Original Research Article

Microspheres and tablet in capsule system: A novel chronotherapeutic system of ketorolac tromethamine for site and time specific delivery

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Abstract

The objective of the present work was to develop a novel delivery system of ketorolac tromethamine (KT) for dual pulse release based on microspheres and tablet in capsule system (MATICS) as a treatment modality for rheumatoid arthritis. The design consisted of an impermeable hard gelatin capsule body, in which a core tablet was (second pulse) placed in the bottom and sealed with a hydrogel plug (HP2). The body was locked with enteric coated cap filled with KT microspheres (first pulse). The microspheres for first pulse were selected by screening the formulations (M1–M6), and M1 with least particle size of 96.38 ± 0.05 μm, highest drug loading of 25.10% ± 0.28% and maximum CDR of 89.32% ± 0.21% was adjudged as the best formulation. The HP2 tablet was selected based on its capability for maintaining a lag period of 6 h. The selection criterion of the second pulse (core tablet: T3) was its disintegration time of 4.02 ± 0.53 min and CDR of 99.10% ± 0.32% in 30 min. All the optimized formulations were assembled in accordance with the proposed design to form pulsatile MATICS and evaluated for *in vitro* release. MATICS displayed delayed sustained CDR of 80.15% in 8 h from the first pulse (microspheres) after a lag time of 2 h, followed by 97.05% KT release from second pulse (core tablet) in simulated colonic fluid within 10 h. Conclusively, *in vitro* pulsatile release was a rational combination of delayed sustained and immediate release of KT that has the potential to combat the pain at night and morning stiffness. Incorporation of two pulses in one system offers a reduction in dose frequency and better pain management.

Key words: Bifunctional capsule, in vitro release, ketorolac tromethamine, microspheres and tablet in capsule system, variable multipulse delivery

INTRODUCTION

Chronopharmacotherapy for rheumatoid arthritis has been approved to ensure highest blood levels of the drug that matches with peak pain and stiffness.[1] In patients suffering from rheumatoid arthritis, circadian rhythm in the plasma concentration of C-reactive protein and interleukin-6 has been documented. The level of the endogenous substances opioid peptides are higher in the morning and lower in the evening both in adults and neonates.[2] Due to the presence of endogenous substances, patients with rheumatoid arthritis usually have peak pain high in the early morning, and this pain subsides as the day passes. Chronotherapy thus is the preferred therapy for rheumatoid arthritis.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are considered as the first-line drugs for the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis.[3] Ketorolac tromethamine (KT), a potent NSAIDs, deploys its effect due to inhibition of both COX-I and II.[4] It is a weak acid, well absorbed from the proximal part of small intestine. KT presents two major problems when administered orally; it causes drastic gastrointestinal side effects as bleeding, peptic ulcer, perforation. Second, it has a short half-life (4-6 h), so it requires persistent administration. Based on these pitfalls various dosage forms of KT have been cited in the literature that describe single unit dosages forms. Of lately modified release dosage forms such as enteric-coated or sustained-release formulations of NSAIDs have been developed to improve therapeutic efficacy and reduce the severity of upper gastrointestinal tract side effects.[5] Controlled release matrix tablets of KT have been reported to overcome the gastrointestinal side effects associated with the oral administration

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Access this article online

Quick Response Code:

Website: www.jpionline.org

DOI: 10.4103/2230-973X.160854

of NSAIDs. The research reports on single/multiple unit systems of KT include, matrix tablet,[6] Eudragit S coated microcapsules,[7] sustained release pellets twice a day[8] and fast dissolving tablets.[9] While conventional release dosage forms (single unit dosage) are associated with limitations of drug tolerance, dose dumping, patient incompliance and resistance due to long-term constant drug concentrations in the blood and tissues, a modified-release drug preparation is expected to improve patient compliance and reduce dosing frequency. Thus to modulate the drug level in synchrony with the circadian rhythm, multiparticulate system microspheres and tablet in capsule system (MATICS) is proposed for relieving both early morning and night pain that eventuate in the patients of rheumatoid arthritis. This chronotropic system may release the drug in sustained manner to normalize circadian changes at night after which immediate release will occur to resolve early morning pain after a predetermined lag time.

The objective of the present study was to develop a new pulsatile release capsule device for time and site specific delivery. The proposed design of the system and its expected functionality has been clearly depicted in Figure 1. Briefly the system will consist of sustained release microspheres (first pulse) filled in enteric coated capsule cap that will lock the impermeable capsule body (coated with EC) consisting of swellable hydrogel plug (HP2) of HPMC K4M at its mouth beneath, which an immediate release KT loaded core tablet will be nestled to provide the second pulse (immediate release). Both the pulses and the HP2 will be selected after appropriate screening of corresponding formulation. The assembled system MATICS would be assessed for *in vitro* release and compared with the marketed formulation of KT.

MATERIALS AND METHODS

Materials

Ketorolac tromethamine was gift sample from Dr. Reddy's Laboratories, Ahmadabad, India. Ethyl cellulose (lot no. 02129) was obtained from Central Drug House (P) Ltd, New Delhi, India. Eudragit RS100 was obtained from Evonik Roehm (Germany). Microcrystalline cellulose, magnesium stearate, methanol, and dichloromethane were purchased from S.D. Fine Chemicals (Mumbai, India). All other chemicals were purchased from local sources.

Design of microspheres and tablet in capsule system

In order to design MATICS, the hard gelatin capsule shell was subjected to two different treatments to confer bifunctional characteristics. The exterior of the capsule cap was enteric coated, and the body was made impermeable by ethylcellulose internal coating. The core tablet of KT (second pulse) along with HP2 tablet was lodged in the impermeable body and closed by enteric coated cap containing microspheres to obtain MATICS. The stepwise procedure is represented diagrammatically in Figure 2 and is detailed below.

