BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

FORMULATION AND EVALUATION OF PULSATILE DRUG DELIVERY SYSTEM FOR ANTI-INFLAMMATORY ACTIVITY BY USING MELOXICAM

Corresponding Author: [1]Amol.U.Gayke
Author: Prof Preetam L.Nikam, Miss Prachi Telgote, Mrs. Radhika Kotme
Corresponding Address: S.N.D College of Pharmacy,Babhulgaon,Yeola
Matoshri Institute of Pharmacy,Dhanore,Yeola

ABSTRACT
The aim of the present work was to formulate and evaluate an oral pulsatile drug delivery system to achieve time release of melonixcam, based on Chronopharmaceutical approach for the treatment of antiinflammatory agent. Pulsatile delivery system is capable of delivering drug when and where it required most. Time-delayed tablets, designed to release drug after a predictable lag time, are intended for oral chronotherapy. The basic design consists of a core tablets prepared by wet granulation method. The tablets were coated with an inners well able layer containing karaya gum and sodium alginate. The entire device was enteric coated with 3% cellulose acetate phthalate solution, so that the variability in gastric emptying time can be overcome. The prepared pulsatile tablets were evaluated for the drug content, thickness and in-vitro release profile, etc. In-vitro release profiles of pulsatile device during six hours studies were found to have very good sustaining efficacy. During the first five hours it shows minimum drug release and at the end of six hours immediate release was observed. Increasing the level of the rupturable layer increased mechanical strength and retarded the water uptake and thus prolonged the lag time. Stability studies proved that coating of tablets seems to decrease the effect of temperature and moisture on the degradation of melonixcam. The programmable pulsatile release has been achieved from tablet overa 7-8 hr period, consistent with the demands of chronotherapeutic drug delivery.

Key words: Melonixcam, chronotherapeutic drug delivery, Pulsatile delivery system.

INTRODUCTION:

Pulsatile drug delivery system

With the advancement of technology in the pharmaceutical field, drug delivery systems have
drawn an increasing interest over the last few decades. Nowadays, the emphasis of pharmaceutical formulation research has turned towards the development of more efficacious
drug delivery systems with already existing molecules rather going for new drug discovery because of the inherent hurdles posed in drug discovery and development process 1 . The oral
route of drug delivery is typically considered the most favored and user friendly means of drug administration having the highest degree of patient compliance, as a result of which great efforts are made to identify orally active candidates that would provide reproducible and effective plasma concentrations in vivo.

Traditionally, drug delivery is meant for getting a simple chemical absorbed predictably from the gut or from the site of injection. A second-generation drug delivery goal has been the perfection of continuous, constant rate delivery of bioactive agents. However, the requirement or response to drugs of living organisms is not „zero-order" all the time as they
are predictable resonating dynamic systems, which require different amounts of drug at predictably different times within the circadian cycle which will maximize desired and minimize undesired drug effects.. A recent review on developmental timing underlined the importance of chronobiologic processe. 7 Till early nineties efforts have been made to design the drug delivery system which will release the drug at fairly constant rate. In fact, these systems turned out to be one of the most successful systems in delivering the drug molecule. 8 But still for many of the drugs, use of conventional sustained release systems is not suitable because of following reasons:

1- **First pass metabolism**: Some drugs, such as beta blockers, and salicylamide, undergo extensive first pass metabolism and require rapid drug input to saturate metabolizing enzymes in order to minimize pre-systemic metabolism. Thus, a constant /sustained oral method of delivery would result in reduced oral bioavailability.

2- **Biological tolerance**: Continous release drug plasma profiles are often accompanied by a decline in the pharmacotherapeutic effect of the drug, e.g., biological tolerance of transdermal nitroglycerin.
3- **Special chronopharmacological needs:** Circadian rythms in certain physiological functionsare well established. It has been recognized that many symptoms and onset of diseaseoccur during specific time periods of the 24 hr day, e. g., asthma and angina pectoris attacks are most frequent in the morning hours.
4- **Local therapeutic need**: For treatment of local disorders such as inflammatory bowel disease, delivery of compounds to the site of inflammation with no loss due to absorption in small intestine is highly desirable to achieve the therapeutic effect and to minimize side effects.
5- Drugs which exhibit tolerance should not be delivered at a constant rate, since the drug effect decreases with time at constant drug level. In addition, drug toxicity increases with time when drug levels are held constant. In such cases it is preferable to opt for dosage form which will provide desired concentration of drug at particular time point only.

The effectiveness and toxicity of many drugs vary depending on the relationship between the dosing schedule and the 24-h rhythms of biochemical, physiological and behavioral processes. In addition, several drugs can cause alterations to the 24- hr. rhythms leading to illness and altered homeostatic regulation. The alteration of biological rhythm is a new concept of adverse effects. It has been demonstrated that the latter can be minimized by optimizing the dosing schedule. 10 Circadian rhythm regulates many body functions in human viz., metabolism, physiology, behavior, sleep patterns, hormone production, etc. It has been reported that more strokes and heart attacks occur during morning hours..

Table 1-1: Diseases requiring pulsatile drug delivery

Disease	Chronological behavior	Drugs used
Peptic ulcer	Acid secretion is high in the afternoon and at night	H2 blockers
Asthma	Precipitation of attacks during night or at early morning hour	β2 agonist, Antihistaminics
Cardiovascular diseases	BP is at its lowest during the sleep cycle and rises steeply during the early morning awakening period	Nitroglycerin, Calcium channel blocker, ACE inhibitors etc.
Arthritis	Pain in the morning and more pain at night	NSAIDs, Glucocorticoids
Diabetes mellitus	Increase in the blood sugar level after meal	Sulfonylurea, Insulin, Biguanide

Pulsatile delivery systems aim to deliver a drug via the oral route at a rate different thanconstant, (i. e., zero order relaease). Ideally such systems aim to match drug release rate to a biological requirement of a given disease while minimizing the treatment‟s side effects.Pulsatile drug delivery systems (PDDS) are characterized by at least two distinctive drugrelease phases following a predetermined lag time. Drug release may be controlled by time,by site or a combination of both the parameters. A delayed release delivery system (wheretime controls the release) would meet the needs of chronopathology with symptoms mostlyrecurring at night time or early in the morning whereas site-specific delivery into the colonregion might enable an improvement in the treatment of inflammatory bowel disease and,hopefully, in the oral bioavailability of peptide drugs.

Classification of PDDS

PDDS can be broadly classified into four classes;

I. Time controlled pulsatile release
   a. Single unit system
   b. Multi-particulate system
II. Stimuli induced
   a. Thermo-responsive pulsatile release
   b. Chemical stimuli induced pulsatile systems
III. External stimuli induced pulsatile release
   a. Electro responsive pulsatile release
   b. Magnetically induced pulsatile release

Pulsatile drug delivery systems are not the only oral drug delivery systems used to achieve tailor made drug delivery. Liposomes, currently used for intravenous delivery of small molecules, are also investigated for oral sustained release delivery of drugs. However, stability issues while in the gastrointestinal environment as well as difficulty in their transport via the intestinal membrane are two of their major disadvantages compared to polymeric vehicles.

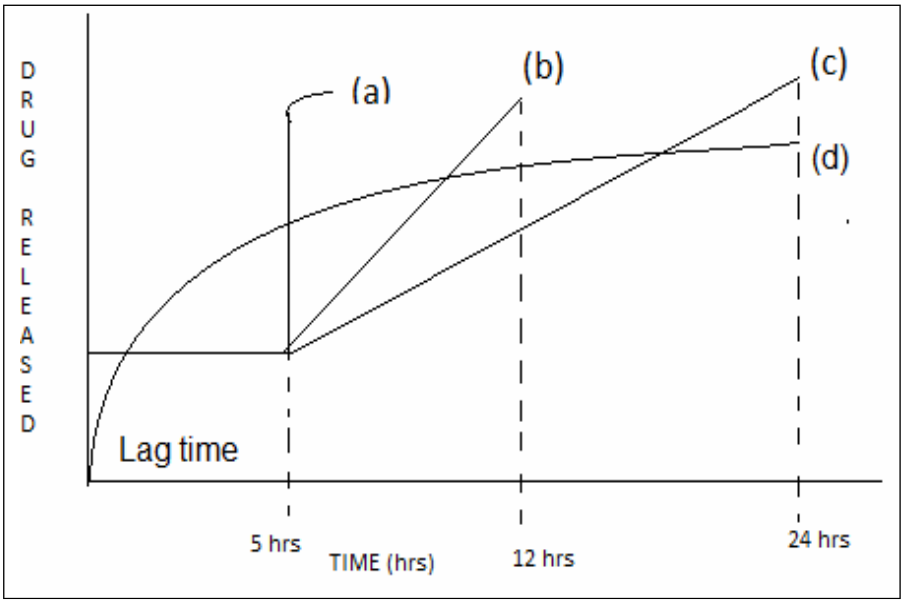


Figure 1-1: Schematic representation of different drug delivery systems

(a) = sigmoidal release after lag time, (b) = delayed release after lag time,
(c) = sustained release after lag time, (d) = extended release without lag time.

MATERIALS AND METHOD

Drug and Acetonitrile was purchased from Merck industries, Mumbai. Corn Starch and HPMC was gift sample from Loba Chemie Pvt. Ltd., Mumbai. Magnesium stearate was purchased from S.D. Fine chemical Ltd., Mumbai. Cyclohexane was purchased from Merck Limited.

METHOD

Drug Quantification

Spectroscopy method

Meloxicam 100 mg was dissolved into dimethylformamide to obtain 1000 µg /ml (firststock) solution which was further diluted to 100 µg /ml in methanol (second stock). Final dilutions from second stock were made in 0.1N HCL and phosphate buffer pH 6.8 ton obtain 2, 4, 6, 8, 10, 12 and 14 µg/ml of meloxicam solutions (n=3) separately. Absorbance of these samples was determined at λ max 364 nm against respective blanks. Regression analysis was performed to find the linearity of the sample in the working range for analysis of samples and the standard curve was constructed.

