M>guar gum>xanthan gum. The designed pulsatile delivery systems exhibited first order diffusion controlled drug release. The study conclusively proved that the designed pulsatile capsule is a promising chronotherapeutic delivery system for rheumatoid arthritis.

KEYWORDS: Ketorolac trometamol; rheumatoid arthritis; enteric coating; polymeric plug; chronotherapeutic delivery.

INTRODUCTION

Several disease states have been proven to follow biological rhythms, expressed by short, intermediate and long-period oscillations. Circadian (24 hours) time structure is the most common oscillation in a number of pathological cases such as asthma where the crisis is mostly happening late at night, osteoarthritis where the pain is more intense during night, rheumatoid arthritis where the pain peaks at the morning and duodenal ulcer where the gastric secretion increases during the night.[1] Morning stiffness associated with pain at the time of awakening is a diagnostic criterion of the rheumatoid arthritis and the clinical circadian symptom is supposed to be outcome of altered functioning of hypothalamic–pituitary–adrenocortical axis. Chronopharmacotherapy for rheumatoid arthritis has been recommended to ensure the highest blood levels of the drug coincide with peak pain and stiffness.[2]

Drug pharmacokinetic also shows circadian variation for various anti-inflammatory drugs like indomethacin, ketoprofen and diclofenac sodium which have greater absorption in morning as compared to evening, and site-specific absorption from small intestine. Therefore, the desired drug release should be time specific as well as site specific to develop a dosage form for chronopharmacotherapy.[3]

Ketorolac trometamol is a nonsteroidal agent with analgesic and anti-inflammatory activity acting by inhibiting prostaglandin synthesis.[4] Unlike a narcotic analgesic, ketorolac does not alter gastric motility or hemodynamic variables or adversely affect respiration, it is not associated with adverse central nervous system effects, abuse, or addiction potential. Therefore, ketorolac is a relatively more preferred therapeutic agent for the management of moderate to severe pain.[5] Ketorolac (as trometamol salt) is administered intramuscularly or orally in divided multiple doses (30 mg four times in a day by IM injection or 10 mg four times in a day as oral tablet). The frequent dosing due to the short half-life of the drug (4-6 hours) results in unacceptable patient compliance[6] To avoid invasive drug therapy using injection or to eliminate frequent dosing regimen, the novel formulation of ketorolac trometamol is need of the time which may exhibit time specific and site specific drug release.

Pulsatile drug delivery systems are gaining more interest as they deliver the drug at the site of action at the right time and in the right amount. The use of pulsatile release is ideal for the drugs where a constant drug release (zero-order release) is not desired. The pulsatile effect,

i.e., the release of drug as a "pulse" has to be designed in such a way that drug release should follow the lag time. These systems are beneficial for drugs which have high first-pass effect, drugs administered for the diseases that follow chronopharmacological behavior, drugs having specific absorption site in GIT, targeting to colon; and cases where night time dosing is essential.[7,8]

The designed pulsatile capsule consisted of a non-disintegrating capsule body and a soluble cap. The microencapsulated drug formulation was coated using pH sensitive methacrylic acid polymers, e.g., eudragit L-100 and S-100. Eudragit coated microspheres were filled in capsule body and separated from the cap by a hydrogel plug (i.e. Guar gum, xanthan gum or HPMC K100M). The entire capsule was enteric coated with HPMC Phthalate to prevent variable gastric emptying. On reaching the small intestine pH, the capsule lost its enteric coating and the hydrogel polymer plug inside the capsule swelled to create a lag phase that equals the small intestinal transit time. This plug ejected on swelling and released the microencapsulated drug from the capsule in the colon. The controlled release of ketorolac trometamol was achieved for up to 24 hours. With this system our goal was to avoid drug delivery in the upper GIT and target drugs to the terminal ileum and colonic region to achieve the chronotherapy of rheumatoid arthritis.

MATERIALS AND METHODS

Materials

Ketorolac trometamol was gifted from Symed labs limited, India. Redson Pharmaceuticals Pvt Ltd, India has supplied (#00) size hard gelatin capsules as gift. Eudragit L-100 and S-100 were gifted by Rohm Pharma, Germany. Xanthan gum was obtained as a gift sample from Five star pharmaceuticals, India. Guar gum was gifted from Krystal colloid, India. HPMC K100M was supplied by Colorcon India Pvt. Ltd. HPMCP and ethyl celluloses were provided by Sunrise remedies, India. Cellulose acetate and the rest of chemicals were obtained from S D Fine Chem Ltd, India and used as received without further purification.

Methods

Method for preparation of ketorolac trometamol loaded cellulose acetate microspheres

Ketorolac trometamol loaded cellulose acetate microspheres were formulated and evaluated in the earlier research work.[9] Core microspheres were prepared by emulsion solvent evaporation technique using liquid paraffin (light/heavy). Accurately weighed quantity of cellulose acetate was dissolved in 10 ml solvent blend of acetone and ethanol (8:2) to get a

homogenous polymer solution. 100 mg ketorolac trometamol was dissolved in the polymeric solution. The resulting solution was added drop wise into 100 ml liquid paraffin containing Span 80 or Tween 80 as surfactant. 10 ml of acetone was added to the external phase to produce a stable emulsion. The system was maintained under constant stirring using an overhead stirrer at room temperature to allow complete solvent evaporation. The microspheres were filtered, washed, dried in a vacuum oven and stored in a closed container.

