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Original Article

FORMULATION AND EVALUATION OF MODIFIED PULSATILE CAPSULE FOR CHRONOPHARMACOTHERAPY OF RHEUMATOID ARTHRITIC PAIN

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ABSTRACT

Objective: The objective of the present study was to design and evaluate pulsincap system for chrono pharmacotherapy of rheumatoid arthritis that combines advantages of both immediate and sustained release technology with the suitable delay of drug release.

Methods: Pulsatile drug delivery system based on pulsincap® technology was designed using mucoadhesive microspheres and Tramadol bilayer tablet plugs. The drug-excipient interaction was carried out by FTIR. Hardness, thickness, lag time, and swelling index all play a role in optimizing tablet plugs. The microspheres were examined for parameters such as particle size, surface morphology, encapsulation efficiency, swelling index, % mucoadhesion, and *in vitro* dissolution.

Results: In modified pulsincaps, bilayer tablet plug shows drug release within 40 min and hydrogel plugs shows good swelling index and suitable lag time. In microspheres formulations, MF9 is the most suitable among them as it shows better drug content, particle size, surface morphology, *in vitro* drug release, and release kinetics. The drug is released right away, followed by the dissolution of the enteric coating at pH 6.8, followed by a suitable delay of 6 hours, and then the maximum amount of drug release is 99.62% at the end of 10th h.

Conclusion: The pulsatile delivery system developed with HPMCK4M and tamarindus gum as plugging material showed a satisfactory lag period when compared to Tamarindus gum alone. Drug release can be achieved from treated gelatin capsule and hydrogel plug for a prolonged period by MF9 formulation.

Keywords: Chrono pharmacotherapy, Pulsincap®, Mucoadhesive microspheres, Rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory autoimmune disorder. The word Arthritis means inflammation of the joint ("artho" means the joint and "it is" meaning inflammation of the joint). RA occurs when our immune system attacks the tissues near joints. This is due to the release of certain chemicals and enzymes that begin to eat away the cartilage and bones. RA affects women more often than men [1, 2]. Hormonal changes are also related to an increased risk of RA. The risk increases with age, it commonly develops between ages 40 to 60. Anyone can get RA at any age. 24-hour rhythms in cytokines play central roles in the pathogenesis of RA. The symptoms of rheumatoid arthritis are always worse in the morning. The patient has to get up early for the medication. This drawback can be overcome by formulating these modified pulsincap systems [3]. Tramadol hydrochloride is a centrally acting analgesic that has a relatively low affinity for opioid receptors and also appears to modify the transmission of pain impulses by inhibiting nor-epinephrine and serotonin uptake. In comparison with other opoids, it has limited side effects in the treatment of acute and chronic pains. A slow drug release system is particularly suitable for analgesic agents, which may be advantageous due to a reduction in the frequency of administration while maintaining the analgesic effect for a longer duration, for example, overnight [4-6].

To achieve a chronomodulated drug delivery for rheumatoid arthritis with drug release at two time points, offers greater benefits. In this study, pulsatile (modified pulsincap) drug delivery system containing a bilayer tablet and microspheres was prepared for time-controlled release. It was developed by Tramadol HCl as a pulsincap® system for diseases with circadian rhythm in the early morning hours to maintain the analgesic affect for longer period with a suitable delay in drug release.

MATERIALS AND METHODS

Tramadol HCL was obtained from Hetero labs, Hyderabad. Sodium Starch Glycolate, Cross povidone, pre-gelatinized starch, HPMCK100, sodium alginate were obtained from yarrow chem products. Microcrystalline cellulose, Magnesium Stearate, Talc, and all other reagents were of analytical grade. Tamarindus seeds were collected at Vikas college of pharmacy, for future reference, a voucher specimen (Voucher no. VP-20-87) has been kept in the herbarium at VCOP. Tamarindus gum was extracted by a suitable analytical procedure given below.

Calibration curve of tramadol HCl

100 mg of Tramadol HCL was taken in a 100 ml volumetric flask and dissolved with 100 ml of 0.1 N HCl to give the concentration of 1000µg/ml. From the above stock solution, aliquots of concentrations of 40 to 300 µg/ml were prepared for pH 1.2 buffer. When this solution was scanned in the UV range, i.e. from 200 nm to 800 nm,λ_{max} was found to be 271 nm for Tramadol HCl. The absorbance of these solutions was measured at 271 nm and a graph of concentration versus absorbance was plotted.

