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Research Article

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Studies on pulsincap formulation of aceclofenac and serratiopeptidase for treatment of inflammatory disorders

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ABSTRACT

The aim of this study was to develop and evaluate pulsincap of Aceclofenac and serratiopeptidase using different amount of HPMC K15 M and di calcium phosphate as plugging materials. This formulation is planned to use for better therapy of inflammatory pain in patients with various inflammatory disorders with a predetermined lag time. Granule equivalent to 100 mg of Aceclofenac and 10 mg serratiopeptidase were accurately weighted and added into the treated formaldehyde bodies by manual hand filling method. The bodies were then plugged with different polymers like hydroxylpropylmethylcellulose K 15 M and dicalcium phosphate. Plugs were punched by rotary compression machine called as tablet plugs. Then capsule body and cap is joined and sealed with a small amount of ethanolic ethyl cellulose solution. Plug composition was optimized by using 2 factor 3 level factorial designs. Plug Amount and % of Polymer in plug were taken as independent factor in the present study. Lag time of rupture of PRTs as dependent variable in this study. FT IR study is used for concluding compatibility of drugs with excipients. The prepared formulation was tested for Rupture Test, In vitro dissolution study and in vivo study. Results of all in vitro study and in vivo study would also justify pulsatile release of drugs from dosage form.

Keywords: Chronotherapy**,** Pulsatile formulation, Aceclofenac, Serratiopeptidase, pulsincap

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INTRODUCTION

Rheumatoid arthritis is a systemic inflammatory disorder that causes pain, stiffness, and progressive disability in high percentages of the patients. It can disturb the function of many tissues and organs, but mainly attack the joints producing an inflammatory synovitis. Rheumatoid arthritis is a disorder based on symmetrical pattern that means if one hand or knee is affected, then other knee or hand also affected. Wrist joints and the finger joints closed to the hand are highly affected by such disorders. An inflammatory disorder also affects other parts of the body besides the joints. [1-4] The goal of present research work is to develop a formulation that meets therapeutic need relating to particular pathological conditions. Specifically, symptoms of various inflammatory disorders have a peak during night or early in the morning. Both variations in plasma drug concentration and disease condition need to be taken in consideration in developing a meaningful dosage form. [5-8]

The objective of this work was to develop and evaluate a pulsincap formulation intended for treatment of various inflammatory disorder condition like rheumatoid arthritis, osteoarthritis etc. The Granule equivalent to 100 mg of Aceclofenac and 10 mg serratiopeptidase were accurately weighted and added into the treated capsule bodies by manual hand filling method. The then plugged with different amounts of polymer like hydroxylpropylmethylcellulose K 15 M and dicalcium phosphate. Plugs were punched by rotary compression

machine called as tablet plugs. Then capsule body and cap is join/sealed with a small amount of the ethanolic ethyl cellulose solution. Plug composition was optimized by using 2 factor 3 level factorial designs. Plug Amount and % of Polymer in plug were taken as independent factor in the present study. Lag time of rupture of PRTs as dependent variable in this study.

EXPERIMENTAL SECTION

2.1. MATERIALS

Aceclofenac was gifted by Pramukh Pharmaceuticals, Wadhwan (GIDC), Surendranagar and Waist Cost Pharma, Ahmedabad. Serratiopeptidase was gifted from J.C. Biotech, Hyderabad. HPMC K 15 M, Dicalcium Phosphate, Starch were purchased from SD Fine chemicals.

2.2. METHODS

2.2.1. Preparation of Cross-Linked Gelatine Capsules

2.2.1.1. Formaldehyde Treatment

Formaldehyde treatment is given to modify the solubility of gelatine capsules (To reduce solubility of capsule shell). Exposure of formaldehyde vapours results in an dramatically decreases in solubility of gelatine because crosslinkage of aldehyde group of formalin and amino group in the gelatine. Hard gelatine capsule of size 0 number taken in present research work. Their bodies were separated from the caps. To generate formalin vapours, 15% (v/v) formaldehyde (20 to 25 ml) was taken into desiccators and a small pinch of potassium permanganate was added to it. The empty bodies were kept above the wire mesh. The wire mesh then exposed to formalin vapours. The caps were not exposed to formalin vapours leaving them water-soluble. The desiccators were tightly closed to allow complete reaction to occur. This cross linking reaction was allow to carried out for 12 h. After 12 hours of exposure of formaldehyde vapour, capsule bodies were removed and dried at $60^{O}C$ for 30 min to ensure completion of reaction between formaldehyde vapours and gelatine. To facilitate removal of residual formaldehyde, bodies were dried at room temperature. These treated capsule bodies were capped with untreated caps and stored in a zip bag. [9-13]

Figure 1: Formaldehyde treatment capsule body shell

2.2.1.2. Quality Control Tests for Treated Formaldehyde Hard Gelatine Capsules[9-13]

Formaldehyde treated and untreated capsules were evaluated for various physical and chemical tests.