Based on the optimization of formulation and process parameters by applying 3^2 full factorial design, maximum % practical yield (87.00%) and maximum % Encapsulation efficiency (88.69%) were achieved with the microspheres at +1 level of X_1 (250 mg of cellulose acetate) and 0 level of X_2 (1.0 g of Span 80) in the factorial optimization study. The formulation of the optimized batch based on the preliminary study and factorial design of earlier work were presented in Table 1.

Table 1: Formulation of ketorolac trometamol loaded cellulose acetate microspheres.

Amount of ketorolac trometamol	100 mg
Amount of cellulose acetate	250 mg
Amount of Span 80	1.0 g
Dispersion medium	Light liquid Paraffin
Batch size	100 ml
Stirring speed	500 RPM
Stirring time	3 hours

Preparation of eudragit coated ketorolac trometamol loaded cellulose acetate microspheres[10]

Ketorolac trometamol loaded cellulose acetate microspheres of earlier research work were used as core microspheres and were coated with different amounts of the eudragit L-100 and S-100 combination (ratio 1:1) using the solvent evaporation technique. Weighed quantity of eudragit L-100 and eudragit S-100 were dissolved in 5 ml ethanol, ketorolac trometamol loaded cellulose acetate microspheres were suspended in the ethanolic solution of eudragit L-100 and eudragit S-100 (1:1) and emulsified into 100 ml of light liquid paraffin containing 1 g of span 80 as surfactant. The system was maintained at room temperature under continuous stirring at 500 rpm using an overhead stirrer to allow complete solvent evaporation. The coated microspheres were filtered, washed, dried in a vacuum oven and stored in a closed container. The formulation details were given in Table 2.

Table 2: Formula of eudragit coated microspheres of ketorolac trometamol.

Batch	Core: Coat	Amount of core microspheres (mg)	Coating material (mg)	
			Eudragit L-100 (mg)	Eudragit S-100 (mg)
E_1	1:2	100	100	100
E_2	1:3		150	150
E_3	1:4		200	200
E_4	1:5		250	250

Evaluation parameters of eudragit coated ketorolac trometamol loaded cellulose acetate microspheres

Particle size analysis of microspheres[11]

Prepared microspheres were separated by sieving method using a range of standard sieves 12/16, 16/20, 20/30, 30/40 and the amount retained on each sieve was weighed. The average size of the microsphere was calculated.

Scanning electron microscopy (SEM) of microspheres[12]

The particle size, shape and surface morphology of microspheres were examined by scanning electron microscopy. SEM allows investigations of the microspheres surfaces. Microspheres were fixed on aluminum studs and coated with carbon using a sputter coater SC 502, under vacuum (0.1 mm Hg). The microspheres were analyzed by SEM (Model LEICA S-430, London, U.K.).

% Practical yield

The total amount of microspheres obtained were weighed and the % practical yield was calculated by taking into the consideration the weight of drug and excipient.

$$\% \text{ Practical yield} = \frac{\text{Amount of microspheres obtained X 100}}{\text{Total weight of drug and excipient}} \quad \ldots\ldots \text{(Equation 1)}$$

% Encapsulation efficiency[13]

Crushed microspheres containing equivalent to 25 mg of ketorolac trometamol were dissolved in 10 ml of acetone. The solvent was allowed to evaporate and the residue left behind was vortexed with 100 ml of methanol for 30 minutes to extract the ketorolac trometamol. The dispersion was filtered, 1 ml of filtered solution was withdrawn and diluted to 10 ml with methanol. The content was analyzed spectrophotometrically at 323 nm against methanol as blank. The drug content was estimated in triplicate using calibration curve constructed in the methanol.

The encapsulation efficiency was calculated using the equation 2:

$$\% \text{ Encapsulation efficiency} = \frac{\text{Estimated drug content X 100}}{\text{Theoretical drug content}} \ldots\ldots \text{(Equation 2)}$$

In vitro dissolution study

In vitro dissolution profile of ketorolac trometamol from the eudragit coated formulation was determined by employing USP XXIV rotating basket type apparatus (Model TDT-08L-Electrolab). Microspheres equivalent to required quantity of ketorolac trometamol were filled into muslin cloth and loaded into the basket of the dissolution apparatus. Dissolution study was carried in acid buffer of pH 1.2 for 2 hours followed by phosphate buffer of pH 6.8 till the end of the study. A 900 ml of buffer medium was taken, temperature of 37 ± 0.5°C and speed 100 rpm were maintained. 5 ml of the sample was withdrawn at different time intervals and the same amount was replaced with respective buffer. Withdrawn sample was filtered using filter paper and the absorbance of the filtrate was determined at 317 nm for dissolution conducted in acid buffer of pH 1.2 and at 323 for dissolution conducted in phosphate buffer of pH 6.8 by using UV-Visible spectrophotometer, using respective buffer as blank (n=3). The cumulative percent of ketorolac trometamol present in the aliquot was calculated from the calibration curve.

In vitro release kinetics

The *in vitro* release data of the optimized formulation of eudragit coated microspheres was tabulated and zero order equation, first order equation, Higuchi's model Korsmeyer-Peppas' models of drug release were used.