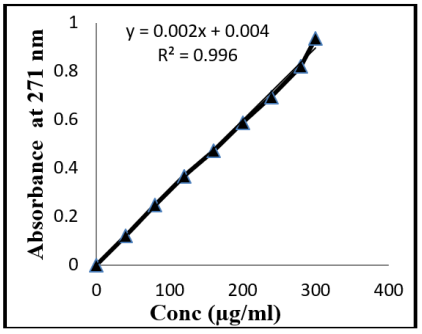

Fig. 1: Calibration curve of tramadol hcl at pH 1.2

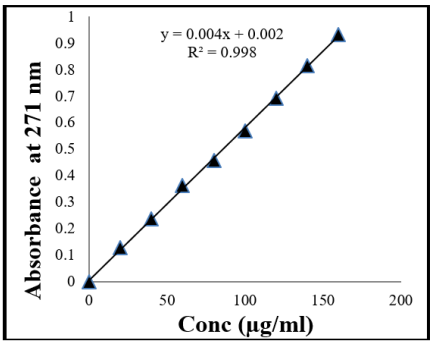

Fig. 2: Calibration curve of tramadol hcl at pH 6.8

Methods

Isolation of tamarindus gum

Tamarindus seeds (Botanical name: *Tamarindusindica* Family: Caesalpiniacea) collected from local markets. Botanica identification of Tamarindus indica was carried by dept of Pharmacognosy, vikas college of pharmacy and voucher specimen, VCOP/VS/2232 of plant material has been procured at college level for future study. Collected seeds were cleaned dried, then peel off the seed coat and crush the remaining white part of the seed. These crushed seeds of Tamarindus were soaked in water for about 24 h, then filter it into a muslin cloth and pressed for the release of gum. The marc was removed from the gum, and to the extracted gum, an equal quantity of absolute ethyl alcohol was added to precipitate the gum and it was separated by filtration. The marc was for multiple extractions with decreasing quantity of extracting solvent, i.e., water with an increase of the number of extractions. The isolation process was continued until the material was free of gum. The separated gum was dried in a hot air oven at 40 °C. Then the extracted dried gum was powdered and stored in an airtight container at room temperature [7, 8].

Preparationofbilayer tablet plug

Drug, super disintegrant, diluent, binder accurately weighed and blended for 15 min. Fast release layer prepared by direct compression method. HPMCK4M, Tamarindus Gum, and Sodium alginate were used to preparation of hydrogel plug by direct compression method. The optimized fast-release layer blend was separately compressed lightly using a rotary tablet compression machine using 6 mm punches. Over this compressed layer optimi zed erodible tablet plug was placed and compressed to form a bilayered tablet plugs [9].

Table 1: Formulation of an fast release layer and bilayer tablet plug

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)	F9 (mg)
Tramadol HCl	100	100	100	100	100	100	100	100	100
SSG	6	-	-	8	-	-	10	-	-
Cross povidone	-	6	-	-	8	-	-	10	-
Pregelatinized Starch	-	-	6	-	-	8	-	-	10
Microcrystalline cellulose	16	16	16	14	14	14	12	12	12
Magnesium stearate	1	1	1	1	1	1	1	1	1
Talc	2	2	2	2	2	2	2	2	2
weight (mg)	125	125	125	125	125	125	125	125	125
The composition of hydrogel plug:									
Ingredients	PF1 (mg)	PF2 (mg)				PF3(mg)			
HPMCK4M	100	-				50			
Tamarindus Gum	-	100				50			
The total weight (mg)	225								

Preparation of microspheres

Batches of mucoadhesive microspheres were developed using mucoadhesive polymers such as sodium alginate, tamarindus gum and HPMCK4M. These are taken in different ratios where the combination of HPMCK4M concentration is maintained as constant. All the data related to formulations was represented in table 2. The Orifice-Ionic Gelation Method was used for the preparation of microspheres as follows: sodium alginate, tamarindus gum, and HPMCK4M were dispersed in purified water (50 ml) to form a homogeneous polymer mixture (table 2), to which Tramadol HCl was dispersed. Then the whole dispersion was dropped into Cacl2 solution using insulin syringe and then cross-linked for 10-15 min for further gelation of alginate: Tamarindus gum beads. The microspheres formed were filtered through Whatman filter paper using Buckner funnel, washed 4-5 times with 50 ml of dichloromethane, dried at room temperature.