Bifunctional capsule shell (impermeable capsule body with enteric cap)

Coating of capsule cap

A 5% w/v solution of cellulose acetate phthalate was prepared in acetone: Ethanol (8:2 v/v) as the solvent system and dibutyl phthalate (0.75% v/v) was incorporated as the plasticizer. Dip coating was used to develop the acid insoluble cap. The caps were dipped in cellulose acetate phthalate solution and dried at room temperature at 37°C ± 0.5°C for 24 h. Coating was repeated until the cap resisted disintegration in 0.1N HCl buffer, pH1.2 for a period of least 2 h. The coated capsule shell thickness was measured with the vernier caliper (Mitoyuto, Japan).

Impermeable capsule body

Impermeable gelatin capsule body was fabricated using ethyl cellulose as a coating polymer by varying its strength [Table 1]. Ethyl acetate, dichloromethane, and ethanol were mixed in a ratio of 4:0.8:0.2 and ethyl cellulose was added to produce 40 g/l, 80 g/l and 120 g/l of ethyl cellulose solution. A quantity of 0.55 ml of ethyl cellulose solution was poured into uncapped gelatin capsule body (size 0). The solvent was allowed to evaporate overnight in a refrigerator at 4°C. The impermeable capsules bodies were stored in desiccator till further use. The thickness of internally coated capsule shell was measured with a screw gauge and expressed in mm ($n = 3$). In addition, the lock length of the capsule was also measured by vernier caliper.

Ketorolac tromethamine loaded microspheres (first pulse)

Solvent evaporation method[10] was used for the preparation of KT loaded microspheres. Eudragit RS100 was dissolved in 8 ml solvent system containing methanol and acetone in ratio of 5:3, followed by the addition of 100 mg of KT and dispersing agent (magnesium stearate; 50 mg). The dispersion was poured in a mixture of ml 60 ml light paraffin oil and 6.8 ml n-hexane continuously stirred on a mechanical shaker at 1500 rpm. Stirring was done until the solvent (acetone and methanol) evaporated to get the microspheres. Formed microspheres were filtered and washed with 50 ml petroleum ether (40-60°C). KT loaded microspheres were dried for

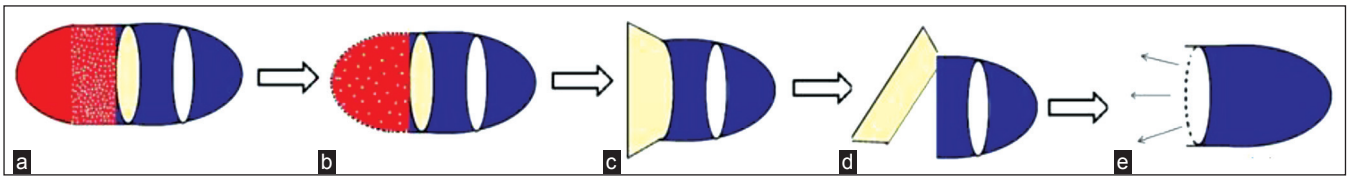

Figure 1: Schematic representation of the proposed release of drug in dual pulse from microspheres and tablet in capsule system. (a) Lag time ($t = 2$ h) with no drug release, (b) sustain drug release from first pulse (microspheres, $t = 8$ h), (c) swelling of plug tablet in simulated intestinal fluid no drug release, (d) ejection of plug tablet in colonic fluid (e) immediate drug release from second pulse ($t = 10$ h)

24 h at room temperature. The microspheres were then stored in a desiccator over fused calcium chloride till further use. A total of six formulations were prepared by varying the amount of eudragit RS100, whereas the amount of drug, magnesium stearate and organic solvents were kept constant [Table 2]. The microspheres were optimized on the basis of particle size, percent entrapment efficiency and percent cumulative drug release (CDR).

Particle size and percentage yield

The optical microscope was used for measurement of particle size of microspheres. The sample was mounted on a clean slide and observed under microscope using calibrated eye piece micrometer. Around 300 microspheres were measured for particle size and the mean size was calculated.[11] The percent yield of KT loaded microspheres was determined by dividing the weight of the prepared microspheres by total amount of drug and excipients used.

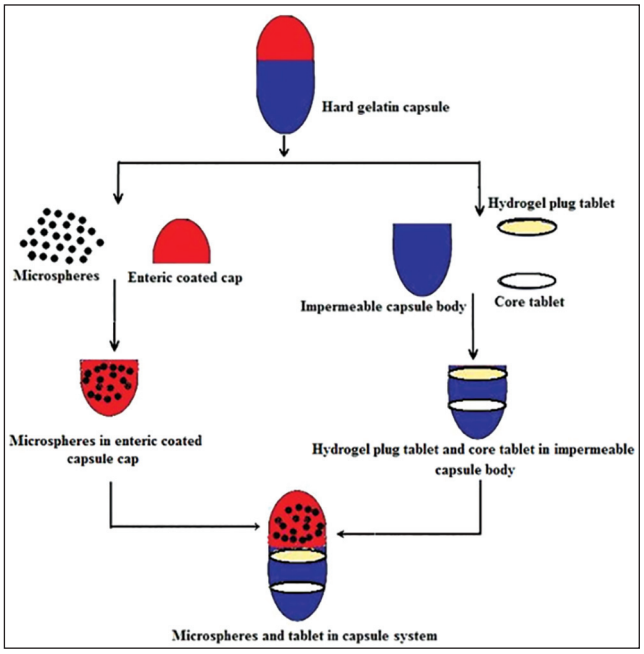

Figure 2: Formulation approach of microspheres and tablet in capsule system

Entrapment efficiency and drug loading

Ketorolac tromethamine loaded microspheres were crushed in a glass mortar and pestle and a quantity theoretically equivalent of 10 mg of drug was extracted with 5 ml of phosphate buffer, pH 7.4 by vortexing for 30 min. After complete mixing the solution was filtered through 0.45 μm membrane filter paper. One milliliter of the solution was diluted up to 10 ml and analyzed using ultraviolet spectrophotometer (Shimadzu Pharmaspec1700, Kyoto, Japan) at 324 nm. The entrapment efficiency and drug loading were calculated using following equations:

$$\text{Percent entrapment efficiency} = \frac{\text{Practically entrapped amount of drug}}{\text{Total amount of drug}} \times 100 \quad \text{Eq.1}$$

$$\text{Percentloading efficiency} = \frac{\text{Drug loaded in microspheres}}{\text{Total weight of microspheres}} \times 100 \quad \text{Eq.2}$$