Design of pulsatile capsule of ketorolac trometamol

Pulsatile capsules were designed adopting the method reported by Mastiholimath *et al.*[14] Hard gelatin capsule bodies were treated with formaldehyde solution to render them insoluble in gastro intestinal fluids, 25 ml of 15% (v/v) formaldehyde aqueous solution was taken into desiccator and potassium permanganate was added to it to generate formalin vapors. About 100 numbers of empty bodies of hard gelatin capsule (# 00) were placed over wire mesh and then exposed to formaldehyde vapors. The desiccator was tightly closed, exposed for 12 hours and kept at 50°C for 30 minutes to ensure complete reaction between gelatin and formaldehyde vapors. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. Then, the eudragit coated microspheres equivalent to 40 mg of drug was loaded in to the bodies by hand filling and followed by plugging with different amounts

(15, 30 or 45 mg) of polymers, like xanthan gum, guar gum and HPMC K100M. The body and cap joined and sealed with a small amount of ethyl cellulose solution (5% w/v ethanolic aqueous solution). The sealed capsules were coated with 5% w/v HPMC Phthalate solution (prepared in 8:2 mixture of acetone: ethanol) plasticized with 0.75% v/v dibutylphthalate using dip coating to prevent variable gastric emptying. Coating was carried out until an increase in weight around 8-10% was obtained.

The % weight gain of capsules before and after coating was determined. The composition of the batches was given in Table 3.

Table 3: Composition of pulsatile drug delivery capsule of ketorolac trometamol.

Batch	Wt. of empty body (mg)	Weight of eudragit coated microspheres* (mg)	Plug material	Wt. of plug material (mg)	Total weight of capsule (mg)	Weight after HPMC Phthalate Coating (mg)
E_3X15	78	203	Xanthan Gum	15	344	367
E_3X30	78	203		30	359	383
E_3X45	80	203		45	376	401
E_3G15	79	203	Guar Gum	15	345	368
E_3G30	78	203		30	359	385
E_3G45	78	203		45	374	399
E_3H15	79	203	HPMC K100M	15	345	368
E_3H30	80	203		30	361	385
E_3H45	79	203		45	375	400

*microspheres equivalent to 40 mg of ketorolac trometamol

Evaluation parameters of pulsatile drug delivery capsule of ketorolac trometamol:

Test for formaldehyde treated empty capsule body[14]

Various tests such as identification attribute, visual defect, dimension change, solubility study are carried out on the formaldehyde treated empty capsule bodies.

Qualitative chemical test for free formaldehyde[14]

Formaldehyde solution (0.002 w/v) was used as standard solution and sample solution was formaldehyde treated capsule bodies (about 25 in number); bodies were cut into small pieces and taken into a beaker containing distilled water. It was stirred for 1 hour with a stirrer to solubilize the free formaldehyde. The solution was then filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with distilled water. For 1 ml of sample solution, 9 ml of distilled water was added. 1 ml of resulting solution was taken into a test tube and mixed with 4 ml of hot water and 5 ml of acetone reagent. The test tube

was warmed in a water bath at 40 °C and allowed to stand for 40 min. The solution should be not more intensely colored than a reference solution.

In vitro dissolution study and *in vitro* release kinetics for pulsatile drug delivery capsule

In vitro release of ketorolac trometamol from the designed pulsatile drug delivery capsule was investigated by placing it in the basket and followed the procedure described under *in vitro* dissolution study of microspheres. However, to mimic the physiological conditions of the GIT, dissolution studies was carried out using acid buffer of pH 1.2 for first 2 hours, phosphate buffer of pH 7.4 for the next 3 hours and phosphate buffer of pH 6.8 till the end of the study.[15]

The *in vitro* release kinetics of pulsatile capsule was performed as per the method described in evaluation of eudragit coated ketorolac trometamol loaded cellulose acetate microspheres.

RESULTS AND DISCUSSION

Evaluation parameters of eudragit coated ketorolac trometamol loaded cellulose acetate microspheres Selection of stirring speed

The eudragit coated microspheres of batches E_1 to E_4 were evaluated for % yield, % encapsulation efficiency, size analysis, *in vitro* dissolution study and scanning electron microscopy.

% yield and % encapsulation efficiency

The results were presented in Table 4 (n=3).

Table 4: Results of % yield and % encapsulation efficiency for eudragit coated cellulose acetate microspheres of ketorolac trometamol (Batches E_1 to E_4).

Batch	% Yield	% Encapsulation efficiency
E_1	88.77 ± 0.72	80.76 ± 0.17
E_2	91.74 ± 0.61	84.92 ± 0.11
E_3	94.89 ± 0.45	89.08 ± 0.07
E_4	94.35 ± 0.53	88.67 ± 0.09

Batch E_3 showed higher % yield (94.89 ± 0.45) and higher % encapsulation efficiency (89.08 ± 0.07). Hence, batch E_3 was selected for designing the pulsatile drug delivery capsule of ketorolac trometamol.

Size analysis

Size analysis of selected batch E_3 was carried out and the results of size analysis were presented in Table 5.

Table 5: Results of size analysis of eudragit coated cellulose acetate microspheres of ketorolac trometamol (Batch E_3).

Size range		Arithmetic mean size (Xi) (µm)	Percent* retained (Fi)	Weight Size (Xi Fi)
Mesh	µm			
50/45	300-355	327.5	33.9	11102.3
45/40	355-425	390	66.1	25779
D avg			368.81 µm	

*Average of 3 determinants.

The size distribution of the microspheres was found to be narrow with an arithmetic mean size of 368.81 µm. The average size of the major fraction of microspheres obtained was in the size range of 327.5-390 µm.

***In vitro* dissolution study**

In vitro dissolution profile of ketorolac trometamol from eudragit coated cellulose acetate microspheres was carried out in acid buffer of pH 1.2 for the first 2 hours and phosphate buffer of pH 6.8 till the end of the study (n=3). The results were shown in Figure 1.