Table 2: Composition of mucoadhesive microspheres

Ingredients (mg)	MF1	MF2	MF3	MF4	MF5	MF6	MF7	MF8	MF9
Tramadol HCl	1000	1000	1000	1000	1000	1000	1000	1000	1000
Sodium alginate	600	800	1000	600	800	1000	600	800	1000
Tamarindus gum	200	200	200	400	400	400	600	600	600
HPMCK4M	200	200	200	200	200	200	200	200	200

Preparation of pulsincap systems

Hard gelatin capsule of size 0 was taken. Bodies were separated from cap, 25 ml of 15% (v/v) formaldehyde was taken into desiccators and a pinch of potassium permanganate was added Toit to generate form alinvapours. The wire mesh containing the empty bodies of capsule was then exposed to form aldehyde vapours. The caps were not exposed leaving them water-soluble. The desiccators were tightly closed. There action was carried out for 12 h after which the bodies were removed and dried at50°C for 30 min to ensure completion of reaction between gelatin and formaldehyde vapours. The bodies were then dried and capsule bodies were capped with untreated caps and stored in a polythene bag [10, 11].

Evaluation of microspheres

Particle size determination and surface morphology by SEM analysis

The size of the prepared microspheres was measured by the optical microscopy method using a pre-calibrated stage micrometer and surface morphology is by Scanning Electron Microscopy [12]. Particle size was calculated by using an equation.

$$Xg=10 \times [(ni \times logXi)/N]$$

Xg is geometric mean diameter,

ni is number of particle in range,

Xi is the midpoint of range and

N is the total number of particles.

All the experimental units were analyzed in triplicate (n=3).

Drug encapsulation efficiency

About 100 mg of microspheres was taken and triturated with phosphate buffer pH 6.8 and transferred to 100 ml volumetric flask. The volume was made up to 100 ml and mixed well. The solution was then kept aside for 12 h. It was sonicated in ultra-sonicator and then filtered through membrane filter 0.45 µm and estimated for drug content by measuring the absorbance at 270 nm. The drug entrapment efficiency was calculated using the formula [13].

$$\text{Drug encapsulation efficiency}=\frac{\text{Estimated \% drug content}}{\text{Theoritical \%drug content}} \times 100$$

Degree of swelling

The swelling ability of microspheres in physiological media was determined by swelling them in the Phosphate buffer pH 6.8. Microspheres were suspended in 5 ml of phosphate buffer pH 6.8, the increase in particle size of microspheres was noted up to 10 h and the swelling index was calculated [14]. The degree of swelling was calculated using following formula:

$$\alpha = \frac{Ws-Wo}{Wo}$$

α is the degree of swelling;

Wo is the particle size of microspheres before swelling;

Ws is the particle size of microspheres after swelling.

***In vitro* wash-off test for microspheres**

The mucoadhesive properties of the microspheres are evaluated by *in vitro* wash off test. A 1 cm2 piece of sheep mucosa was tied on a glass slide using thread. About 100 microspheres were spread on to the wet rinsed tissue specimen and the prepared slide was hung onto one of the grooves of a USP tablet disintegration apparatus. The USP disintegration apparatus is operated such that the tissue specimen is given regular up and down movements in a beaker containing 800 ml of phosphate buffer pH 6.8. At the end of 30 min, 1hour and hourly intervals up to 10 h the number of microspheres still adhering to the tissue was counted. [15].

Physico-chemical characterization of hydrogel plug

Hydrogel Plugs were studied for Hardness, Thickness, and Swelling index. Hydrogel plugs were kept immersed in three different pH conditions. Plugs were taken out carefully at 2, 4, 6, 8, 10, 12 h and their weights were determined accurately.

$$\text{\% Swelling index}=\frac{\textbf{wetweight}-\textbf{dryweight}}{\textbf{wetweight}} \times \mathbf{100}$$

***In vitro* dissolution studies**

For fast-release layer

The *in vitro* dissolution was carried out using USP Type I (Paddle) dissolution apparatus under sink condition. The dissolution medium was 900 ml of a 0.1M HCl solution (pH=1.2), at 37 ˚C±0.5 ˚C and the stirring speed of 50 rpm. The *in vitro* release studies were carried out for 2 h. 5 ml of samples were taken at 10 min intervals for 1 h. The absorbance of the solution was recorded at UV-spectrophotometer.