Assay

Accurately weighed working standard (WS) of meloxicam (Batch no. QC/WS/ML/01) 10mg was dissolved in 10 ml of chloroform to get stock solution of 1000 µg/ml. One ml ofthe stock solution was diluted to 100 ml to get a solution of 10 µg/ml (n=3). The absorbance of these solutions was measured with UV spectrophotometer at 364 nm.The test sample was prepared by accurately weighing 10 mg of meloxicam sample anddissolving it in 10 ml of chloroform to get stock solution of 1000 µg/ml. One ml of thestock solution was diluted to 100 ml to get a solution of 10 µg/ml (n=3). Absorbance ofthese solutions was measured with UV spectrophotometer at 364 nm.

Compatibility studies

Drug–excipient compatibility study was carried out to find out effect of temperature and humidity on drug excipient mixture, by placing drug alone and drug along with individual excipients in certain ratio of formulation in Stoppard vials at 40 0 C/75% RH for two months.

Samples were physically observed and degradation was examined by FTIR spectroscopy at the end of the studies.

Table 5-3: Composition of samples for compatibility studies

Sr. No.	Composition	Ratio
1	API	1
2	API:Spray dried lactose	1:2.8
3	API :Croscarmellose	1:0.1
4	API :Magnesium stearate	1:0.1
5	API :Talc	1:0.1
6	API :Ethyl cellulose	1:10

Analytical method validation

Stability indicating assay method (HPTLC)

Stability Testing On New Drug Substances and Products‟ (Q1A) requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances. It suggests that the degradation products that are formed under a variety of conditions should be identified and degradation pathways should be established. The testing should include the effect of temperature, humidity where appropriate: oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values.

The stress conditions used for the study of decomposition in acid conditions reveals that hydrochloric acid at strength of 0.1 N was mostly used. A few reports also indicated the use of 1N HCl and even higher normalities. There are a few instances where sulphuric acid in varying normalities was used. The stress conditions used for the drug degradation in alkaline conditions uses sodium hydroxide of strength 0.1N and 1N. Potassium hydroxide is used in a few cases. The stress conditions for studying oxidation employs hydrogen peroxide in various strengths from 1% to 30%.

A. Experimental conditions

A stock solution of meloxicam (1mg/ml) was prepared in chloroform. A standard solution of 100 µg /ml was

used for the analysis. The samples were spotted on HPTLC aluminium plates (10 x10 cm) precoated with silica gel 60 F_{254} (layer thickness 0.2 mm, E-Merck). Spotting was done using Camag Automatic TLC Sampler 4 (ATS4) model. The samples were spotted in the form of narrow bands of length 8.0 mm, 15 mm from the bottom edge, 10 mm from the margin, 14.0 mm apart at a constant rate of 150 nl/s using a nitrogen aspirator. The chamber was saturated for a period of 15 min with the mobile phase ethyl acetate: cyclohexane: glacial acetic acid (6.5:3.5:0.02% v/v/v) and the plate was allowed to run a length of 7 cm. The separation was observed under short length (254 nm) ultraviolet lamp. Densitometric analysis of the separated components was carried out using Camag TLC Scanner 3 in the absorbance/reflectance mode at 361 nm. The slit dimensions were 6.00 x0.45 mm and the sensitivity was kept at the auto mode. Scanning speed was 20.00 mm/s. Integration of the chromatogram was carried out using the Camag TLC scanner/integrator system (Perkin Elmer, USA). Appropriate volumes of standard solution (100µg/ml) were spotted to obtain meloxicam in the concentration range of 60 ng -180 ng (n=3) and calibration curve was obtained by plotting peak area versus concentration.

B. Precision and accuracy

To evaluate system precision, six spots of standard solution (100 µg /ml) were applied to get concentration of 150 ng. Method precision was carried out by applying the spot (150 ng) from six different standard solutions (100 µg /ml). The LOD and LOQ were calculated from the signal to noise ratio

Table 5-4: Sample preparation for stress studies

Sr.No.	Degradation	Sample	Description
1	Acid degradation	Blank 1	1 ml chloroform +1ml 1N HCl stored under normal condition, neutralized, reconstituted to 10ml by chloroform
		Blank 2	1ml chloroform +1ml 1N HCl subjected to refluxed for 3 hrs., cooled to R. T., neutralized, reconstituted to 10 ml by chloroform
		Zero time	1 ml drug solution in chloroform + 1 ml distilled water under normal condition, reconstituted to 10 ml by chloroform(100 µg/ml)
		Sample	1 ml drug solution in chloroform + 1ml 1N HCl subjected to refluxed for 3 hrs., reconstituted to 10 ml by chloroform(100 µg/ml)
2	Base degradation	Blank 1	1ml chloroform +1ml 1N NaOH stored under normal condition, neutralized, reconstituted to 10 ml by chloroform
		Blank 2	1ml chloroform +1ml 1N NaOH subjected to refluxed for 3 hrs., cooled to R. T. , neutralized, reconstituted to 10ml by chloroform.
		Zero time	1 ml drug solution in chloroform + 1 ml distilled water under normal condition, reconstituted to 10 ml by chloroform (100 µg/ml)

		Sample	1 ml drug solution in chloroform + 1ml 1N NaOH subjected to refluxe for 3 hrs., reconstituted to 10 ml by chloroform (100 µg/ml)
3	Photodegradation	Sample	10 mg drug sample dissolved in 100 ml chloroform (100µg/ml)
4	Heat degradation	Sample	10 mg drug sample dissolved in 100 ml chloroform (100µg/ml)
5	Oxidative degradation	Sample	1 ml drug solution in chloroform reconstituted to 10 ml by 1%, 3% and 5% H_2O_2 separately under normal conditions. (100 µg/ml)

Bioanalytical method validation

A reported method for the determination of meloxicam in the plasma samples was studied. The drusg was extracted from plasma into organic phase via Liquid-Liquid Extraction (L.L.E.) by precipitating the proteins with ACN, separated organic phase was evaporated under nitrogen stream and reconstituted with known volume of mobile phase. The drug was analysed using HPLC. A RP-HPLC-UV method was validated using mobile phase with composition 0.02M Potassium dihydrogen phosphate: Acetonitrile in the ratio 60:40. Cosmosil C18 (150mm x 4.6 mm, ID) 5µ column was used to estimate meloxicam in pig plasma samples and Lornoxicam was used as internal standard (IS). Constant flow rate of 1.0 ml/ minute was maintained throughout the study at fixed detection wavelength of 360 nm at ambient temperature. The injection volume of 20 µl and 10 minutes run time was kept constant during analysis. The rentention time for lornoxicam (IS) and meloxicam was found to be 5.03 and 8.89 min respectively. The bioanalytical method was further validated for specificity, linearity, precision and accuracy, recovery (% extraction yield), stock solution stability.

Mobile phase preparation

- **Preparation of 0.02 M Potassium Di-hydrogen Phosphate buffer**

  Potassium Di-hydrogen Phosphate (KH_2PO_4) (1.3609 G) was dissolved in 1000ml of distilled water and the pH was adjusted to 3.5 with orthophosphoric acid (OPA).

- **Mobile phase composition**

  The solvent mixture of 0.02M potassium dihydrogen phosphate and acetonitrile (ACN) in the ratio of 60:40 (v/v) was prepared.

Sample preparation

- **Blank**

  Heparinised pig plasma (500µl) was taken in different tarson tubes.

- **Pig plasma sample**

Blood samples from pigs were collected at predetermined time intervals in tarson tubes containing heparin. The plasma was separated by centrifugation at 5000 rpm for 5 min. The heparinised plasma (500 µl) was taken in different ependorf tubes.

Extraction procedure

Choloform (5ml) was added in tarson tube with solvent dispenser. The tubes were kept on rotary shaker for 10 min. at 12 rpm. Then the tubes were centrifuged at 5000 rpm for 10 mins. The upper layer of the plasma was aspirated and 4 ml clear supernatant was transferred in low volume evaporator tubes. The solvent was evaporated at 60^0C under steady nitrogen stream till dryness. The residue was reconstituted with 100 µl mobile phase and was votexed for 30 sec. Reconstituted residue (20 µl) was injected into the HPLC

system.

Table 5-5: Summary of validation parameters

Sr no.	Parameter	Description
1	Analyte	Meloxicam
2	Analytical technique	RP HPLC-UV detector
3	Equipment used	Jasco H.P.L.C. PU 980 pump fitted with AS- 1555-10 Auto sampler
4	Software used	Borwin Integrator Software, version 1.21
5	Scan type	Jasco UV 970
6	Column type	Cosmosil C_{18} (150mmX4.6mm, I.D. 5µ)
7	Mobile phase	0.02 M Potassium dihydrogen phosphate : Acetonitrile (60:40)
8	Flow rate	1.0 ml/minute
9	Biological matrix	Pig plasma
10	Anticoagulant used	Heparin
11	Sample extraction	Liquid-Liquid extraction
12	Linearity range	20 ng/ml-3000ng/ml
13	Equation type	Linear, y = mx+c
14	Weighting factor	1/x
15	Validated LQC	30.00 ng/ml for meloxicam
16	Validated MQC	400.00 ng/ml for meloxicam
17	Validated HQC	2000.00 ng/ml for meloxicam
18	Autosampler stability	For 24 hours at 4^0C
19	Freeze Thaw stability	3 cycles at -20± 5^0C
20	Short term Stock solution Stability	For 12 hrs. at 2-8^0C
21	Long term stock solution stability	For 30 days at 2-8^0C
22	Long term stability in matrix	For 30 days at -20± 5^0C

Formulation of core tablets

From the study of powder characteristics of meloxicam powder it was observed that meloxicam is having poor flow properties and compressibility. To improve the flow properties of the powder it was decided to use spray dried lactose as one of the excipients. Meloxicam immediate release tablets are available in two strengths, i.e., 7.5 mg for osteoarthritis twice a day and 15 mg for chronic artriyic conditions like rheumatoid arthritis once a day. The pharmacokinetic properties of meloxicam allow once a daily administration, which is important factor in compliance, especially in the treatment of chronic rheumatic diseases. The elimination half life of meloxicam is 20 hrs. and the steady state is achieved within 3 to 5 days with small peak-through fluctuations. This makes meloxicam well suited for once –daily

administration without a longer lasting accumulation of drug levels. Oral pulsatile release tablet is intended for once a daily preparation. Hence, the core tablet containing 15 mg meloxicam was prepared by direct compression method using 5mm flat bed punches on rotary tablet punching machine.