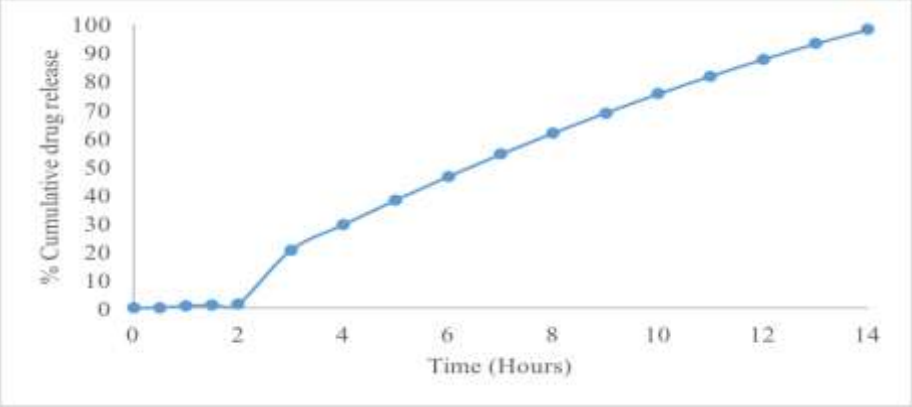


Figure 1: *In vitro* release of ketorolac trometamol from eudragit coated cellulose acetate microspheres (Batch E_3) in pH 1.2 and pH 6.8 buffers.

The results of *in vitro* dissolution study of the eudragit coated microspheres revealed a controlled drug release (1.41% at the end of 2 hours) in pH 1.2 indicating the complete coating of the core microspheres by the adopted technique and also the efficiency of eudragit L-100 and eudragit S-100 combination as enteric coating polymers. The microspheres

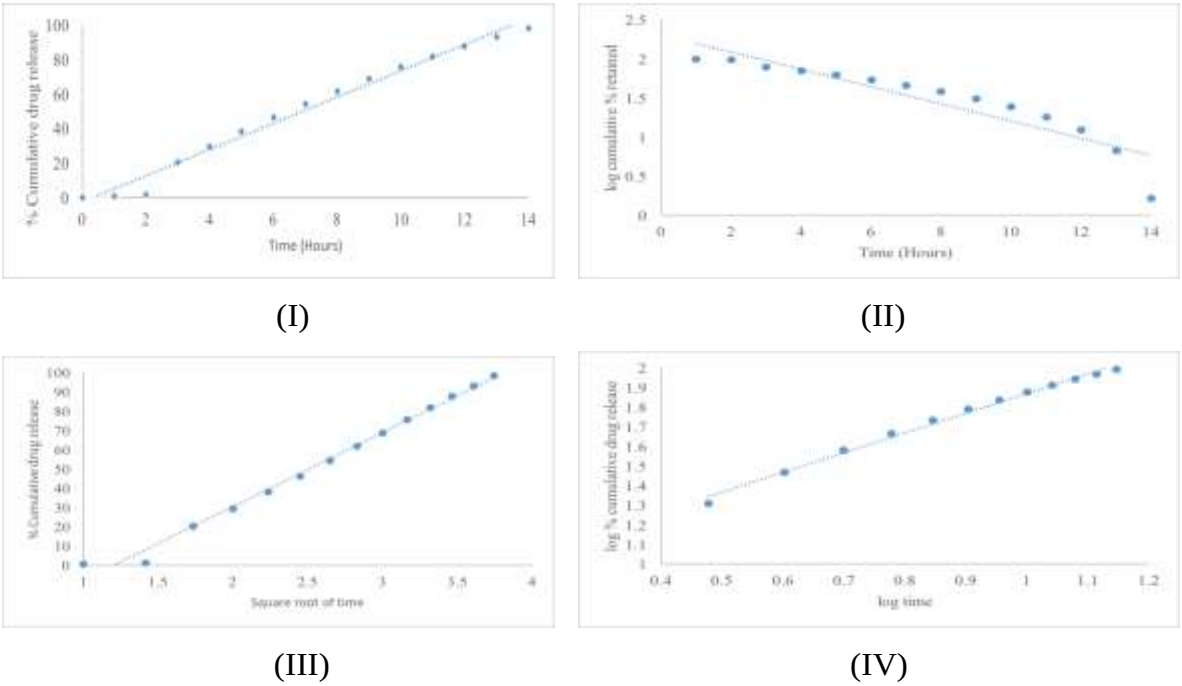
exhibited initial burst effect with a drug release of 18.92% in 1 hour as the eudragit coat was dissolved when exposed to phosphate buffer of pH 6.8. It was observed that once the eudragit coat was dissolved, cellulose acetate cores effectively exhibited controlled the drug release (98.36%.) up to 14 hours. The dissolution study showed that formulated eudragit coated cellulose acetate microspheres exhibited both pH sensitive and controlled release property.

In vitro release kinetics

In order to determine the mechanism of drug release, *in vitro* data was subjected to various kinetic models such as zero order, first order, Higuchi's and Korsmeyer-Peppas' equation (Table 6 and Figure 2).

Table 6: *In vitro* release kinetics of ketorolac trometamol from eudragit coated cellulose acetate microspheres (Batch E_3) in acid buffer of pH 1.2 and phosphate buffer of pH 6.8.

Kinetics	n	r^2
Zero Order	7.6339	0.9848
First Order	-0.1104	0.8452
Higuchi's	38.31	0.9907
Korsmeyer-Peppas	1.008	0.9927



(I) (II)

(III) (IV)

Figure 2: Plots for release of ketorolac trometamol from eudragit coated cellulose acetate microspheres (Batch E_3) (I) Zero order (II) First order (III) Higuchi's plot (IV) Korsmeyer-Peppas' plot.