For microspheres

The *in vitro* dissolution was carried out using USP Type I (Basket) dissolution apparatus under sink condition. The dissolution medium was 900 ml of a phosphate buffer pH 6.8 at 37 ˚C±0.5 ˚C and the rotating speed was 50 rpm. 5 ml of samples were taken at 1h intervals for subsequent hours and were replaced with freshd is solution medium. The absorbance of the solution was recorded at UV-Spectrophotometer.

***In vitro* drug release of pulsatile capsule**

In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used (sequential pH change method). The pH 1.2 was first used for 1 h then removed and the fresh phosphate buffer pH 7.4 was added. After 3 h the medium was removed and colonic fluid phosphate buffer pH 6.8 was added for subsequent study. 900ml of the dissolution medium was used at each time. The rotation speed was 100rpm and temperature was maintained at 37±0.5 ˚C. 10 ml of dissolution medium was withdrawn at predetermined time intervals and fresh dissolution media was replaced every time. The withdrawn samples were analyzed by UV absorption spectroscopy and the cumulative percentage drug release was calculated [16, 17].

RESULTS AND DISCUSSION

Properties of gum

The isolated gum was physico-chemically evaluated and these parameters are represented in table 6. Tamarindus gum is soluble in hot water and swells in cold water. Particle size of tamarindus gum is 0.168 mm on average. pH values are 6.0, shows good flow properties. Carbohydrates, proteins, alkaloids and glycosides presence is confirmed by their respective tests for tamarindus gum. Gum produces mucilage and shows positive results for the ruthenium red test. With this extracted gum powder able for producing formulation. The swelling property of gum is responsible for considering them as one of the excipients or polymers with special properties.

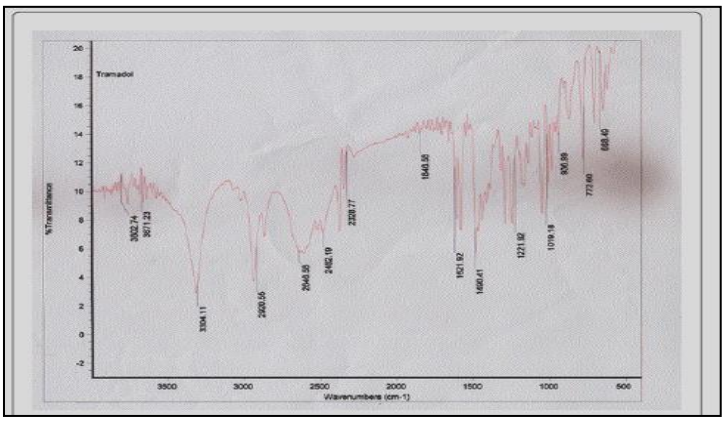

Fig. 3: IR spectrum data of pure tramadol HCl

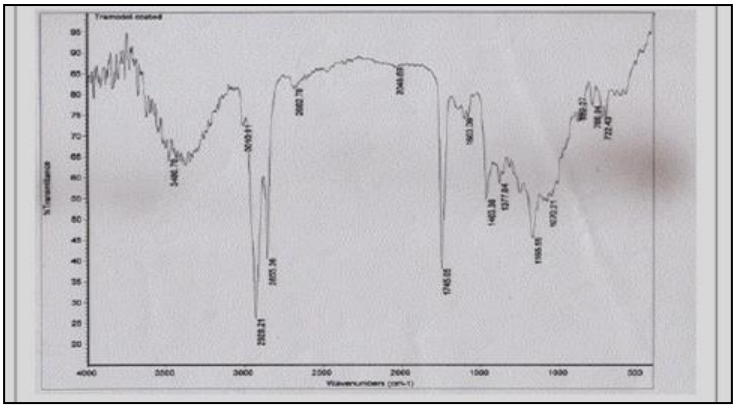

Fig. 4: IR spectrum of tramadol HCl with polymers

Compatibility studies by FTIR

Compatibility studies by FTIR has been evidenced by its characteristic peaks that the drug is compatible with polymer, no new peaks were observed, indicating there is no interaction of the drug with excipients, and also drug does not undergoes any degradation and were represented in fig. 3 and fig. 4 by its characteristic peaks at 3300, 2900, and 1400 cm^{-1}.