In vitro drug release

Microspheres equivalent to 10 mg of KT were placed in USP I basket (mesh #230 = 63 μm) apparatus and the study was performed in the 900 ml of phosphate buffer, pH7.4 for 6 h at 50 rpm, maintained at 37°C ± 0.5°C. An aliquot of 5 ml was withdrawn at regular periodic intervals for 6 h and sink conditions were maintained by replacing equal volume of fresh release medium. Samples were analyzed spectrophotometrically at λ max of 324 nm and % CDR versus time plots were constructed. The release data was subjected to zero order, first order and Higuchi's model to understand the release kinetics of KT from microspheres. The model with highest correlation coefficient was considered to be the best fitted for release profile. The data

Table 1: Characterization of enteric capsule cap and impermeable capsule body of MATICS

Enteric capsule cap			Impermeable capsule body code	Coating solution (ethyl cellulose; %w/v)	Impermeable capsule body			Height (lock length) (mm)
Outer diameter (mm)	Inner diameter (mm)	Thickness (mm)			Outer diameter (mm)	Inner diameter (mm)	Thickness (mm)	
7.065±0.62	7.0±0.98	0.005±0.44	IC1	4	7.004±0.37	7.00±0.51	0.011±0.21	22.5±0.23
7.105±0.34	7.0±0.66	0.055±0.54	IC2	8	7.005±0.78	6.01±0.92	0.048±0.41	22.6±0.32
7.122±0.91	7.0±0.38	0.062±0.38	IC3	12	7.003±0.82	6.20±0.47	0.072±0.37	22.9±0.16

MATICS: Microspheres and tablet in capsule system

Table 2: Formulation design and characterization parameters of KT loaded microspheres (first pulse)

Formulation code	Drug: Polymer (by weight)	Yield (%)	Particle size (μm)	Entrapment efficiency (%)	Drug loading (%)	CDR (%) (6 h)	Higuchi model (best fit) (R^2)
M1	1:2	78.85	96.38±0.05	65.26±0.32	25.10±0.02	89.32±0.21	0.9995
M2	1:3	80.30	115.67±0.06	71.32±0.04	23.77±0.45	63.82±0.11	0.9989
M3	1:4	84.28	125.37±0.03	76.57±0.27	16.79±0.31	53.66±0.32	0.9983
M4	1:5	81.11	139.26±0.08	80.27±0.66	15.08±0.12	45.06±0.11	0.9975
M5	1:6	85.64	153.33±0.12	86.59±0.37	13.21±0.91	35.29±0.34	0.9986
M6	1:7	89.99	212.92±0.13	90.07±0.09	10.37±0.23	30.08±0.54	0.9975

CDR: Cumulative drug release, KT: Ketorolac tromethamine

was also fitted in Korsemeyer Peppas model and the value of diffusion exponent (n) was calculated in order to analyze the mechanism of drug release.

Selection of optimized formulation

The formulation experimentally approaching minimum particle size, maximum percent entrapment efficiency and percent CDR was selected as optimized formulation and was characterized by differential scanning calorimetry and scanning electron microscopy (SEM).

Differential scanning calorimetry

The thermal behavior of KT, eudragit RS100, their physical mixture, and microspheres (M1) was recorded using a scanning calorimeter equipped with a thermal analysis data system (NETZSCH DSC 200F3-240-20-427-L, USA) to estimate the interaction between drug and polymer and the state of drug inside the polymer matrix in microspheres. Accurately weighed the amount of samples were hermetically sealed in an aluminum pan, heated at a constant rate of 10°C/min, over a temperature range of 0-350°C. The flow rate of the nitrogen purge (50-80 mL/min) was maintained as an inert atmosphere throughout heating. The thermograms were recorded and analyzed.

Scanning electron microscopy

The SEM analysis of optimized KT microspheres (M1) was carried out using JEOL-6360A, analytical scanning electron microscope (JEOL 6360A, Tokyo, Japan). The dried sample was coated with gold in an ion sputter. The microspheres were viewed at an accelerating voltage of 20 KV under various magnification and pictures were taken by random scanning of the stub.

Core tablet (second pulse)

Core tablets of KT were prepared using hand operated single punch machine (HICON® Grover Enterprises, New Delhi, India) equipped with 6 mm biconcave-faced punches. For preparation of tablets, the ingredients were passed through a #60 mesh sieve and prerequisite amounts were mixed for 15 min in a polybag. To the resulting mixture, magnesium stearate and talc were added and made into 100 mg tablets. The dose of KT was 10 mg, in each tablet. A total of six formulations were prepared as detailed in Table 3.

Evaluation of core tablet

The core tablets were subjected to selected pharmacopoeial (uniformity of weight, uniformity of content) and non-pharmacopoeial tests (thickness, hardness, friability). The hardness and thickness ($n = 6$) were determined by Monsanto hardness tester and Vernier caliper respectively. The friability was determined on a Roche friabilator ($n = 6$) by the method described in <1216> general information, USP 27/NF 22.[12] Uniformity of weight was determined by the IP 2007 methodology with a sample size of 20 tablets.[13] The drug content was assessed by crushing 10 tablets and extracting 100 mg powder with 10 ml methanol, followed by filtration through Whatman filter no.1 and assay at 324 nm. The drug content was determined using validated calibration curve.