For the formulation of eudragit coated Cellulose Acetate microspheres (E_3), the best fit with highest correlation coefficient were observed for Korsmeyer-Peppas >Higuchi > zero order > first order equation as given in Table 6. The rate constants were calculated from the respective plots. When the data was plotted as per zero order equation, linear plot was obtained with correlation coefficient value of 0.9848. When the data was plotted as per first order equation, fairly linear plot was obtained with correlation coefficient value of 0.8452. Further, when the release data was fitted in to Higuchi model, linear plot was obtained with high correlation coefficient value of 0.9907. The drug release was found to be proportionate to square root of time, indicating the diffusion controlled release mechanism (Figure 2-III). The drug release data was fitted in Korsmayer-Peppas' model in order to find out n values, which described the diffusion was whether Fickian or non-Fickian. The n value was 1.0. Thus, the mechanism of drug release was found to be Case II transport (zero order release), as slope value was found to be 1.0. This could be indicative of drug release mechanism involving combination of diffusion and chain relaxation mechanism.[16] Hence, it might be concluded that the mechanism of drug release from the formulated microspheres followed non-Fickian diffusion controlled release kinetics.

Scanning electron microscopy

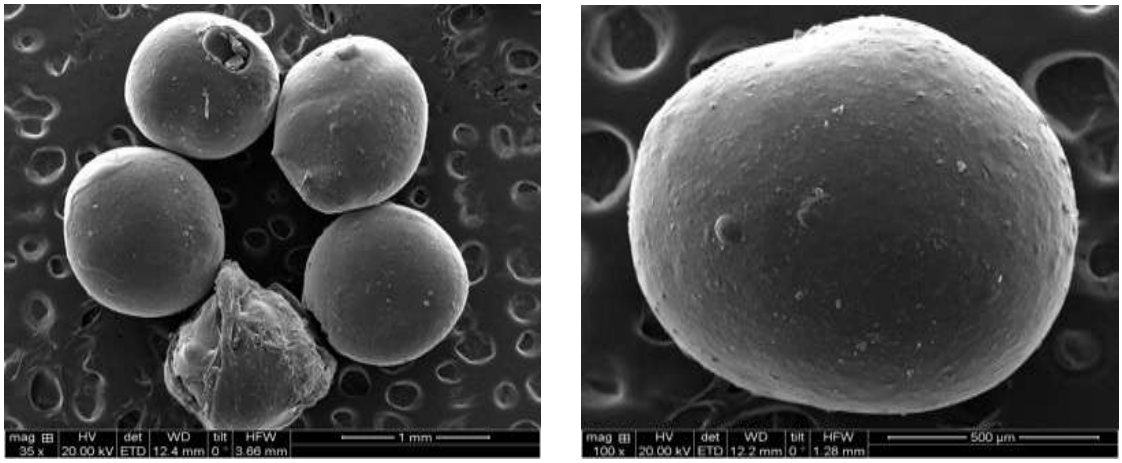


Figure 3: SEM photographs of eudragit coated ketorolac trometamol loaded cellulose acetate microspheres (Batch E_3).

The eudragit coated cellulose acetate microspheres were found to be discrete, uniform and spherical, with a smooth and dense surface in SEM study (Figure 3).

Evaluation parameters of pulsatile capsule of ketorolac trometamol

Evaluation of formaldehyde treated empty capsule body

Formalin treatment has been employed to modify the solubility of gelatin capsules. Exposure to formalin vapors results in an unpredictable decrease in solubility of gelatin owing to the cross-linkage of the amino groups in the gelatin molecular chain with aldehyde groups of formaldehyde by Schiff's base condensation.[14] The formaldehyde treated empty capsule bodies were evaluated for visual appearance, change in shape, solubility study and qualitative chemical test for free formaldehyde. The capsule bodies after formaldehyde treatment slightly reduced in the size and no other visual defects were observed. The formaldehyde treatment of the capsule bodies significantly altered their solubility compared to the untreated cap of the capsule. The untreated caps were dissolved within 12 minutes and the treated bodies remained intact over a period of 24 hours and thus indicating the suitability for the colonic delivery. The qualitative chemical test for the free formaldehyde was carried out. The sample solution was not intensely colored than the standard solution, inferring that less than 20µg of free formaldehyde was present in 25 capsules used for the test.

***In vitro* dissolution study of formulated pulsatile drug delivery capsule of ketorolac trometamol (Batches E_3X15 to E_3H45)**

In vitro release of ketorolac trometamol from the formulated pulsatile drug delivery capsule was investigated by placing it in the basket and followed the procedure described under *in vitro* dissolution study of microspheres. However, to mimic the physiological conditions of the GIT, dissolution studies were carried out using acid buffer of pH 1.2 for first 2 hours, phosphate buffer of pH 7.4 for the next 3 hours and phosphate buffer of pH 6.8 till the end of the study.[15]