Physico-chemical characterization of hydrogel plug

Hydrogel plug bilayered tablets were studied for Hardness, Thickness, and Swelling index. The hydrogel plugs show good swelling properties at different pH values and were represented in table 3. There is no drug release within 6 h as shown in table 4, which indicates the suitable lag period. Among them the plug with HPMC K4M and tamarind us gum shows excellent results.

Table 3: Hardness, thickness and swelling index of hydrogel plugs

Formulation code	Hardness (n=3)			Thickness (n=3)		
PF1	3.12±0.22			5.14±0.11		
PF2	3.14±0.11			5.08±0.14		
PF3	3.52±0.24			5.11±0.18		
	Swelling index of hydrogel plugs (n=3)					
	Time in h					
	2	4	6	8	10	12
	pH1.2					
PF1	28.26±0.22	32.64±0.28	48.22±0.18	56.55±0.28	78.22±0.16	95.66±0.12
PF2	21.16±0.36	28.66±0.16	42.52±0.22	54.42±0.16	72.32±0.24	94.42±0.18
PF3	29.36±0.18	34.46±0.21	50.22±0.36	59.34±0.18	79.24±0.32	98.88±0.26
	pH7.4					
PF1	26.28±0.24	30.54±0.18	49.56±0.28	58.82±0.12	79.98±0.62	96.68±0.52
PF2	22.12±0.16	28.98±0.24	44.46±0.16	55.22±0.26	73.32±0.24	95.56±0.56
PF3	30.22±0.26	35.56±0.12	52.26±0.26	60.56±0.16	80.24±0.18	98.28±0.32
	pH6.8					
PF1	28.66±0.28	30.62±0.32	50.56±0.33	56.52±0.36	78.66±0.18	96.22±0.16
PF2	24.42±0.12	27.68±0.26	44.48±0.18	54.46±0.38	74.42±0.24	94.46±0.28
PF3	31.16±0.62	36.58±0.66	53.32±0.54	61.52±0.16	81.28±0.54	98.72±0.68

All the mean values±SD, (n=3)

Particle size determination and SEM analysis

All the microspheres were spherical in nature its surface was smooth, as observed in SEM report. Here, when increase the drug: polymer ratio, particle size of microspheres was increase. As particle size of microspheres was increase, decrease the surface area of microspheres, so finally, drug release was decrease. Here particle size of microspheres decrease with increase the volume of external phase, more surface are available for drug release, so finally, drug release was increase and all the values were represented in table 4.

Table 4: Evaluation studies of mucoadhesive microspheres

Formulation code	Angle of repose (θ) (n=3)	Particle size(µm)		Swelling index	% Mucoadhesion	Percentage Yield	(%) Encapsulation efficiency (n=3)
		Dry (n=3)	Wet (n=3)				
MF1	29.12±0.60	1.82±0.20	2.92±0.28	78.82	68.26	73.44	74.32±0.22
MF2	29.38±0.91	1.62±0.12	2.82±0.24	79.64	70.24	79.05	78.66±0.15
MF3	31.56±0.38	1.78±0.14	2.86±0.22	80.62	72.68	88.62	80.36±0.02
MF4	31.69±0.65	1.80±0.11	2.90±0.28	78.86	70.60	94.34	80.26±0.24
MF5	29.67±0.78	1.84±0.21	2.92±0.26	84.22	73.32	75.41	88.28±0.15
MF6	30.06±0.55	1.78±0.12	2.84±0.32	86.36	78.28	76.48	90.18±0.22
MF7	29.76±0.65	1.82±0.14	2.82±0.54	88.24	81.26	77.82	85.52±0.20
MF8	29.65±0.80	1.80±0.22	2.80±0.24	96.22	84.32	93.38	90.16±0.16
MF9	29.09±1.38	1.78±0.22	2.90±0.28	98.28	88.26	94.5	94.28±0.08

All the mean values±SD, (n=3)