Table 5-6: Formulation of core tablet

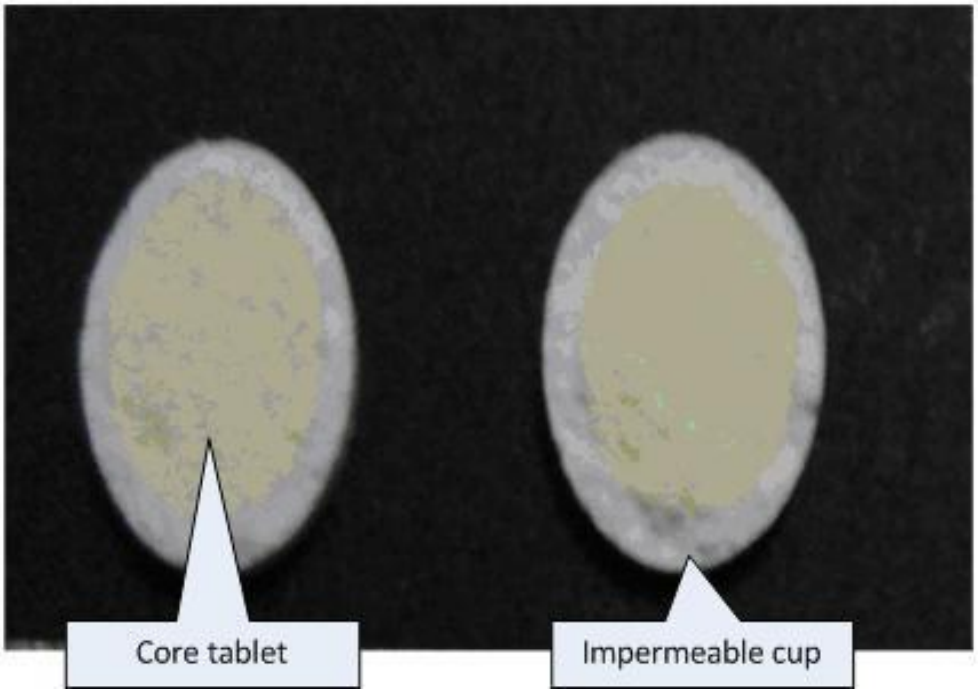
Sr. No.	Ingredients	Batches		
		F 1	F 2	F 3
1	Meloxicam	15 mg	15 mg	15 mg
2	Spray Dried Lactose (Pharmlactose)	45.5 mg	43.8 mg	42.5 mg
3	Croscarmellose	1.3 mg	2.5mg	3.7 mg
4	Talc	1.3 mg	1.3 mg	1.3 mg
5	Magnesium stearate	1.9 mg	2.4 mg	2.5 mg
	Total weight	65 mg	65 mg	65 mg

Evaluation of Core tablet:

- The core tablets of meloxicam were subjected to quality control tests for tablet. Tablets were evaluated for hardness (n=6) using Monsanto type hardness tester, friability (n=10) using Roche Friabilator, weight variation (n=10) using an electronic balance and thickness (n=10) using vernier caliper.
- Content uniformity of tablets was determined where 10 tablets were powdered and powder quantity equivalent to 15 mg (label claim) meloxicam i.e., 65 mg was extracted with 100 ml of chloroform. The resultant suspension was shaken for 15 min. and was subjected to filtration through 0.45 micron filter. Suitably diluted samples were estimated for content uniformity using validated HPTLC method and recovery study for assay was carried out.
- **In- viro dissolution test**

  The core tablet was tested for dissolution as per *USP 32* using USP II paddle dissolution apparatus, using 900 ml 0.1N HCl, phosphate buffer pH 6.8, and pH 7.2 as dissolution media separately ,at 75 rpm and at $37^0C \pm 0.5^0C$. The aliquots were removed at 5, 10, 15, 30, 45 and 60 mins and were analyzed by UV spectrophotometer at 364 nm.

Formulation of Swellable top layer core in cup pulsatile drug delivery system



Hydrophilic polymer, polyethylene oxide (PEO) 1105 and hydrophobic polymer, Cellulose acetate propionate (CAP) 482-0.5 were used to develop directly compressed core in cup type tablet. Hundred milligrams of CAP482-0.5(#40) was filled in the 8mm flat bed die and slightly compressed to get a flat bed. The core tablet was kept over the flat bed and remaining quantity of CAP 482-0.5 (40 mg) was poured on the sides of the core tablet. It gave core in cup tablet. PEO was added as top swellable layer and was compressed. The swellable top layer composition was varied with different weights of PEO and stearic acid (SA). The mixtures of SA and PEO were prepared by dissolving SA in ethanol. Ethanolic solution of SA was used to prepare granules with PEO. The PEO - SA granules were dried at room temp. over night and passed through sieve # 40. PEO-SA mixture was also prepared by melting SA at 60^0c.

Table 5-7: Formulation of swellable top layer PDDS

Sr. No.	Ingredients	Batch Description				
		SW1 (mg)	SW2 (mg)	SW3 (mg)	SW4 (mg)	SW5 (mg)
1	Core tablet	65	65	65	65	65
2	CAP	100	100	100	100	100
3	PEO WSR 1105	35	65	85	---	---
4	PEO + SA(1:1 granulation)	---	---	---	95	85
5	PEO + SA(1:1 fusion)	---	---	---	---	
	Total weight	200	230	250	260	25

Evaluation of swellable top layer PDDS

- ***In vitro* dissolution tests**

Formulated pulsatile release tablets were subjected to dissolution studies using USP II paddle dissolution apparatus, with 900 ml, 0.1 N HCL as dissolution medium at 75 rpm and 37 ^{0}C ± 0.5^{0}C for 2hrs., followed by 900 ml phosphate buffer pH 6.8 ,at 75 rpm , 37 ^{0}C ± 0.5^{0}C for rest of the period. Aliquots were removed at predetermined time intervals and analyzed by using UV spectrophotometer at 364 nm. The lag time was estimated by plotting average % cumulative release vs average time in hours.

Formulation of capsular pulsatile drug delivery system

Time- delayed dosage forms permit the delivery of drugs after a predetermined lag –time. This can have clinical significance where the disease state has shown circadian rhythm dependency. A pulsed- release capsule delivery device is developed where the release time can be controlled by varying the properties of the erodible tablet used to seal the drug inside an impermeable capsule body.

Size 0 hard gelatin capsule was used to formulate the capsular PDDS. The capsule body was coated with EC N45. An 8 % w/v solution of EC N45 was prepared in ethanol. The capsule bodies were coated by dipping method. The coated capsule bodies were air dried overnight. A hydrophilic plug of HPMC (methocel K10) and lactose was prepared by wet granulation.

In vitro dissolution tests

Formulated pulsatile release coated capsules were tested for dissolution studies using USP II paddle dissolution apparatus, using 900ml, 0.1 N HCL as dissolution medium at 75 rpm and at 37 ^{0}C ± 0.5^{0}C for 2hrs., followed by 900 ml phosphate buffer pH 6.8, at 75 rpm, and at 37 ^{0}C ± 0.5^{0}C for rest of the period. Aliquots removed at predetermined time intervals were analyzed by UV spectrophotometer at 364nm. Lag time was found out by plotting the average % cumulative drug release vs average time in hours.

Table 5-8: Formulation of capsular PDDS

Sr. No.	Ingredients							
	(mg)	C1	C2	C3	C4	C5	C6	C7
1	Core tablet	65	65	65	65	65	65	65
2	Croscarmellose	200	200	200	200	---	---	---
3	L-HPC	---	---	---	---	200	200	200
4	EP*(HPMC 15% +lactose) Wet granulation	65	---	---	---	55	---	---
5	EP*(HPMC 30% +lactose) Wet granulation	---	60	---	---	---	60	---
6	EP*(HPMC 15%+lactose) Direct compression	---		60	---	---	---	60
7	EP*(HPMC 30%+lactose) Direct compression	---	---	---	60	---	---	---
	Total wt (mg)	330	325	325	325	320	325	325

Stability studies

Stability testing is an integral part of the pharmaceutical development process. It is routinely performed on

the drug substances and the drug products. The primary purpose of stability testing in an industrial setting is to provide supporting evidence on stability behavior of chemical or biological entities as well as to study the stability behavior of pharmaceutical products. Stability testing is a function of time against environmental factors. Stability testing is conducted so as to determine storage conditions, retest periods, expiry dates and shelf lives of pharmaceutical products. Primary stability studies are intended to show that the drug product stored in the proposed container/closure for marketing will remain within specifications if stored under storage conditions that support the proposed shelf life.

Stability study of optimized batch of rupturable pulsatile release tablets was conducted for three months as per ICH guidelines. HDPE bottles were used as containers for stability studies. Each bottle containing 20 units & 2G silica bag were kept for real time (25^0C / 60% RH), 30^0C /65% RH, and accelerated condition (40^0C /75% RH). The samples were withdrawn at predetermined time intervals as per ICH guidelines and analysed for % drug release and total drug content.