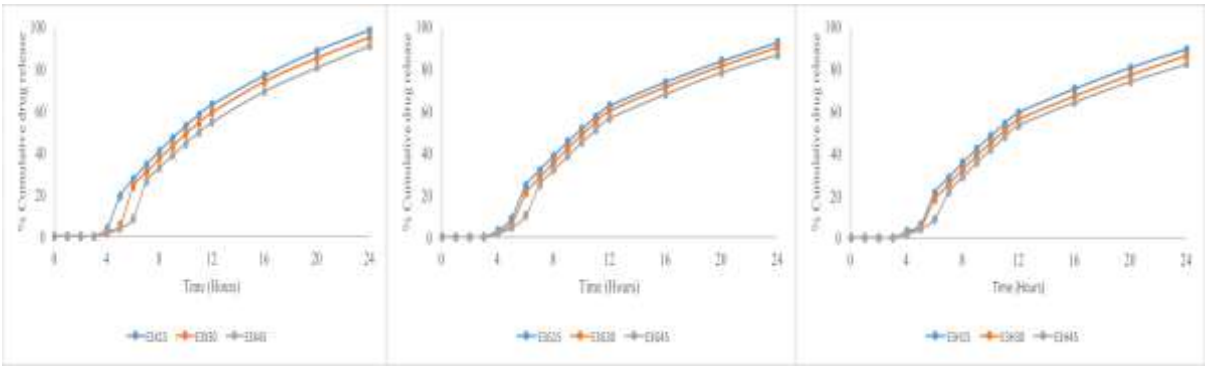


Figure 4: *In vitro* release of ketorolac trometamol from pulsatile drug delivery capsules (Batches E_3X15 to E_3H45).

The results of the dissolution studies showed that the formulated pulsatile drug delivery capsules remained intact in pH 1.2 for 2 hours and indicating the efficiency of HPMC Phthalate as an enteric coating material. The enteric coat started to dissolve when the pH of medium was changed to 7.4, leaving the soluble cap of the capsule which also dissolved after few minutes. The exposed polymeric plug absorbed the surrounding fluid then swelled and released the drug through the microsphere matrix. When polymeric plug was completely wetted by the dissolution fluid, a soft mass was formed which was then easily ejected out of the capsule body, thereby liberating the microspheres in to the simulated colonic fluid (pH 6.8 phosphate buffer). The drug release was not observed in first three hours and negligible drug was released in the fourth hour in almost all pulsatile drug delivery capsules. It indicated that the formulated pulsatile drug delivery capsule can maintain the lag time of no drug release for minimum of first 4 hours which is ultimately desired for the chronotherapeutic delivery of ketorolac trometamol in the treatment of rheumatoid arthritis.

Effect of plugging material on *in vitro* drug release

Pulsatile drug delivery capsules were designed using different natural or semi synthetic polymers like xanthan gum, guar gum or HPMC K100M as plugging materials at concentrations of 15, 30 or 45 mg and their effect on drug release from the pulsatile capsule was investigated using the dissolution media mimicking the pH conditions (without enzymes) of the GI tract.

Effect of Xanthan gum

Xanthan gum as a hydrogel plugging material at three different concentrations of 15, 30 and 45 mg were used in the formulations E_3X15, E_3X30 and E_3X45 respectively. The cumulative drug release at the end of 4^{th} hour was almost negligible and found to be 3.76, 2.71 and 1.46% for batches E_3X15, E_3X30 and E_3X45 respectively. The negligible amount of drug released at the end of 4^{th} hour might be due to the less diffusion of drug from the microspheres through the swollen hydrogel plug. With all the formulations, the concentration of the plugging material was found sufficient to maintain the minimum lag period of 4 hours. In between the 4^{th} and 5^{th} hour of the dissolution study, the hydrogel plug after complete wetting and swelling was ejected for batch E_3X15, whereas the ejection of the hydrogel plug was observed at the end of 5^{th} and 6^{th} hour for batch E_3X30 and E_3X45 respectively, thereby releasing the drug containing microspheres in the colonic region (pH 6.8). The delay in the ejection of plugging material for the remaining two formulations could be attributed to

delayed wetting and swelling of the hydrogel material at the higher concentration. At the end of 24 hours, 98.20, 94.67 and 90.67% drug release was observed with batches E_3X15, E_3X30 and E_3X45 respectively. With all the formulations containing xanthan gum as a plugging material, a desired lag period of 4 hours was achieved and the release of drug in a controlled manner began after the 5th hour and spread over a period of 24 hours.

Effect of Guar gum

In another set of formulations, guar gum was used as a hydrogel plugging material at concentrations of 15, 30 or 45 mg for batches E_3G15, E_3G30 and E_3G45 respectively. The cumulative drug release at the end of 4th hour was negligible and found to be 3.13, 2.96 and 1.88% with batches E_3G15, E_3G30 and E_3G45 respectively. This could be due to diffusion of drug from the microspheres through the swollen hydrogel plug. The different polymeric concentrations used in the formulations were found sufficient to maintain the lag period for a minimum period of 4 hours. After 5th hour, the hydrogel plug after complete wetting and swelling was ejected for batches E_3G15 and E_3G30 and thus releasing the drug containing microspheres in the colonic fluid (pH 6.8). In case of batch E_3G45, the polymeric plug was ejected after the 6th hour in the dissolution study. This could be attributed to delayed wetting and swelling of the hydrogel material at the higher concentration. At the end of 24 hours, 92.36, 90.02 and 86.53% drug release was observed with batches E_3G15, E_3G30 and E_3G45 respectively. Overall, with the formulations containing guar gum as a plugging material, a desired lag period of 5 hours was achieved and the drug was released in a controlled manner after the 6th hour and continued till the end of 24th hour.