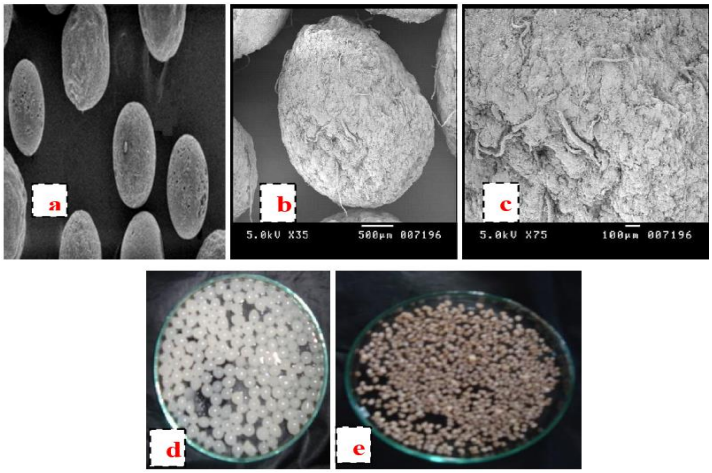

Fig. 5: Surface morphology of microspheres by SEM a. normal b. low magnification(x35) c. High magnification (x75) d. Prepared microspheres e, dried microspheres

Table 5: *In vitro* dissolution studies and *in vitro* wash-off test (%) of formulations

CODE	Cumulative % drug release (n=3)						
	0 min	10 min	20 min	30 min	40 min	50 min	60 min
F1	0	26.8±1.22	47.96±1.43	68.13±1.58	81.6±1.48	92.6±1.58	101.4±1.80
F2	0	28.5±1.40	52.3±1.58	70.43±1.24	86.4±1.58	98.6±1.52	
F3	0	28.5±1.4	35.6±1.61	55.6±1.20	78.2±1.46	87.8±1.36	92.65±1.56
F4	0	32.8±1.22	50.96±1.43	70.13±1.5	84.6±1.48	96.6±1.58	
F5	0	34.5±1.40	54.23±1.58	73.43±1.24	88.4±1.58	99.6±1.52	
F6	0	34.8±1.25	47.6±1.30	58.3±1.34	80.7±1.48	90.21±1.47	98.2±1.48
F7	0	36.8±1.22	53.96±1.42	72.13±1.5	86.6±1.48	99.6±1.58	
F8	0	38.5±1.40	56.23±1.22	76.43±1.2	99.8±1.58		
F9	0	38.8±1.25	57.6±1.30	75.3±1.34	89.7±1.48	99.21±1.47	
	1h	2h	3h	4h	6h	8h	10h
MF1	30.6±1.22	48.26±1.44	60.46±1.22	82.63±1.32	99.66±1.33		
MF2	28.26±1.62	45.25±1.26	58.24±1.32	80.46±1.24	96.4±1.42		
MF3	27.54±1.36	46.28±1.28	56.22±1.44	78.82±1.26	94.33±1.24		
MF4	30.25±1.24	45.25±1.42	60.54±1.28	78.28±1.44	92.46±1.26		
MF5	31.32±1.22	48.42±1.28	61.55±1.26	74.82±1.28	88.14±1.22	98.28±1.2	
MF6	29.96±1.38	47.56±1.22	60.44±1.32	72.52±1.26	86.24±1.40	96.26±1.4	
MF7	28.82±1.22	46.74±1.26	58.06±1.46	71.15±1.24	84.54±1.32	94.46±1.3	
MF8	27.54±1.64	44.28±1.62	56.36±1.22	70.64±1.22	82.62±1.33	97.24±1.2	
MF9	26.54±1.25	42.26±1.46	54.12±1.28	69.96±1.46	80.54±1.62	86.54±1.2	99.62±1.3
	In vitro wash-off test (%)						
	1h	2 h	3h	4Hh	6h	8h	10h
MF1	70±1.22	68±1.42	54±1.32	32±1.22	12±1.24	00	00
MF2	74±1.32	69±1.24	56±1.54	34±1.30	14±1.32	00	00
MF3	76±1.33	71±1.32	58±1.24	36±1.32	16±1.42	00	00
MF4	86±1.22	76±1.24	60±1.28	34±1.28	12±1.26	00	00
MF5	84±1.33	74±1.22	58±1.24	36±1.26	16±1.28	00	00
MF6	82±1.22	72±1.24	56±1.28	38±1.20	18±1.22	00	00
MF7	90±1.36	80±1.33	62±1.22	48±1.24	32±1.30	15±1.28	00
MF8	94±1.32	84±1.44	66±1.26	50±1.26	46±1.32	36±1.22	16±1.32
MF9	96±1.26	88±1.26	72±1.30	52±1.30	48±1.22	40±1.24	24±1.24