In vitro release

In vitro release study was conducted using USP II dissolution apparatus containing 900 ml phosphate buffer, pH 6.8 as a dissolution medium stirred at 100 rpm and maintained at a temperature of 37°C ± 0.5°C. Five milliliters of the sample was withdrawn at predetermined time intervals of 0, 5, 10, 15, 20 and 30 min and replaced with an equal volume of fresh medium. The samples were analyzed spectrophotometrically at λ max of 324 nm.

Hydrogel plug tablet

Hydrogel plug tablet for sealing the capsule body was prepared by compressing equal amounts of HPMC K4M and lactose using 6 mm single punch machine. A total of three formulations (HP1-3) were prepared by varying the weight of plug tablet [Table 4] and evaluated for the following parameters.

Table 3: Formulation design and evaluation parameters of core tablets (second pulse)

Code	KT (mg)	Superdisintegrant		Mannitol (mg)	Mean weight (mg ± SD)	Thickness (mm ± SD)	Hardness (kg/cm²)	Drug content (%)	Friability (% ± SD)	Disintegration time (min)	CDR (%) (30 min)
		CCM (mg)	CPV (mg)								
TI	10	2	—	50	99.50±0.47	3.09±0.06	2.90±0.21	97.16±0.83	0.66±0.15	5.76±0.54	96.78±0.32
T2	10	4	—	48	98.98±0.89	3.16±0.08	2.06±0.36	97.88±1.03	0.57±0.13	4.43±0.43	98.45±0.41
T3	10	6	—	46	99.94±0.10	3.13±0.06	2.76±0.27	99.55±0.39	0.16±0.03	4.02±0.53	99.10±0.60
T4	10	—	2	50	98.95±0.04	3.06±0.06	2.00±0.29	98.63±0.80	0.63±0.15	5.960±0.63	98.56±0.32
T5	10	—	4	48	99.5±0.87	3.18±0.09	2.96±0.24	96.16±0.59	0.50±0.20	5.560±0.54	97.00±0.20
T6	10	—	6	46	99.90±0.52	3.10±0.06	2.86±0.35	97.14±1.68	0.14±0.07	4.330±0.56	98.60±0.51

CCM: Croscarmellose sodium, CPV: Crospovidone, CDR: Cumulative drug release, SD: Standard deviation, KT: Ketorolac tromethamine

Table 4: Formulation design and characterization of HP tablet

HP tablet code	Spray dried lactose (mg)	HPMC K4M (mg)	Talc (mg)	Total weight (mg)	Hardness (kg/cm²)	Thickness (mm)	Swelling index (%)	Lag time (h)
HP1	44	44	2	90	2.3±0.87	3.02±0.14	47.66±0.40	4.28±0.48
HP2	49	49	2	100	2.5±0.34	3.24±0.55	51.62±0.26	6.76±0.96
HP3	54	54	2	110	2.4±0.58	3.91±0.21	65.20±0.26	7.35±0.12

HPMC: Hydroxypropylmethyl cellulose, HP: Hydrogel plug

Hardness and thickness

The thickness ($n = 3$) and hardness of the HP2 tablet was evaluated using vernier caliper and Pfizer hardness test apparatus (HICON® Grover Enterprises, New Delhi, India). Five tablets were selected randomly from each batch, and their hardness was determined and measured in terms of kg/cm^2.

Swelling index

Hydrogel plug tablets from each batch were sequentially immersed in media of three different pH. Weighed plug tablet (W1) was placed in a glass beaker containing 200 mL phosphate buffer, pH 7.4 for 6 h. Plug tablets were taken out at 6 h and the excess surface liquid was carefully removed by a filter paper. The swollen tablet was then reweighed (W2). The swelling index (SI) was calculated using the following equation.

$$SI = \frac{\text{Wet weight (W2)} - \text{Dry weight (W1)}}{\text{Wet weight (W2)}} \times 100 \qquad \text{Eq. 3}$$

Lag time

Lag time was determined indirectly by measuring the time required for ejection of HP2 based on the fact that the drug from core tablet (second pulse) would be released only after the swollen HP2 got ejected from the impermeable capsule body mouth completely. For the study, an assembly of impermeable capsule body consisting of core tablet in the bottom and HPMC K4M HP2 tablet snugly fitted in the mouth of the impermeable capsule body was fastened to the paddle of the USP apparatus II and suspended in phosphate buffer, pH 7.4 for 8 h. The lag time was measured as the time required for complete ejection of plug tablet from the mouth of impermeable capsule body. In addition, the drug release from core tablet was also monitored at regular intervals of 30 min for a period of 8 h. At the end of the test period, surface topography of impermeable capsule body was determined by SEM and compared with the micrograph of capsule body shell before the test.

Assembly of microspheres and tablet in capsule system

Microspheres and tablet in capsule system were designed to achieve two successive variable site specific pulse release. For achieving the same, the core tablet equivalent to 10 mg of KT was lodged in the bottom of the impermeable body and plugged with HP2 tablet at the capsule body mouth. Microspheres equivalent to 10 mg of KT were placed in enteric coated capsule cap and finally the capsule body was closed with a cap to get the MATIC. The system was subjected to *in vitro* release.

In vitro release

As the preparation was designed to traverse through the entire gastrointestinal tract to safely reach colon after oral administration, three dissolution media were sequentially used. The assembled device MATICS was subjected to *in vitro* drug release test using USP apparatus II stirred at 100 rpm. For complete immersion and avoidance of capsule floating in dissolution media, sinkers were used. *In vitro* drug, release was first performed in 900 ml of hydrochloric acid buffer, pH 1.2 at 37°C ± 1°C for 2 h. An aliquot of the dissolution fluid was removed every hour, filtered through Whatman filter paper no.1 and assayed for the amount of KT at 324 nm. At the end of 2 h, the media was replaced by phosphate buffer, pH 7.4 and sampling was continued for another 6 h by withdrawing 5 ml sample every hour and assayed at 324 nm. After 8 h, the medium was removed and replaced with phosphate buffer, pH 6.8 to continue release studies for further 2 h. An aliquot (5 ml) of the dissolution fluid was removed at an interval of 0, 5, 10, 15, 20, 30 and 60 min, filtered and analyzed spectrophotometrically at 324 nm. Each sample withdrawal was replaced by corresponding fresh dissolution medium. The percent CDR versus time plots was made and analyzed.