Table 5-9: Protocol for stability testing of optimized batch

Sr. No.	Sampling interval / Conditions	15 Days	1 month	2 months	3 months	Parameters tested	
						Dissolution (at the end of 3 months)	Drug Content
1	RT (25^0C /60%RH)	√	√	√	√	√	√
2	30^0C/65% RH	√	√	√	√	√	√
3	Accelerated (40^0C/ 75% RH)	√	√	√	√	√	√

X-ray imaging studies for gastric emptying in rabbits

Table 5-10 : Study protocol for assessment of gastric emptying of optimised PR tablet in rabbits

1	**Animals used**	Rabbits
2	**Weight**	1.5-2.0 Kg
3	**Gender**	Male/Female
4	**Dose of barium**	15 mg/tablet
5	**PR tablet weight**	220 mg
6	**PR tablet diameter**	7.5 mm
7	**Number of test formulations**	1
8	**Total number of animals**	2
9	**Route of administration**	Oral

10	**Imaging intervals**	0, 1, 2, 4, 6, 8 hrs.

***In -vivo* studies of optimized formulation**

Pharmacokinetic data obtained from test species are useful in interpretation of drug effects and formulation performance especially for modified release dosage forms. Blood samples after administration of the drug are collected at specific times to assess the concentration – time profile. Depending on the dosage form primary parameters in the pharmacokinetic studies include estimation of area under the concentration curve (AUC), maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), elimination half life ($t_{1/2}$), absorption rate constant (K_a) and elimination rate constant (K_e)

Table 5-11: Protocol for evaluation of pharmacokinetic parameters of the optimised formulation

Sr. No.	Description	Number of formulations	Number of animals	Total number of animals
1.	Test formulation	1	3/group	3
2.	Reference group (positive control)	1	3/group	3
3	Total number of animals	6		

Results and discussion

Preformulation studies

1. **Organoleptic properties of meloxicam**

Sr. No.	Parameter	Specification	Observation	Remark
1.	Colour	A pale yellow powder	A pale yellow powder	Complies with pharmacopoeial monograph in BP
2.	Odour	No characteristic odour	No characteristic odour	Complies with pharmacopoeial monograph in BP

2. Solubility

Parameter	Specification	Observation	Remark

Solubility	Practically insoluble in water, slightly soluble in acetone, soluble in dimethylformamide, very slightly soluble in ethanol (96%) and in methanol	Practically insoluble in water, slightly soluble in acetone, soluble in dimethylformamide, very slightly soluble in ethanol (96%) and in methanol	Complies with pharmacopoeial monograph in BP

2. **UV spectroscopy**

- The UV spectra of 0.00015% w/v solution of meloxicam in methanol showed an absorption of 0.6850 at wavelength of 364 nm as per BP.

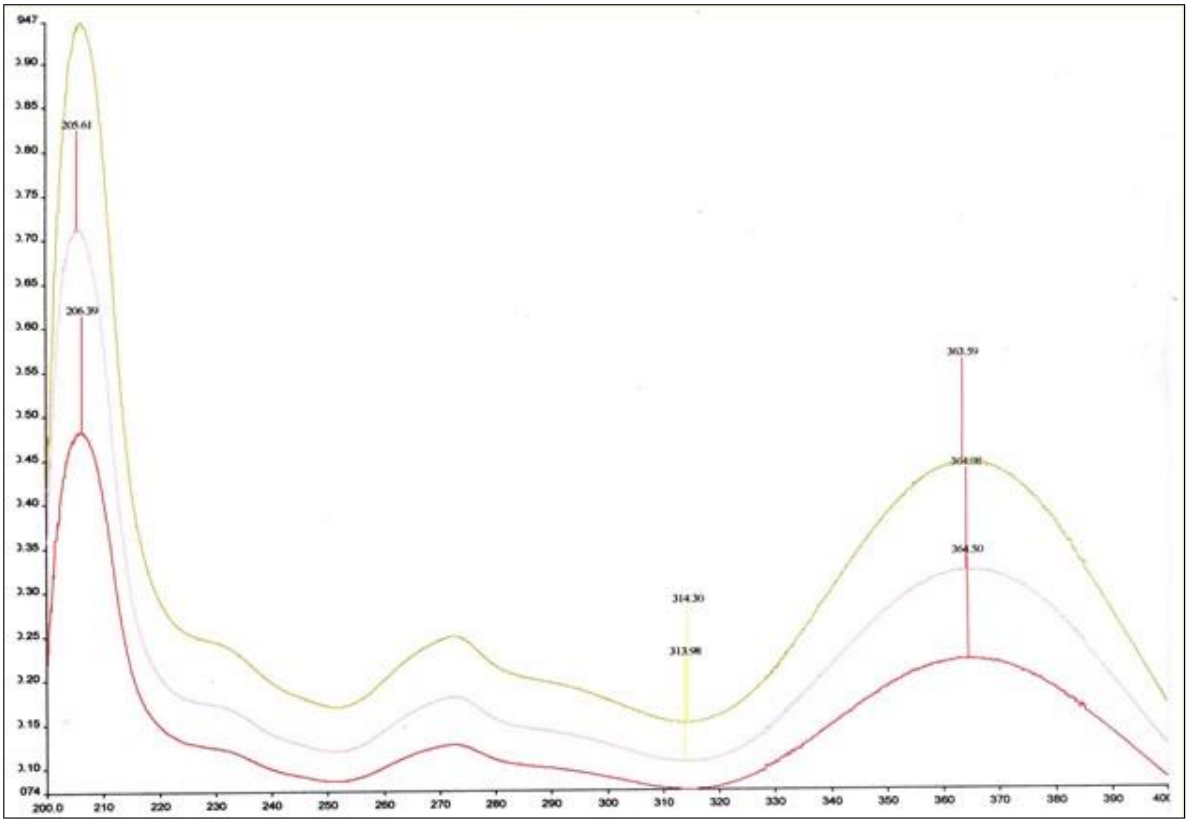


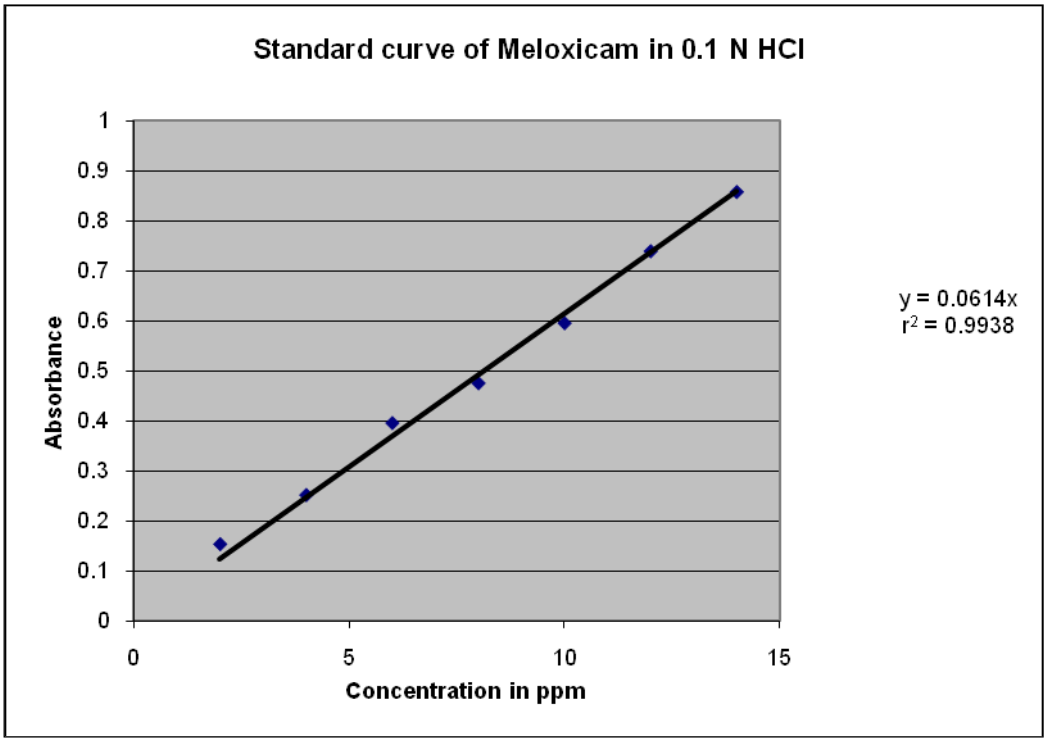
Figure 5-12 : UV spectra of meloxicam in methanol

Drug quantification

- **Spectroscopy method**

Table : Standard curve of meloxicam in pH 1.2 (0.1 N HCl)

Sr. No	Concentration (ppm)	Absorbance
1	0	0
2	2	0.1547
3	4	0.253
4	6	0.3969
5	8	0.4765
6	10	0.5966
7	12	0.7405
8	14	0.8589