All the mean values±SD, (n=3)

Percentage yield and drug encapsulation efficiency

The percentage yield of coded batches varies from 74.32 to 94.28%. The minimum percentage yield was 74.32% of batch MF1, whereas the maximum 94.28% for MF9 batch. The results are given in table 4. The entrapment efficiency of batches varies from 74.32 to 94.28% as shown in table 4. The maximum entrapment efficiency was 94.28% of batch MF9. It shows entrapment efficiency is increased due to an increase in the concentration of sodium alginate and tamarindus gum. The entrapment efficiency depends on the type and amount of polymers used. It was found that, if increasing the amount of sodium alginate and tamarindus gum then entrapment efficiency was increased [18].

Degree of swelling

In the microsphere formulation, use of different natural polymers and their combinations have shown an excellent degree of swelling and it ranges from 78.82 to 98.28 % and the formulation with high concentrations of sodium alginate and tamarindus gum shows better swelling than their other formulations. With these studies, we can conclude that these polymers were suitable for manufacture for their extended release in colon and shows swelling of up to more than 10 h. Because of this, targeted drug delivery to colon is made possible for chrono-modulated systems.

Table 6: *In vitro* drug dissolution of optimized formulation

Dissolution medium	Time (h)	Cumulative % drug release
0.1MHCL	20 min	56.23
	40 min	99.81
PH6.8	7	26.54
	8	42.26
	9	54.12
	11	69.96
	13	80.54
	15	86.54
	17	99.62

In vitro dissolution and wash-off test

The untreated caps were dissolved within 10 min while treated bodies remained intact over a period of 24 h; this is due to the exposure to formalin vapors, which results in decrease in solubility of gelatin and is hence suitable for colonic delivery which was desired; arthritis by circadian biorhythms [19]. Apparently, the colon has a lower pH value (6.5) than that of the small intestine (7.0–7.8). Based on the concept that a formulation on leaving the stomach arrives at the ileocaecal junction in about 6 h after administration and difference in pH throughout GIT, a time and pH-dependent pulsatile device proposed for colonic targeting was designed for achieving the selective delivery of drugs to colon, which is a chrono modulated approach for the treatment of arthritis.

In the pulsincap formulations, bilayered tablets were optimized for immediate release layer with F8 formulation which releases 99.4% drug within 40 min. Cross povidone as a super disintegrant shows good results and the plug was evaluated for swelling studies with good swelling as individual polymer and shows excellent results in high concentration in F8. In hydrogel plug formulations, tamarindus gum as a hydrogel plugging material in three formulations with individual HPMCK4M, tamarindus gum as well as their combination in ratio 1:1 were formulated as PF,1 PF2 and PF3. The different polymeric concentrations used in the above formulations were found sufficient to maintain the lag period for a minimum period of 4 h. During 5th h, the hydrogel plug after complete wetting and swelling was ejected from bodies of capsules and, thus releasing the drug-containing microspheres in the colonic fluid (pH6.8). In case of PF3, the polymeric plug was ejected during the 6th h of the dissolution study. This could be attributed to delayed wetting and swelling of the hydrogel material at that concentration.

With all the formulations negligible amount of drug was released at the end of 6th h, and was in a range of 26.54 to 31.32 %, respectively. At the end of 6th h, the hydrogel plug, after complete wetting and swelling was ejected from capsule and thus releasing the drug-containing microspheres in the colonic region (pH6.8). Due to higher water-absorbing capacity of tamarindus gum

With all the formulations of microspheres, the formulation MF9 that containing high concentration of sodium alginate achieves 17 h of drug release with a desired lag period of 6 h. This could be due to diffusion of drug from the microspheres through the swollen hydrogel plug. At the end of 17 h 96.39 % drug release was observed with MF9 formulation. Overall, with all the formulations containing sodium alginate in combination with HPMCK4M as a mucoadhesive material, release the drug in a controlled manner began after the 6th h and spread over a period of 17 h.