RESULTS AND DISCUSSION

Bifunctional capsule

The bifunctional capsule was aimed to achieve delayed site specific pulsatile delivery. Correspondingly, the capsule shell was separated, and the cap was enteric coated, whereas the capsule body was coated with ethyl cellulose to achieve unidirectional release at the desired site (colon). In order to ensure locking of the capsule, the enteric coating was applied to the exterior of the shell, whereas ethylcellulose was used for internal coating [Figure 2]. The coating was accomplished by dip coating wherein the coating was precariously optimized. The coated capsule parts were evaluated for thickness. The thickness of the enteric coated cap narrowly ranged between 0.005 and 0.062 mm, and with increased number of coatings the thickness increased. Hence, triple coated enteric cap had a thickness of 0.062 ± 0.38 mm.

On the other hand, the thickness of the ethylcellulose coated impermeable capsule body ranged between 0.011 and 0.072 mm again depending on the strength of the coating composition. On increasing the concentration of ethyl cellulose, thicker impermeable capsule body was formed. The thickness of impermeable capsule body was considered important for mechanical strength and avoidance of premature drug release from the capsule body. Thus, IC3 was adjudged as most impermeable capsule body that was confirmed by *in vitro* release studies detailed later. The optimized enteric coated capsule cap and impermeable capsule body were assembled and measured for lock length. The lock length of was 22.9 ± 0.16 mm.

Ketorolac tromethamine loaded microspheres

The particle size of the KT loaded microspheres ranged between 96.38 ± 0.05 and 212.92 ± 0.13 μm [Table 2]. Mean microsphere size increased with increasing the polymer concentration due to a significant increase in viscosity leading to increased droplet size and hence the microspheres size.[14] Correspondingly an increase in percent yield and entrapment efficiency of microspheres was recorded with an increase in drug: Polymer ratio [Table 2]. M6 showed highest percent yield and entrapment efficiency because the increase in the polymer concentration leads to increase in viscosity, resulting in the formation of large size microspheres, and increasing the product yield.[15] In contrast, drug loading efficiency

displayed a reverse order. With the increase in drug: Polymer ratio the drug loading efficiency decreased from 25.1 ± 0.20 to 10.37% ± 0.23%. Formulation M1 showed highest loading efficiency of 25.1% ± 02%. Loading efficiency increased as the concentration of eudragit RS100 decreased, which may be attributed to the fact that, on increasing the concentration of polymer, amount of entrapped drug increases, at the same time weight of microsphere was also increases, which is inversely related to the loading efficiency.[16]

The *in vitro* release of KT loaded microspheres showed biphasic release pattern [Figure 3], initially burst release for 1 h due to surface associated drug, followed by sustained release till 6 h attributable to the drug entrapped in the matrix of microspheres. Formulation M1 showed highest CDR of 89.32% ± 0.21% whereas least CDR of 30.08 ± 0.54 was recorded for M6. Clearly, the concentration of eudragit RS100 had considerable influence on the extent of the release. As the concentration of eudragit RS100 increased the particle size increased and correspondingly, the diffusional path length increased resulting in diminution in drug release.[17] In order to elucidate the drug release mechanism, the release data was fitted to different release models. The data best fitted to Higuchi model and the highest value of coefficient of determination (r^2 = 0.9995) was observed for M1. On fitting the data to Korsemeyer Peppas model the value of n was found to be <0.5 indicating Fickian diffusion as the release mechanism of the drug. Thus on the basis of particle size, entrapment efficiency and %CDR, M1 was selected as optimised formulation.

For determination of residual solvent in the optimized formulation M1 gas chromatography-mass spectrophotometer (Agilent 7890AGC system) 5975C VL MSD equipped with triple axis detector was used. The chromatogram showed no solvent residues and it characteristically displayed a sharp single peak of 2-Amino-4-methylbenzophenone [Figure 4] with Rt value of 13.069 min and area under the curve of 100%.

In order to study the shape and external morphology of M1, SEM was performed. The micrograph [Figure 5a] depicted spherical particles of diameter between 500 and 1000 μm. The magnified micrograph of the outer surface of microspheres showed minute pores [Figure 5b], formed due to evaporation of the organic solvent (acetone and methanol) entrapped within the shell of microspheres. The surface was grossly smooth and free from imperfections.

For further understanding of the state of the drug in microspheres, solid state analysis studies were carried out. The differential scanning thermogram of KT [Figure 6a] exhibited a sharp, intense endothermic peak at 168.4°C and 265.5°C corresponding the melting points of drug and eudragit RS100 [Figure 6b] respectively. DSC thermogram of M1 [Figure 6c] displayed the endothermic peaks at 135.6°C., a broad asymmetric peak at172.3°Cand sharp peak at 260°C. The broadening and shifting of the peak of the drug in M1 was due to the fact that the crystallinity of drug was reduced on its entrapment into the polymer. A sharp endothermic peak was observed at 135.6°C, which is of magnesium stearate used as droplet stabilizer.[18]

Core tablet of ketorolac tromethamine

Various nonpharmacopoeial and pharmacopoeial characteristics of the tablets have been enlisted in Table 3 that suggest acceptable physical characteristics. The thickness of the tablets ranged from 3.09 ± 0.06 to 3.18 ± 0.09 mm. The low standard deviation values indicated thickness uniformity. Hardness which is indicative of crushing strength of tablets varied from 2.00 ± 0.29 to 2.96 ± 0.24 kg/cm^2 and friability was reported within the official limit (<1%).