Saturation solubility studies

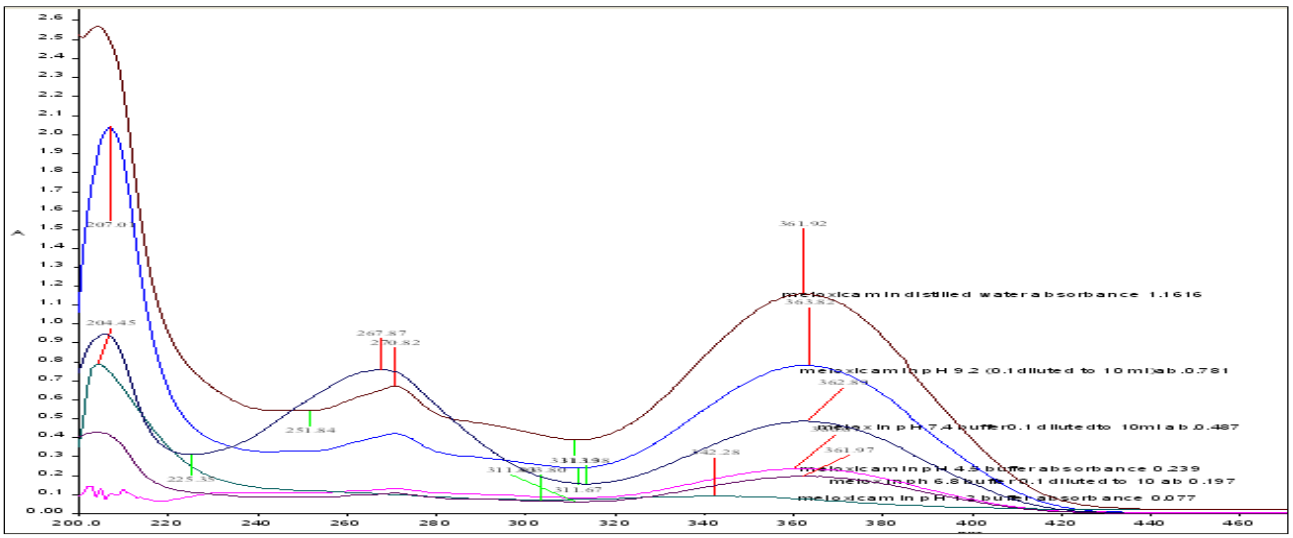
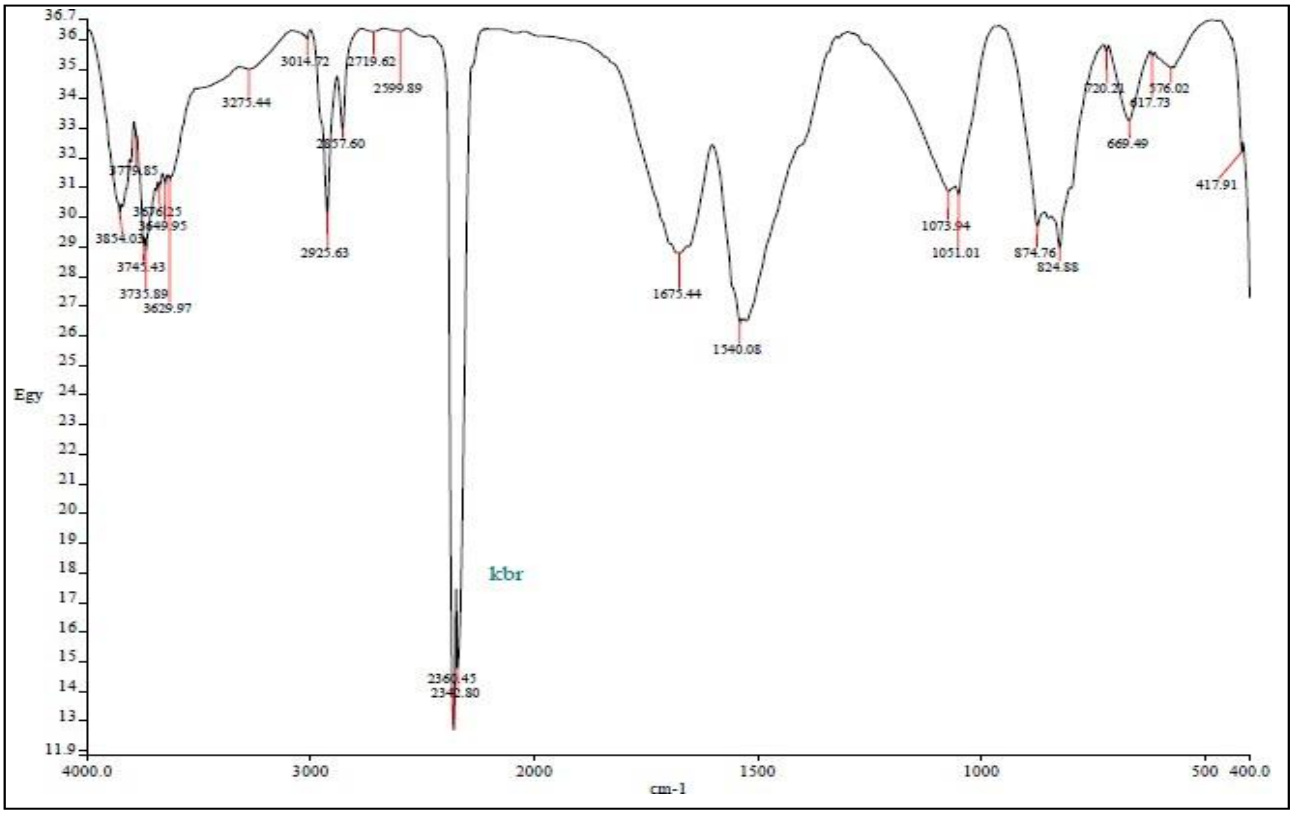


Figure: UV spectra of meloxicam at different



- **FTIR Compatibility studies**

Analytical method validation

Stability indicating assay method (HPTLC)

1.Detection and quantification limits (sensitivity)

LOD: The limit of detection (LOD) was estimated to be 15 ng /ml for meloxicam.

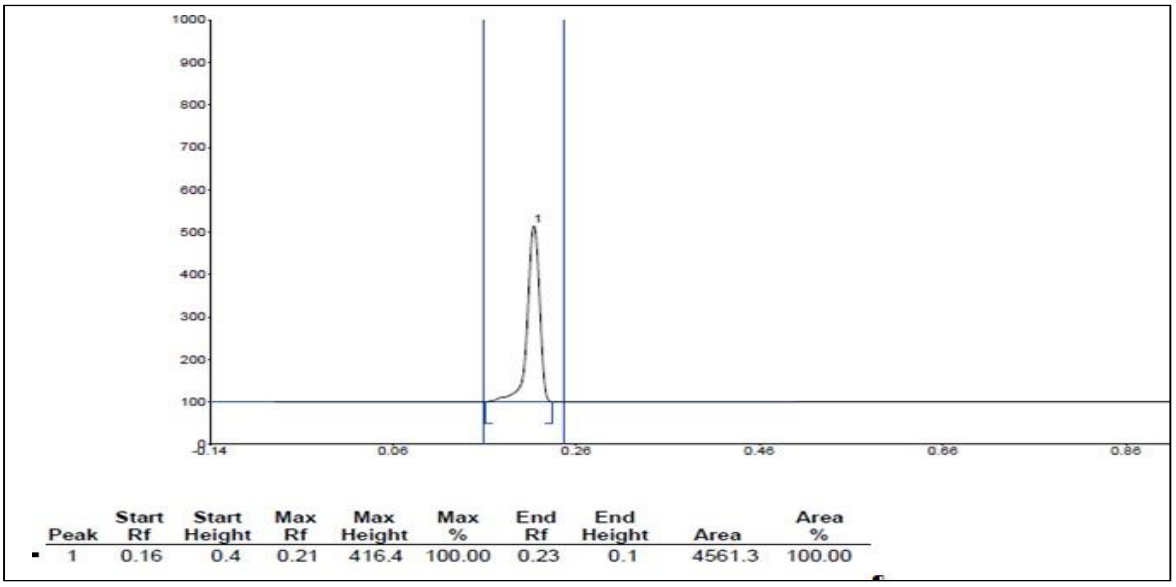
LOQ: The limit of quantification (LOQ) was estimated to be 50 ng/ml for meloxicam.

Stress Studies
1.**Acid hydrolysis Figure**

Physicochemical evaluation of meloxicam core tablet

Formulation code	**Mel1**	**Mel2**	**Mel3**
Average weight (mg)	65	65	64
Thickness (mm)	1.6± 0.01	1.6± 0.01	1.6± 0.01
Diameter (mm)	5± 0.05	5± 0.05	5± 0.05
Hardness (kg/cm^3)	5± 0.05	5± 0.02	5± 0.03
% drug content	99.98	100.2	99.99
Disintegration time (min)	5±0.5	3±0.05	3±0.05

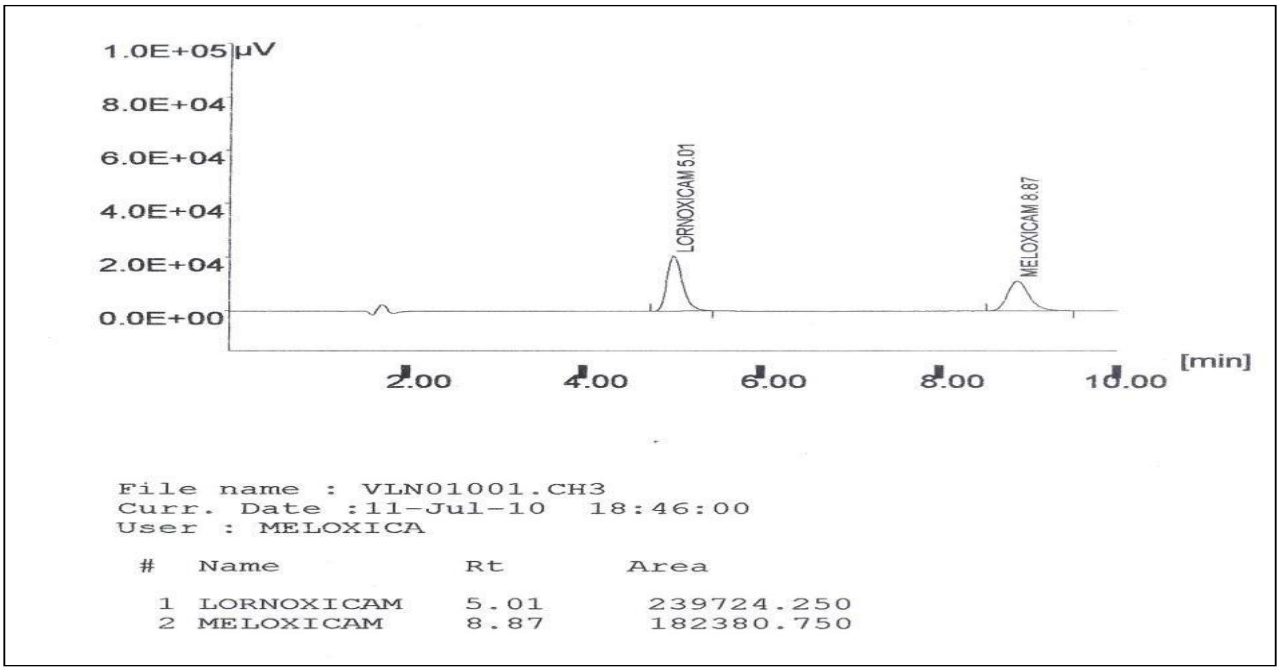
Chromatogram for standard meloxicam solution