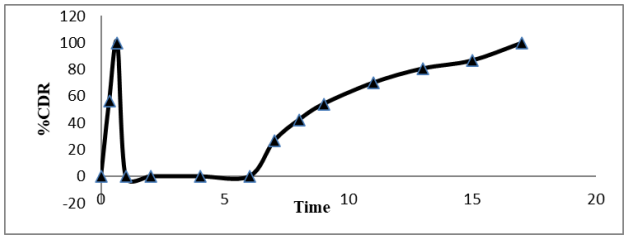

Fig. 6*: In vitro* drug dissolution of optimized formulation

Drug release kinetics

Drug release from these mucoadhesive microspheres was slow manner and for a extended period of time and depends on concentration of polymers. All the formulations followed zero-order kinetics up to 8 h [19]. Drug release from the formulation MF9 was studied by fitting the data into different drug release kinetic models and was represented in table 7and release kinetic plots were shown in fig. 7. By the results it was found that the release pattern was best fitted to Kors-meyer peppas kinetics (R^2 is maximum) [20, 21].

Stability testing

The optimized formulation with MF9 microspheres was selected for stability testing and was carried out at accelerated 40±0 °C, 75±2% RH conditions in desicator, the capsules were packed in muslin cloth and covered with aluminium foil for a period of 3 mo. The formulations were evaluated for surface morphology, swelling, drug content, mucoadhesion and in-vitro drug release and significant results were obtained.

Table 7: Drug release kinetics data of optimized formulation

Time (H)	%CDR	% Drug remaining	√time	Log %DR	Log time	Log % CDR	% DR	Wt	Wo-Wt
0	0	100	0	2	0	0	100	4.64158	0
7	26.54	73.46	2.64575131	1.866051	0.84509	1.423901	73.46	4.188099	0.453481
8	42.26	57.74	2.828429	1.761477	0.95424	1.625929	57.74	3.865083	0.776497
9	54.12	45.88	3	1.661623	1.04139	1.733358	45.88	3.5799294	1.061650
11	69.96	30.04	3.31662479	1.4777	1.3222	1.84485	30.04	3.1086128	1.532967
13	80.54	19.46	3.60555127	1.289143		1.906012	19.46	2.6897646	1.951815
15	86.64	13.36	3.87829833	1.125806		1.937718	13.36	2.3728419	2.268738
17	99.62	0.38	4.1231056	-0.42022		1.998347	0.38	0.72431564	3.9172643

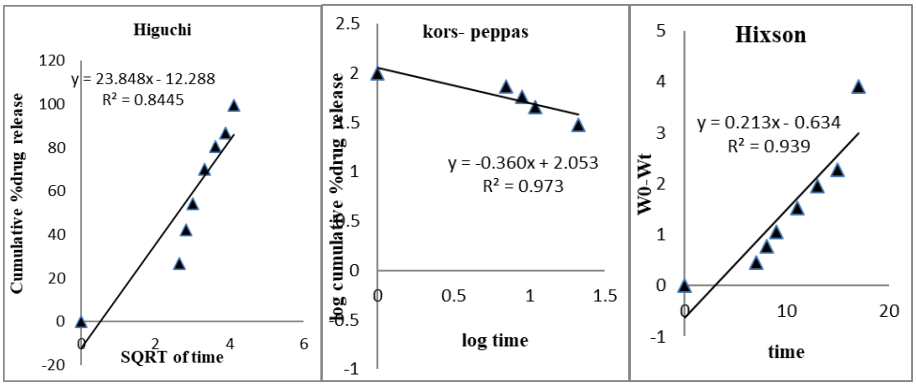

Fig. 7: Drug release kinetics plots of optimized formulation

CONCLUSION

The results of the present study conclude that developed modified pulsincap capsules using modified pulsincap technology along with immediate dose it will release the drug at predetermined lagtime. This helps in achieving drug release at right site, right amount, and in right time. A time-controlled chrono modulated pulsatile drug delivery system of Tramadol HCl containing immediate release dose and Tramadol HCl microspheres for controlled release of the drug after a desired lag time was successfully developed. With the present study, it has been concluded that Modified Pulsincap dosage form of Tramadol HCl could be effectively control the early morning arthritis pain.

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AUTHORS CONTRIBUTIONS

This study was done in collaboration with all authors. PJ (Research Scholar) designed this study. PJ, AB, and DRR participated in the conduct of the study. PJ, AB, and DRR analyzed the data. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

Declared none

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