The weight variation of the tablets (T1-T6) ranged from 99.50 ± 0.47 to 99.94 ± 0.10 mg. Tablets displayed drug content within the pharmacopoeial limit. *In vitro* drug release of tablets T1-T6 revealed 96.43 ± 0.76–99.10 ± 0.60% CDR within 30 min [Figure 7]. No significant difference was documented in the extent of drug release. However, the impact of superdisintegrant was evident as burst release of the drug in the 1st h. As *in vitro* dissolution test did not assist in the selection, therefore, *in vitro* disintegration time was used as selection criteria. Thus tablet T3

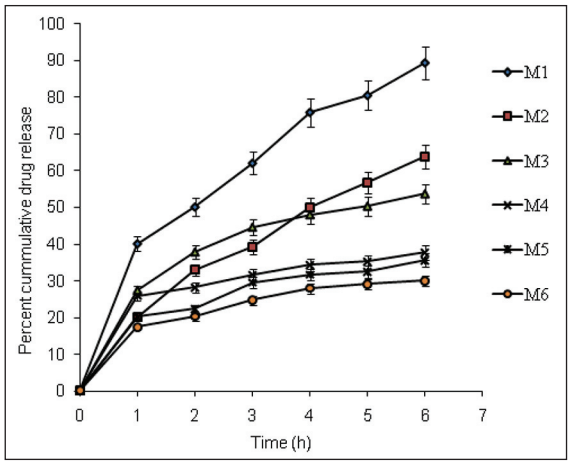

Figure 3: *In vitro* drug release of ketorolac tromethamine from microspheres in phosphate buffer, pH 7.4

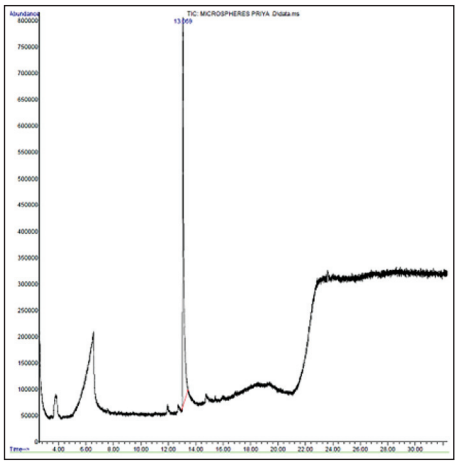

Figure 4: Gas chromatography-mass spectrophotometer analysis of M1 microspheres displaying single peak of the drug

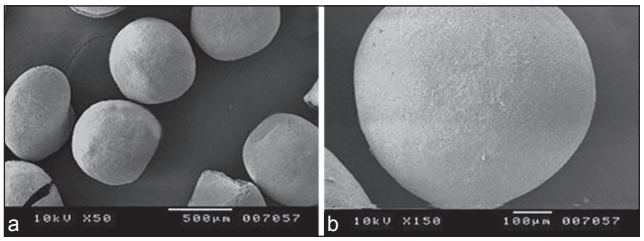

Figure 5: Scanning electron micrographs of (a) optimized microspheres (M1) and (b) magnified view of the surface of microsphere

that displayed least disintegration time of 4.02 ± 0.53 min was selected in the fabrication of MATICS.

To assess the compatibility of excipients used, DSC of T3 was carried out and compared with reference thermograms. The thermogram of physical mixture [Figure 6d] was characterized by four endothermic peaks: One at 91.3°C (corresponding to melting point of mannitol), second at 135.7°C (corresponding to melting point of magnesium stearate), third at 168.8°C (corresponding to melting point of drug) and another at 265.4°C (corresponding to melting point of croscarmellose sodium). The thermogram of optimized formulation T3 [Figure 6e] also displayed the four characteristic peak of mannitol at 92.7°C, magnesium stearate at 137.1°C, drug at 168°C and croscarmellose sodium at 266.4°C. A Slight shift in the peak of mannitol, croscarmellose sodium and magnesium stearate in the optimized formulation was due to the influence of one excipient on another suggesting no chemical interaction between drug and excipients.

Hydrogel plug tablet

The HP2 tablets were formed by direct compression and the evaluation data is tabulated in Table 4. The thickness of plug tablets increased with increase in weight of the plug tablet as the diameter was kept constant (6.19 ± 0.21 mm) by uniform tablet tooling. Variation in weight had insignificant influence on the hardness of the plug tablets that narrowly ranged from 2.3 ± 0.01 to 2.4 ± 0.03 kg/cm^2. While the physical characteristics were insufficient to identify satisfactory plug material, plug tablets were subjected to swelling studies. The swelling indices of HP1-3 varied in the range of 47.66 ± 0.40–65.20 ± 0.26 exhibiting a definite pattern. The Increase in weight of the tablet resulted in an increase in SI, which is quite obvious considering the fact that more the gelling polymer higher would be its fluid absorbing capacity. Throughout the study period, a gradual increase in the swelling indices was achieved with increasing the amount of HPMC K4M and lactose attributable to the ability of HPMC to absorb water due to the presence of hydrophilic groups in its structure.[19] The hydration of these functional groups resulted in water entry into the polymer network leading to the expansion of the plug.