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.16	0.4	0.21	416.4	100.00	0.23	0.1	4561.3	100.00

Bioanalytical method validation parameters

1.System suitability

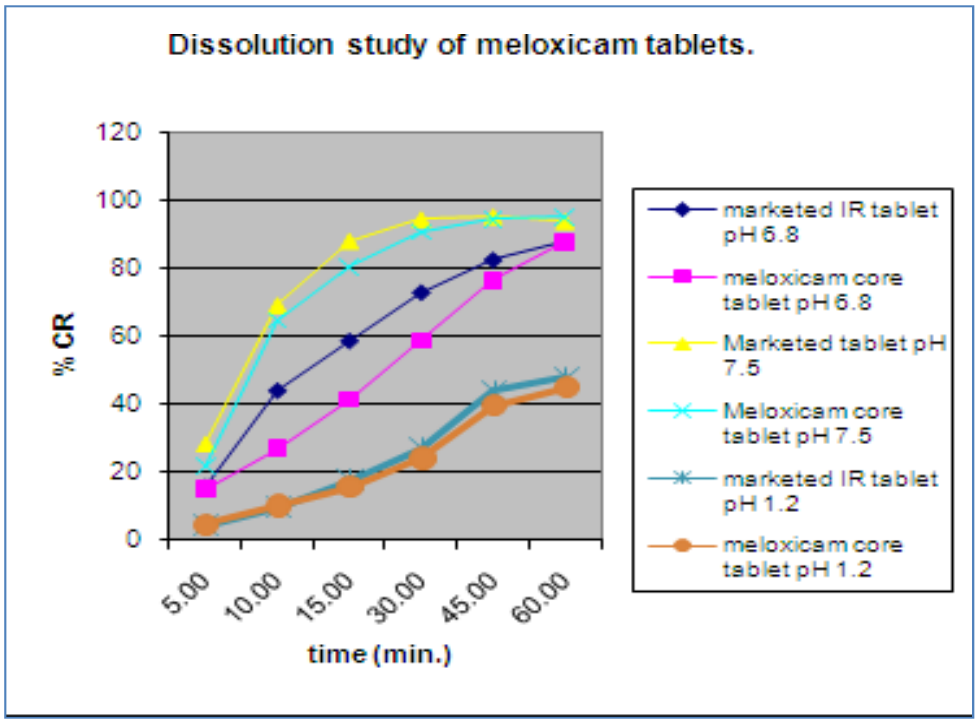


➢ **Dissolution studies of meloxicam core tablet**

Table 5.13: Dissolution of meloxicam IR tablet

Formulation	Time point (min.)	% CR in pH 1.2	% CR in pH 6.8	%CR in pH 7.4
Muvera	5	4.50	15.82	28.08
	10	9.89	44.05	69.07
	15	17.90	58.61	88.09
	30	26.90	72.85	94.53
	45	44.08	82.38	95.31
	60	47.98	87.70	95.31
Meloxicam core tablet	5	5.00	15.05	21.46
	10	10.40	27.17	64.78
	15	15.90	41.47	80.56
	30	24.40	58.89	90.98
	45	40.00	76.80	94.56
	60	45.00	88.09	95.37

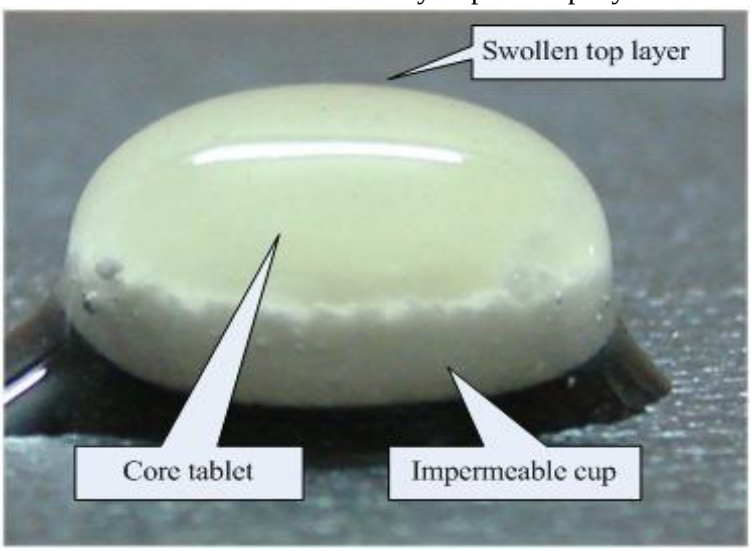
Figure 5-14: Dissolution profile of meloxicam tablets at different physiological pH

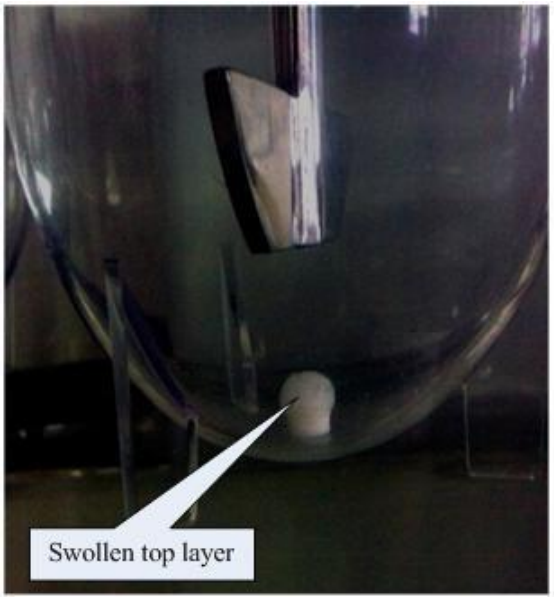


Evaluation of swellable top layer pulsatile drug delivery system

- **In vitro dissolution test**

➢ Top layer comprising PEO gradually uptake dissolution media and undergoes swelling as shown in fig 6.78. It indicates that the impermeable cup does not absorb dissolution media and core table get exposed to the dissolution media only from top of the tablet. Dissolution studies were carried out at pH 1.2 and at pH 6.8. The % CR at different time points is shown in table no 6.38. Fig. 6.79 indicates swelling of top PEO layer and removal of swollen layer. The dissolution studies indicated that the hydrophilic top layer.





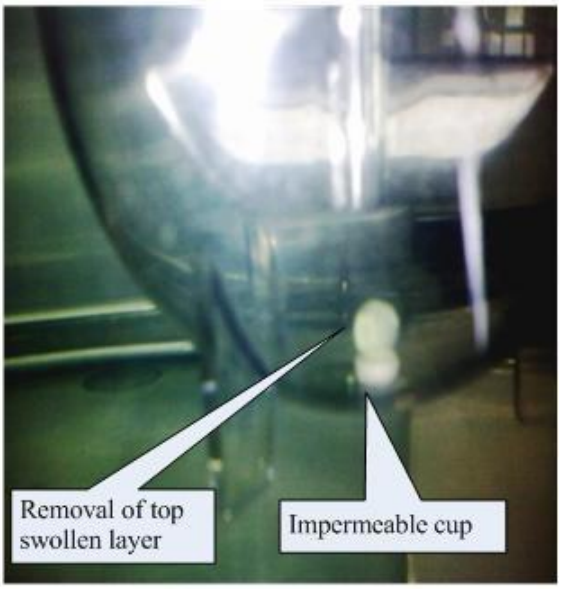


Table 5-15-: In vitro dissolution of swellable top layer oral PDDS

Batch no										
Avg (n=3)	**S1**		**S2**		**S3**		**S4**		**S5**	
	Time (hrs)	%CR	Time (hrs)	%CR	Time (hrs)	%CR	Time (hrs)	%CR	Time (hrs)	%CR
pH 1.2	1	0.00	1	0.00	1	0.00	1	0.00	1	0.00
	2	0.00	2	0.00	2	0.00	2	0.00	2	0.00
pH 6.8	2.3	0.00	2.05	0.68	2.05	0.74	2.05	1.74	2.05	1.74
	3	2.04	2.1	0.12	2.1	0.23	2.1	0.23	2.1	0.23
	3.1	31.12	2.15	0.52	2.15	0.40	2.15	0.40	2.15	0.40
	3.2	62.54	2.3	0.31	2.3	0.92	2.3	0.92	2.3	0.92
	3.3	84.08	2.45	0.16	2.45	0.05	2.45	0.05	2.45	0.05
	3.4	87.23	3	0.21	3	0.64	3	0.64	3	0.64
	4	91.37	3.3	0.25	3.3	0.40	3.3	0.40	3.3	0.40
	4.3	90.71	4	0.12	4	0.22	4	0.22	4	0.22
	5	91.06	4.3	1.73	4.3	1.82	4.3	1.82	4.3	1.96
	5.3	91.65	5	0.75	5	1.96	5	1.96	5	1.96
			5.25	8.76	6	1.96	6	1.96	6	8.74
			5.77	30.83	7	1.77	7	25.89	7	25.89
			5.9	44.00	7.3	8.74	7.3	66.03	7.3	66.03
			6.05	70.50	7.45	25.89	8	86.26	8	77.34
			6.33	80.59	8	66.03	8.15	95.36	8.15	86.26

			6.77	88.87	8.15	77.34			8.3	95.36
			7.15	88.74	8.3	86.26				
			7.3	91.32	8.45	95.36				
			8	93.39	9	95.41				
			8.15	93.40						