Effect of HPMC K100M

HPMC K100M was employed as a plugging material at concentrations of 15, 30 or 45 mg for batches E_3H15, E_3H30 and E_3H45 respectively. Negligible amount of drug (1.67-2.92%) was released at the end of 4th hour with batches E_3H15, E_3H30 and E_3H45. This could be due to diffusion of drug from the microspheres through the swollen hydrogel plug like the batches containing xanthan gum or guar gum as plugging material. The polymeric concentrations used in the formulations were found sufficient to maintain the lag period for a minimum period of 4 hours. After 5th hour, the hydrogel plug after complete wetting and swelling was ejected for batches E_3H15 and E_3H30 formulations and thus released the drug containing microspheres in the colonic fluid (pH 6.8). In case of batch E_3H45, the polymeric plug was ejected after the 6th hour of the dissolution study. This could be attributed to delayed wetting

and swelling of the hydrogel material at the higher concentration. At the end of 24 hours, 89.13, 86.13 and 82.22% drug release was observed with E_3H15, E_3H30 and E_3H45 formulations respectively. Overall, with all the formulations containing HPMC K100M as a plugging material, a desired lag period of 5 hours was achieved and the drug was released in a controlled manner after the 6th hour and continued till the end of 24th hour.

However, when compared to the formulations containing xanthan gum or guar gum as plugging materials, more sustained drug release was observed with HPMC K100M as a plugging material. This could be due to higher viscosity of the HPMC than the xanthan gum or guar gum.

It was found that HPMC K100M or guar gum as plugging material was found better in maintaining the desired lag period compared to xanthan gum. The rank order of sustaining drug release of polymer plug was HPMC K100M>guar gum>xanthan gum. The lag period and the drug release could be efficiently modulated by altering the concentration of plugging material to a certain extent. Thus, the study conclusively demonstrated the efficacy and suitability of the polymers as plugging materials in the design of pulsatile capsule of ketorolac trometamol.

***In vitro* release kinetics of formulated pulsatile drug delivery capsule of ketorolac trometamol (Batches E_3X15 to E_3H45)**

In order to determine the mechanism of drug release, *in vitro* drug release data was subjected to various kinetic models such as zero order, first order, Higuchi's and Korsmeyer-Peppas' equation (Table 7 and Figure 5 to Figure 8).

Table 7: *In vitro* release kinetics of pulsatile drug delivery capsules of ketorolac trometamol.

Formulation Batch	Zero order		First order		Higuchi's		Korsmayer-Peppas	
	n	r^2	n	r^2	n	r^2	n	r^2
E_3X15	4.0774	0.9579	-0.0765	0.9114	29.708	0.9902	0.9974	0.9520
E_3X30	3.8873	0.9656	-0.0601	0.9681	29.041	0.9913	0.9692	0.9633
E_3X45	3.732	0.9736	-0.0505	0.9842	28.498	0.9931	0.9774	0.9693
E_3G15	3.662	0.9409	-0.0526	0.9881	27.504	0.9763	0.9210	0.9418
E_3G30	3.7227	0.9371	-0.0448	0.9935	27.981	0.9738	1.0020	0.9299
E_3G45	3.5034	0.9470	-0.0425	0.9964	26.896	0.9763	0.9612	0.9395
E_3H15	3.6538	0.9410	-0.046	0.9943	27.442	0.9764	0.9835	0.9354
E_3H30	3.6458	0.9420	-0.0413	0.9954	27.374	0.9769	1.0564	0.9276
E_3H45	3.4235	0.9427	-0.0368	0.9961	26.302	0.9733	1.0155	0.9294

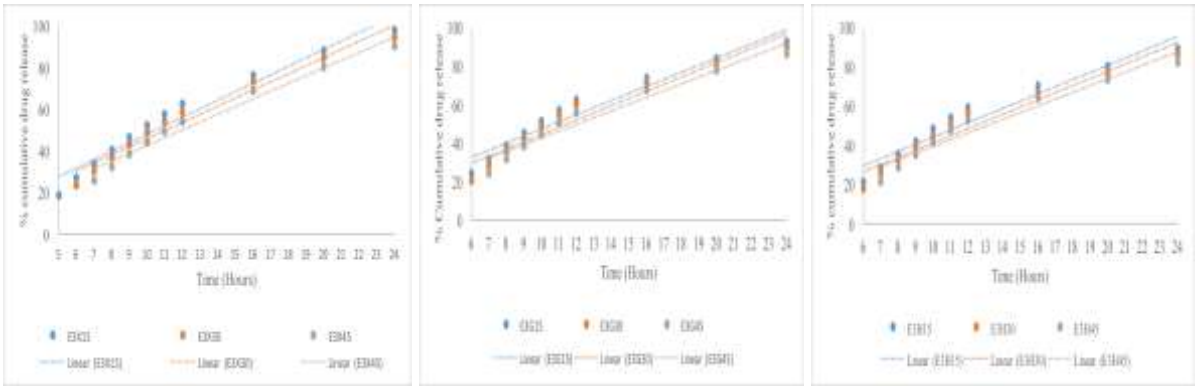


Figure 5: Zero order plots for release of ketorolac trometamol from pulsatile drug delivery capsules (Batches E_3X15 to E_3H45).

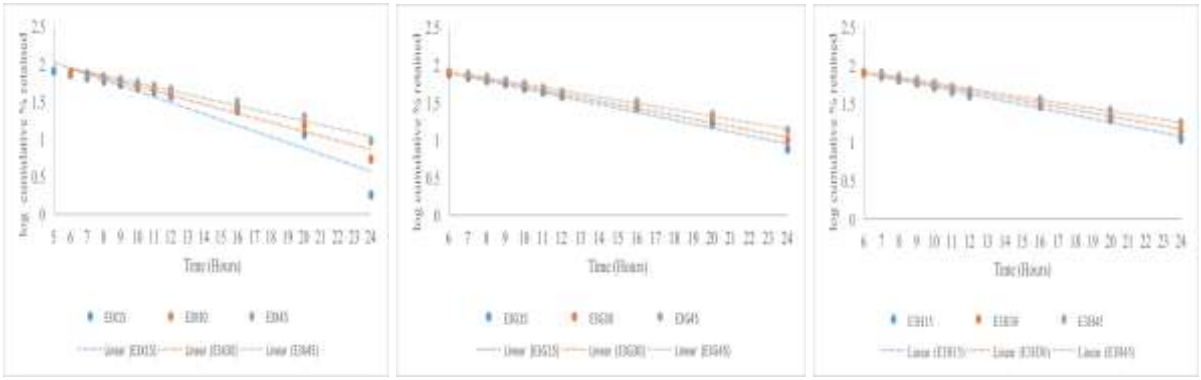


Figure 6: First order plots for release of ketorolac trometamol from pulsatile drug delivery capsules (Batches E_3X15 to E_3H45).