In addition to swelling attributes, lag time was also determined to optimize the HP2 tablet. As mentioned in the methodology section, an assembly of impermeable capsule body consisting of core tablet in the bottom and HPMC K4M HP2 tablet

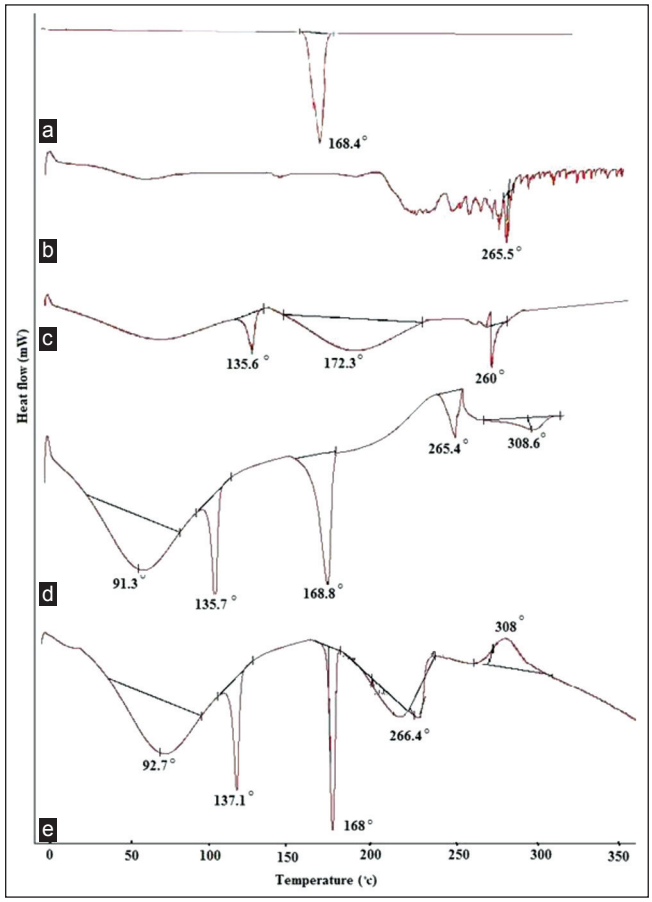

Figure 6: DSC thermogram of (a) pure drug, (b) eudragit RS100, (c) optimized microspheres (M1) (d) physical mixture, and (e) optimized core tablet (T3)

snugly fitted in the mouth of the impermeable capsule body was used to determine the lag time by monitoring drug release. Figure 8 clearly reveals that the lag time for drug release was prolonged with the increasing plug tablet weight and hence HP3 showed highest lag time of 7 h and HP1 displayed least lag time of 5 h. HP2 showed the intermediate value of 6 h and was considered appropriate based on the consideration that the small intestine transit in humans is 6 h. The lag time studies also proved the effectiveness of plug tablet in maintaining unidirectional drug release that could be accomplished due to the impermeability of the capsule body coated internally with EC.

The impermeable capsule body before dissolution and after dissolution was visualized by SEM Figure 9a revealed smooth and uniform surface of impermeable capsule body before the test, whereas the micrograph of the outer surface of impermeable capsule body after the test showed very fine pores [Figure 9b]. However, the micrograph of the inner surface of the impermeable capsule body [Figure 9c] exhibited smooth surface with faint striations but was free from pores suggesting impermeability of the inner layer after dissolution. This clearly indicated that the drug release would happen only when the HP2 tablet got

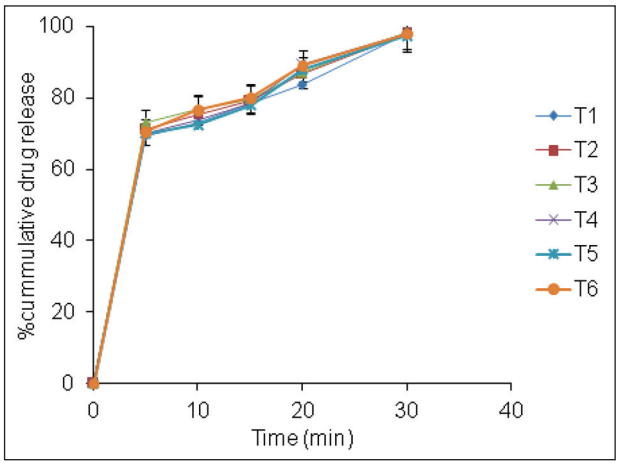

Figure 7: *In vitro* drug release of ketorolac tromethamine from core tablet in phosphate buffer, pH 6.8

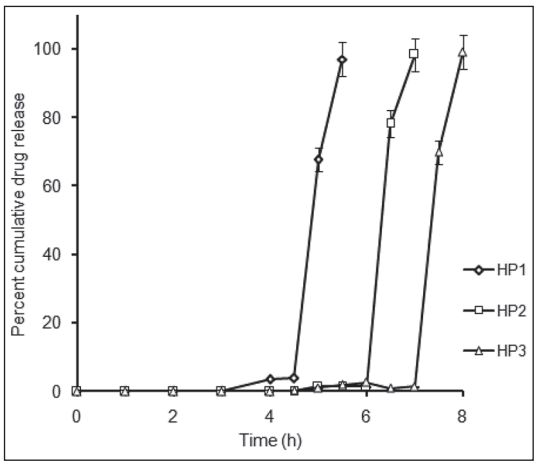

Figure 8: Lag time determination studies from an assembly of impermeable capsule body consisting of core tablet in the bottom and HPMC K4M hydrogel plug tablet snugly fitted in the mouth of the impermeable capsule body fastened to the paddle of the USP apparatus II and suspended in phosphate buffer, pH 7.4

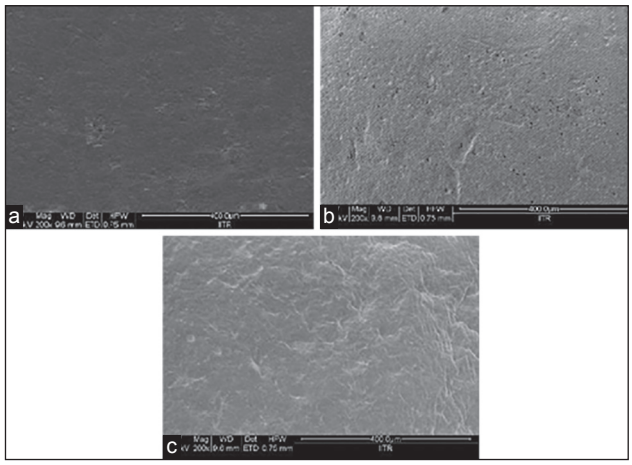

Figure 9: Scanning electron micrographs of impermeable capsule body (a) outer surface before *in vitro* drug release, (b) outer surface after *in vitro* drug release, (c) inner surface after *in vitro* drug release

ejected and that the drug release would predominantly occur uni-directionally.