Formulation of capsular pulsatile drug delivery system

Table 5-16: *In vitro* dissolution of capsular PDDS

	Batch no.															
Avg (n=3)	**C1**		**C 2**		**C3**		**C4**		**C5**		**C6**		**C7**		**C8**	
	Time (Hrs)	% CR	Time (Hrs)	% CR	Time (Hrs)	% CR	Time (Hrs)	% CR	Time (Hrs)	% CR	Time (Hrs)	% CR	Time (Hrs)	% CR	Time (Hrs)	% CR
pH 1.2	1.00	0.98	1	0.20	1.00	0.06	1.00	0.20	1	0.98	1.00	0.20	1	0.67	1.00	0.20
	2.00	0.40	2	0.26	2.00	0.01	2.00	0.20	2	0.40	2.00	0.39	2	0.37	2.00	0.20
pH 6.8	3.00	0.88	3	0.59	3.00	0.00	3.00	0.88	3	0.88	3.00	0.44	3	0.59	3.00	0.44
	3.30	0.47	3.3	0.73	3.30	0.22	3.30	1.76	3.3	0.47	3.30	0.22	3.3	0.38	3.30	0.22
	4.00	5.07	4	0.52	4.00	31.17	4.00	0.23	4	5.07	4.00	0.44	4	13.77	4.00	0.88
	4.10	5.07	4.3	0.66	4.05	48.00	4.30	0.66	4.1	5.07	4.30	0.88	4.08	19.38	4.30	0.44
	4.15	6.17	5	1.39	4.10	62.77	5.00	1.54	4.15	6.17	5.00	0.88	4.13	25.04	5.00	1.76
	5.10	33.27	5.3	1.47	4.15	75.40	5.30	1.98	5.1	42.05	5.30	0.88	4.78	50.24	5.30	1.55
	5.15	49.24	5.81	8.40	4.30	82.05	6.00	1.77	5.15	44.90	5.45	21.88	4.87	58.73	6.00	1.54
	5.30	63.87	6.2	17.66	4.45	87.74	6.30	1.76	5.3	57.26	6.00	49.46	5.02	69.63	6.30	1.76
	5.45	88.00	6.71	33.17	5.00	95.67	7.00	8.78	5.45	85.76	6.15	68.11	5.3	89.81	7.00	22.61
	6.00	88.13	6.91	54.77	5.15	95.72	7.15	20.89	6	88.12	6.45	88.02	5.72	90.65	7.15	55.39
			7.2	69.85			7.30	53.16	6.3	88.19	7.00	88.24			7.30	68.16
			7.35	84.96			7.45	78.63			7.15	88.24			7.45	88.02
			7.7667	88.26			8.00	88.41			7.30	88.24			8.00	88.13
			8.15	88.46			8.15	88.46								

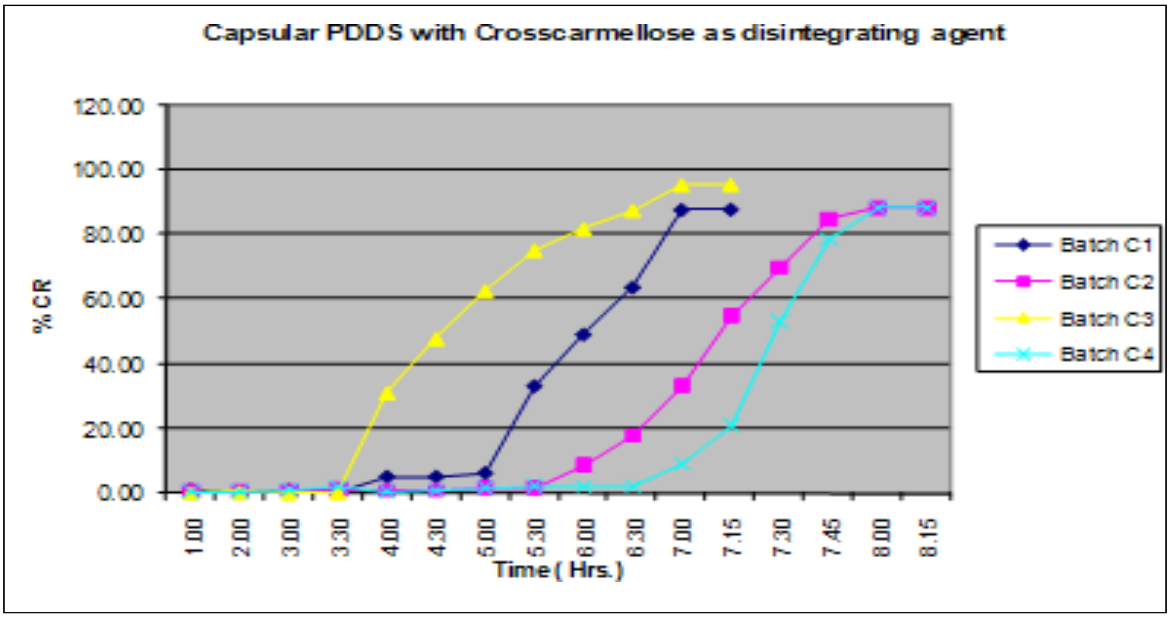


Figure5.17 : Drug release from capsular PDDS with croscarmellose as distegrating agent

Stability studies

- **Stability indicating assay**

Assay of the tablets from batch R5 (n=10) was performed. A validated HPTLC stability indicating assay method was used. It indicates developed HPTLC plate for assay at different conditions of temp. and humidity as per ICH guidelines

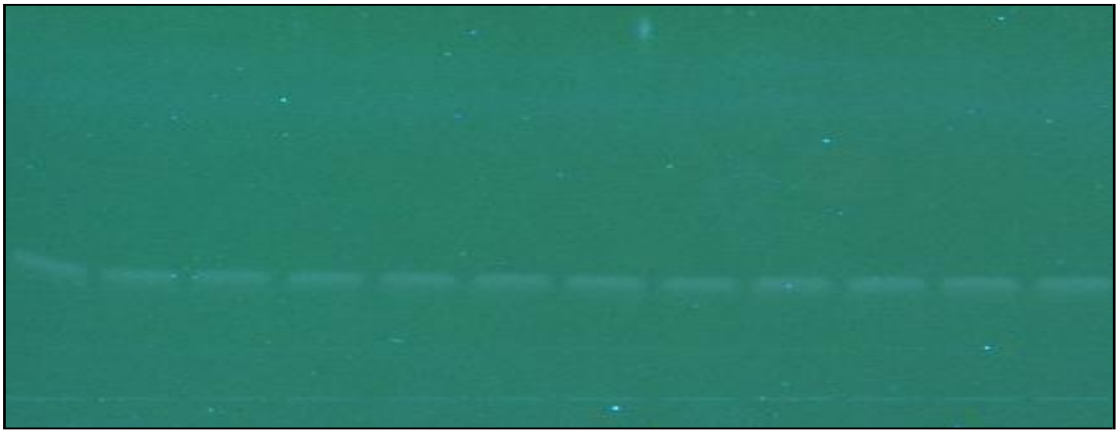


Figure 5-18: Developed HPTLC plate for drug content of the tablets at RT (25^{0}c /60% RH), at 30^{0}C/65% RH and at 40^{0}C/75% RH after 3 months at 361 nm

The HPTLC plate indicates absence of any degradation product formation during stability testing. Hence it can be concluded that the formulation batch R5 was stable during stability

testing.

Table 5-19: Physicochemical properties and % drug content of batch R5

Time points	RT (25^0C/60% RH)		(30^0C/65% RH)		Accelerated (40^0C/75% RH)	
	Appearance	Drug content (%)	Appearance	Drug content (%)	Appearance	Drug content (%)
Initial	White coloured tablets	99.98	White coloured tablets	99.98	White coloured tablets	99.98
15 days	NC	99.98	NC	99.98	NC	99.98
1 month	NC	99.97	NC	99.98	NC	99.93
2 months	NC	99.93	NC	99.92	NC	99.92
3 months	NC	99.86	NC	99.91	NC	99.91

- **In vitro dissolution studies**

Table5-20 : *In-vitro* drug release of batch R5 at the end of stability studies

Sr. No.	Time (hrs.)	Percentage drug release			
		Initial	RT (25^0c /60% RH)	(30^0C/65% RH)	Accelerated (40^0C/ 75% RH)
1	1	0.19	0.1	0.18	0.15
2	2	0.19	0.15	0.18	0.15
3	3	0.43	0.33	0.23	0.2
4	4	0.43	0.46	0.56	0.43
5	5	62.07	63	62.98	61.89
6	6	93.93	92.9	90.76	93.56
7	7	98.42	97.77	96.65	97.87
8	8	98.46	98	97.9	98.67
9	9	98.46	98.46	97.9	98.67

Conclusion

The objective of the present study was to develop a pulsatile drug delivery system synchronized with circadian rhythms of body for effective chronotherapy for rheumatoid arthritis. Patients suffering from Rheumatoid Arthritis experience marked pain in early morning hours due to muscle stiffness. The PDDS with predetermined lag time is supposed to synchronize peak plasma blood levels of the drug with the peak pain in early morning hours.

The work was initiated with thorough literature survey on pulsatile drug delivery, pathogenesis of Rheumatoid arthritis (RA), and treatments available for RA. Most of the treatments available do

not consider the circadian rhythm in pathogenesis of the disease. The available treatment with non-steroidal anti-inflammatory agents shows adverse side effects on long term therapy such as gastric irritation. The adverse effects can be minimised if the therapy is syncronised with the peak time of symptoms. This can be achieved by chronotherapy.

Meloxicam was selected as model drug as it is the selective COX II inhibitor and a drug of choice in the treatment of rheumatoid arthritis. The maximum dose of Meloxicam is 15 mg once daily or 7.5 mg twice daily.Meloxicam was standarised as per British Pharmacopoeia 2007. A suitable UV spectrophotometric method was validated for analysis of the dissolution samples of the formulations developed. A reported HPTLC stability indicating assay method was validated. The method was found to be selective, specific, accurate and precise. Meloxicam was subjected to forced degradation conditions like acidic hydrolysis, alkaline hydrolysis, oxidative degradation, heat and UV exposure. Forced degradation studies indicated meloxicam underwent alkaline degradation showing two distinct peaks in the chromatogram at two different R_f values. Hence it was concluded that the method can be used as stability indicating assay method for meloxicam. A reported RP-HPLC method was validated for bioanalysis of meloxicam in pig blood plasma. The method was found to be selective, specific, sensitive, accurate and precise, and robust.