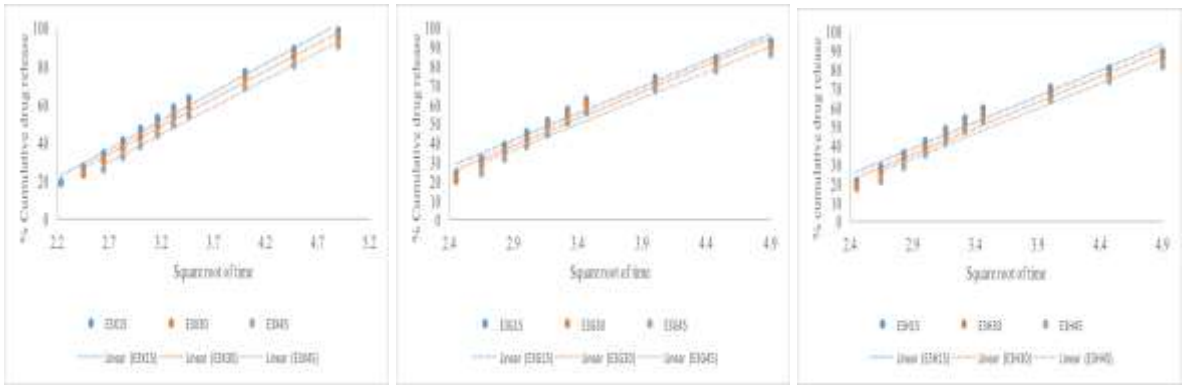


Figure 7: Higuchi's plots for release of ketorolac trometamol from pulsatile drug delivery capsules (Batches E_3X15 to E_3H45).

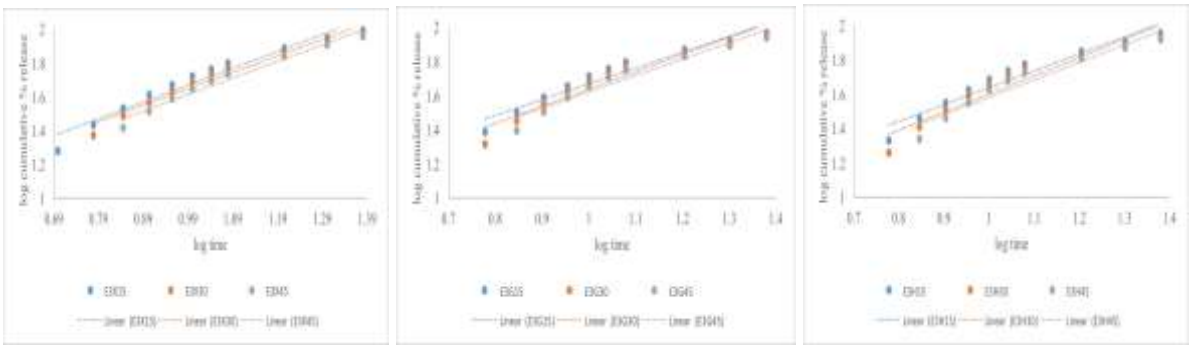


Figure 8: Korsmeyer-Peppas' plots for release of ketorolac trometamol from pulsatile drug delivery capsules (Batches E_3X15 to E_3H45).

The release kinetics of the designed pulsatile drug delivery capsule was found to be complex as compared to the release kinetics of microspheres. The drug release data from 6th hour of the dissolution study was employed for the kinetic analysis, as the systems maintained lag period of 4 to 6 hours, where negligible amount of the drug was released. For all the formulations (Batches E_3X15- E_3X45, E_3G15- E_3G45 and E_3H15- E_3H45), the best fit order with highest correlation coefficient were observed for Higuchi's > First order > Zero-order > Korsmeyer-Peppas' equation as shown in Table 7 and Figure 5 to Figure 8. When the data was plotted as per first order kinetics, linear plots were obtained for all the formulations with high correlation coefficient values ranging from 0.9114 to 0.9964 indicating the first order release kinetics. Further, when the drug release data was also put into Higuchi's equation, high correlation coefficient values ranging from 0.9733 to 0.9931 were obtained, indicating that the drug release was diffusion controlled. To know whether the drug release followed diffusion controlled fickian or non-fickian, the data was plotted into Korsmeyer-Peppas' equation. The formulations exhibited the n values between 0.9210-1.0564, indicating that the drug release was found to be super case II transport, as slope values was found > 0.89. This could be indicative of drug release mechanism involving combination of diffusion and chain relaxation mechanism.[16] In conclusion, the pulsatile drug delivery systems exhibited first order diffusion controlled drug release mechanism.

CONCLUSION

The present study conclusively proved that, the designed pulsatile capsule could be a promising chronotherapeutic delivery system in the treatment of rheumatoid arthritis. Bedtime administration of such a system would be beneficial for the patients in the management of rheumatoid arthritis.

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