Microspheres and tablet in capsule system of ketorolac tromethamine

Microspheres and tablet in capsule system formulation showed no release in the hydrochloric acid buffer, pH 1.2 due to the additive effect of the impermeable capsule body and enteric coated capsule cap. The pH dependent enteric coat of capsule cap remained unionized in the medium and hence prevented drug release. However, the coating of pH-dependent polymer cellulose acetate phthalate dissolved in phosphate buffer, pH7.4 liberating the first pulse (microspheres: M1) and the drug release was initiated [Figure 10]. Simultaneously, the HP2 was exposed to the release medium, and it started to swell. The analysis of drug release from first pulse (microspheres) showed biphasic pattern with14.82% CDR in the 3rd h, followed by and 80.15% of CDR at the end of 8 h. Sustainment of drug release was attributable to the property of eudragit RS100 to form dense matrix structure.[20]

Simultaneously the HP2 tablet absorbed the media and was swollen to frustroconical shape [Figure 11]. In this form, it slowly pulled itself out of the capsule body and after a lag time of 6 h, the HP2 tablet was ejected out from the mouth of impermeable capsule body. Thereafter, the second pulse came in contact with phosphate buffer, pH6.8 and release of the drug from the core tablet was initiated. The second pulse displayed CDR of 76.04% in 8.33 h and 96.65% in 9 h. This can be explained on the basis of highly swelling and water uptake ability of croscarmellose sodium that led to fast disintegration.[21] In addition, the wetting property of microcrystalline cellulose increased the dissolution rate because of its property to ingress large amount of fluid[22] and this influenced the fast release of drug from the core tablet in MATICS.

The *in vitro* release profile of MATICS was compared to the marketed Ketorolac-DT. The result indicated that the MATICS of KT was released the drug in pulsed manner according to body circadian rhythm. This chronotropic system released drug in sustained manner for 6 h in SIF (80.15%) to normalise circadian changes at night, after a predetermined lag time of 6 h fast release core tablet will occur to resolve early morning pain. On the other hand the commercially available tablet (Ketorolac-DT) release 97.67% of the drug in 30 min, the drug was released immediately. So Ketorolac-DT is not suitable for rheumatoid arthritis because of high dose frequency, dose dumping and patient incompliance to administer the drug in the early morning. So to overcome these problems, MATICS was designed for time and site specific delivery.

CONCLUSIONS

The MATICS of KT conceptualized for the treatment of late night pain and morning stiffness associated with

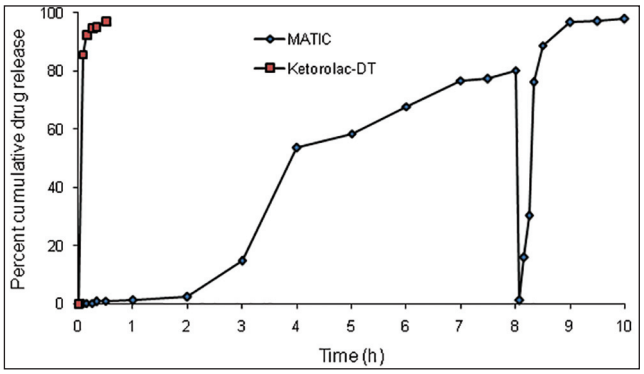

Figure 10: *In vitro* drug release profiles of microspheres and tablet in capsule system in hydrochloric acid buffer, pH 1.2 for 2 h, phosphate buffer, pH 7.4 for next 6 h and finally in phosphate buffer, pH 6.8 for further 2 h; and ketorolac DT in hydrochloric acid buffer, pH 1.2

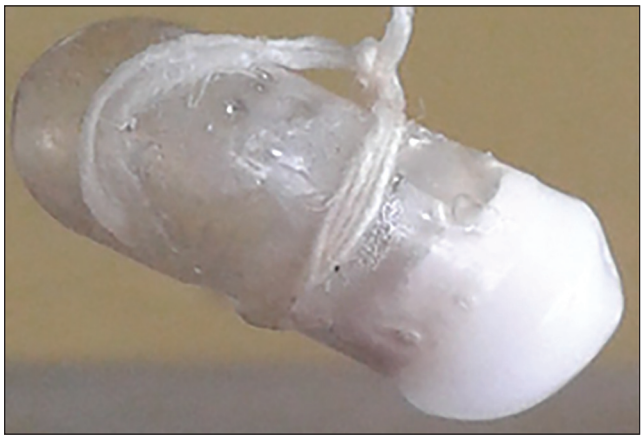

Figure 11: Frustroconical shape of hydrogel plug tablet (HP2)

rheumatoid arthritis was successfully developed. The optimized formulation exhibited sustained and complete release of KT via microspheres after a lag time of 2 h thus avoiding gastric delivery. The HP2 tablet was inserted into the mouth of impermeable capsule body to protect the core tablet in SIF environment. After a predetermine lag time of 6 h the plug was ejected, thereafter, a core tablet that remained intact in the bottom of impermeable capsule body was released in SCF. This presents an opportunity of forbid the need for two-time administration. Conclusively, the MATICS exhibited its variable multipulse nature of slow and fast pulse that appear to be accommodated in accordance with the chronotherapeutic needs of a rheumatoid arthritis patient.

ACKNOWLEDGMENTS

The authors are highly thankful to Professor S K Garg, Dean, DIVASU, Mathura, India for providing the facilities for GCMS analysis.

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How to cite this article: Shahi P, Kumari N, Pathak K. Microspheres and tablet in capsule system: A novel chronotherapeutic system of ketorolac tromethamine for site and time specific delivery. Int J Pharma Investig 2015;5:161-70.
Source of Support: Nil. **Conflicts of Interest:** None declared.