The preformulation studies confirmed the identity and purity of the drug and the excipients. FTIR studies concluded that the procured sample of meloxicam was enol form of meloxicam which is reported to be the therapeutically active form. Particle size analysis of meloxicam sample concluded to have D_{90} and D_{50} values as 8 and 5.5 µ respectively. The results of saturated solubility studies indicated pH dependent solubility for meloxicam. Solubility of meloxicam increases with increasing pH. This suggested that meloxicam can be better absorbed from intestine than from stomach (pH 1.2). Drug excipient compatibility studies indicated no incompatibility ofdrug with excipients on the basis of visual observations and IR spectroscopy. To locate the site of absorption of meloxicam in the GIT x-ray imaging studies were carried out in rabbits. The results of these studies indicated that rupturable, single unit PDDS was retained in the stomach for nearly 8 hrs and ruptured in the stomach. The tablet did not empty into the small intestine. Meloxicam has shown pH dependent solubility with maximum solubility in alkaline pH. For rapid and complete absorption of meloxicam after the lag time it is necessary that the drug dissolves quickly and completely. Hence for this type of dosage form it is necessary that the dosage form should get emptied into the small intestine. Similar x-ray imaging study as was performed in larger animals like Yorkshire pigs. The results indicated gastric emptying of the tablet after 2 hrs and the tablet was disintegrated in the small intestine approximately 4 hrs after oral administration. The pharmacokinetic studies of rupturable PDDS (batch R5) in Yorkshire pigs indicated an average lag time of approximately 4 hrs. Pharmacokinetic parameters of immediate release marketed meloxicam tablet in Yorkshire pigs indicated average T_{max} of 4 hrs. whereas R5 had an average T_{max} 7 hrs. A good IVIVC level A correlation was observed with correlation coefficient of 0.935.

This concludes that if formulation R5 is administred at bedtime (10 pm) will have lag time of 4 hrs. and maximum amount of drug will be available in the blood in earlduringy morning hours. It suggestes that, this type of formulation can be used as platform technology where the lag time can be tailored according to the circadian rythyms of body. The developed formulation as oral, time dependent, single unit compression coated rupturable, pulsatile release tablet showed promising results as chronotherapeutic system for the treatment of rheumatoid arthritis.

References

1. Davis SS, Illum L, Drug delivery systems for challenging molecules. Int J Pharm; 176 (1998) 1-8.
2. Pittendrigh CS, Temporal organization: Reflections of a Darwinian clock-watcher. Annu. Rev. Physiol.; 55 (2022) 16–54.
3. Adkisson PL, Internal clocks and insect diapause, Science; 154 (1966) 234– 241.
4. Kramer A, Yang FC, Snodgrass P, Li X, Scammell TE, Davis FC,Weitz CJ, Regulation of

daily locomotor activity and sleep by hypothalamic EGF receptor signaling. Science; 294 (2001) 2511–2515.
5. Menaker M, Circadian rhythms. Circadian photoreception. Science; 299 (2003) 213– 214.
6. Schultz TF, Kay SA, Circadian clocks in daily and seasonal control of development. Science; 301 (2003) 326–328.
7. Maroni A, Zema L, Cerea M,. Expt.Opinion Drug Del.; 2 (2005) 855-871.
8. Pozzi F, Furlani F, Gazzaniga A, J. Control. Rel; 31 (1994) 99-108.
9. Ohdo S, Koyanagi S, Suyama H, Higuchi S, Aramaki H, Changing the dosing schedule minimizes the disruptive effects of interferon on clock function. Nat. Med.; 7 (2001) 356–360.
10. Gurny R, Junginger HE and Peppas N, Pulsatile Drug Delivery: Current Application and Future Trends, WissenscheflicheVerlagsgesellschaft, Stuttgart, Germany; 36 (1993) 56- 65.
11. Lemmer B, Chronopharmacokinetics: Implications for drug treatment. J. Pharm. Pharmacol.; 51 (1999) 887-890.
12. Siegel RA, Pitt CG, A Strategy for Oscillatory Drug Release: General Scheme and Simplified Theory. J. Control. Rel; 33 (1995) 173-188.
13. Crison JR, Siersma PR, Taylor MD, Amidon GL, Programmable oral release technology, Port Systems A novel dosage form for time and site specific oral drug delivery. Proceed Intern Symp Control Rel Bioact Mater;.22 (2021) 278-279.
14. Binns J, Stevens HNE, McEwen J, Pritchard G, Brewer FM, Clarke A, Johnson ES, McMillan I, The tolerability of multiple oral doses of Pulsincap capsules in healthy volunteers. J Control Rel.; 38 (1996):151-158.
15. Kohn JB, Schachter DM; US20037326425 (2003).
16. Saeger H, Virley P, Pulsincap: Pulsed-Release Dosage Form. Product information from Scherer DDS Ltd; 2004.
17. Bodmeier R, Pulsatile drug release from an insoluble capsule body controlled by an erodible plug. Pharm Res; 15(3) (1998) 474-481.
18. Krgel I, Bodmeier R, Evaluation of an enzyme-containing capsular shaped pulsatile drug delivery system. Pharm Res; 16(9) (1999) 1424-1429.
19. Crison JR, Siersma PR, Taylor MD, Amidon GL, Programmable oral release technology, Port Systems: a novel dosage form for time and site specific oral drug delivery. Proceed Intern Symp Control Rel Bioact Mater; 22 (1995) 278-279.
20. Crison JR, Siersma PR, Amidon GL, A novel programmable oral release technology for delivering drugs: human feasibility testing using gamma scintigraphy. Proceed Intern Symp Control Rel Bioact Mater; 23 (1996) 51-52.
21. Pollock DC, Dong L, Wong P, A new system to deliver a delayed bolus of liquid drug formulation. Proceed Intern Symp Control Rel Bioact Mater; 28 (2001) 6033.
22. Linkwitz A, Magruder JA, Merrill S Osmotically Driven Delivery Device with Expandable Orifice for Pulsatile Delivery Effect; US Patent No. 5,318,558 (1994).
23. Linkwitz A, Magruder JA, Merrill S. Osmotically Driven Delivery Device with Expandable Orifice for Pulsatile Delivery Effect; US Patent No. 5,221,278; (2018).
24. Magruder PR, Barclay B, Wong PSL, Theeuwes F, Composition comprising salbutamol; US Patent No.4751071 (1988).
25. Pozzi F, Furlani P, Orale Feste Pharmazeutische Darreichungsform Mit Programmierter Freisetzung. DE Patent No. 4122039 (1992).
26. Wilding IR, Davis SS, Pozzi F, Furlani P, Gazzaniga A, Enteric coated timed release systems for colonic targeting. Int J Pharm; 111 (1994) 99-102.
27. Gazzaniga A, Iamartino P, Maffione G, Sangalli ME, Oral delayed-release system for colonic specific delivery. Int J Pharm; 2(108) (1994) 77-83.
28. Maroni A, Sangalli ME, Cerea M, Busetti C, Giordano F, Gazzaniga A, Low viscosity

HPMC coating of soft and hard gelatin capsules for delayed and colonic release: Preliminary investigations on process parameters and *in-vitro* release performances. Proceed Int Control Rel Bioact Mater; 26 (1999) 887-888.

29. Ueda Y, Hata T, Yamaguchi H, Ueda S, Kotani M, Time Controlled Explosion System and Process for Preparation for the Same. US Patent No. 4,871,549 (1989).
30. Ueda Y, Hata T, Yamaguchi H, Kotani M, Ueda S, Development of a novel drug release system, time-controlled explosion system (TES). Part 1: Concept and design. J Drug Targeting; 2 (1994) 35-44.
31. Amidon GL, Leesman GD, Pulsatile Drug Delivery System. US Patent No. 5,229,131 (2022.
32. Chen CM, Multiparticulate Pulsatile Drug Delivery System. US Patent No. 5,508,040 (1996).
33. Beckert TE, Pogarell K, Hack I, Petereit HU, Pulsed drug release with film coatings of Eudragit: RS 30D. Proceed Int'l Symp Control Rel Bioact; 26 (1999) 533-534.
34. Narisawa S, Nagata M, Danyoshi C, Yoshino H, Murata K, Hirakawa Y, Noda K, An organic acid-induced sigmoidal release system for oral controlled-release preparations. Pharm Res; 11(1) (1994) 111-116.
35. Narisawa S, Nagata M, Hirakawa Y, Kobayashi M, Yoshino H, An organic acid-induced sigmoidal release system for oral controlled-release preparations. Part II: Permeability enhancement of Eudragit RS coating led by the physicochemical interactions with organic acid. J Pharm Sci; 85(2) (1996) 184-188.
36. Roy, Shahiwala A, Multiparticulate formulation approach to pulsatile drug delivery: Current perspectives. J Control Rel; 134 (2009) 74–80.
37. Lee DY, Chen CM, Anil K, Triggered Release of Bioactive Compounds. Recent Patents on Endocrine, Metabolic & Immune Drug Disc; 1 (2007) 183-190.
38. Sershen SR, Westcott SL, Halas NJ, West NJ, Temperaturesensitive Polymer-nanoshell composites for photothermally modulated drug delivery. J Biomed Mater Res; (2000) 293-298.
39. Dinaravand RD, Emanele A, Use of thermoresponsive hydrogels for on-off release of molecules. J Control Rel; (1995) 221-227.
40. Gutowska A, Bark JS, Kwon IC, Bae YH, Kim SW, Squeezing hydrogels for controlled oral drug delivery. J Control Rel; 48 (2020) 141-148.