Physical tests

Dimensions: Variations in dimensions between formaldehyde treated and untreated capsules were studied. The length and diameter of the capsules were measured before and after formaldehyde treatment, using dial caliper. Capsule body length and capsule length both were considered for this study.

Solubility studies: Normal capsules and formaldehyde treated capsules were evaluated for solubility study for 24 hrs in a beaker. Ten capsules were subjected to solubility studies at room temperatures in 100 ml buffers of pH 1.2

and 6.8. (Selection of capsule on random basis). The time was noted when capsule dissolves or forms a soft fluffy mass.

Disintegration studies for capsules First 10 empty capsules containing both untreated caps and treated bodies were randomly selected. Then the study was carried out by dipping and stirring a single capsule in different buffers of pH 1.2 and 6.8 filled in disintegration apparatus. This testing was carried out at 37 centigrade temperature for a period of 06 hours.

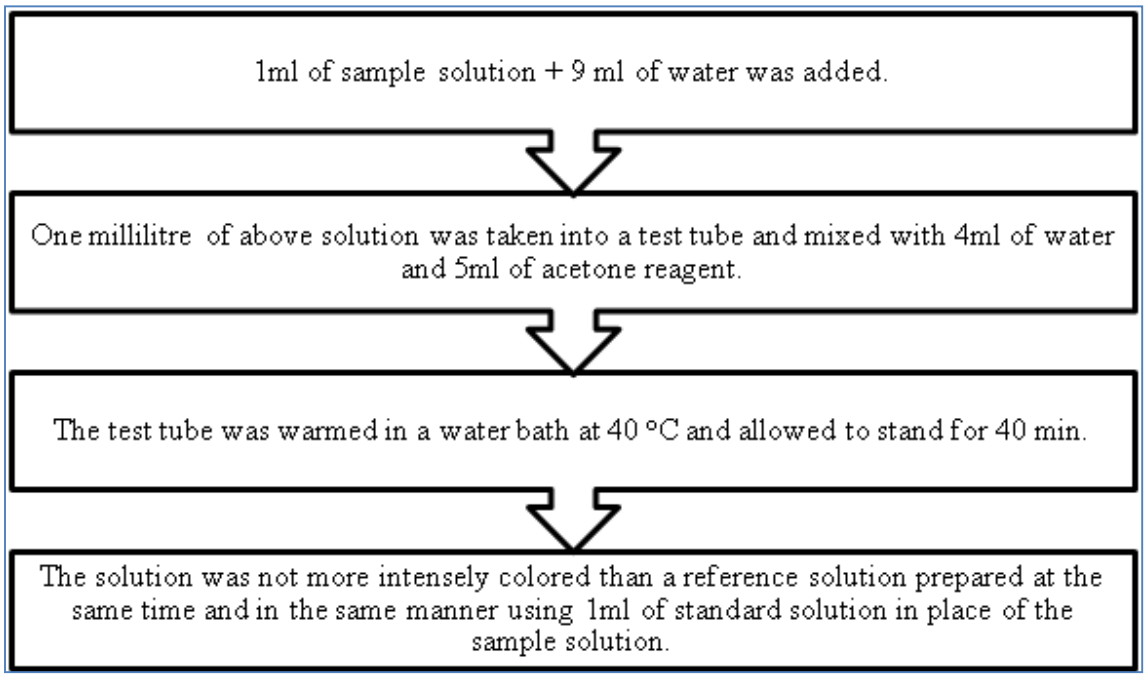
Identification attributes: Various identifiable attributes like colour, odour, stickiness, softness, tackyness were evaluated.

Visual defect: Number of capsule bodies were shrunk or distorted was determined by random selection of 100 treated capsule bodies.

Chemical Test

Qualitative control test for free formaldehyde

Standard formaldehyde solution (0.002, w/v formaldehyde) and sample solution from formaldehyde treated bodies were taken for comparison tests. Formaldehyde treated bodies (About 30 treated bodies) were fragmented in small fragment pieces and added into a beaker containing demineralised water. This solution was agitated for 2 hrs with a magnetic stirrer, to solubilize free formaldehyde. The above stirred solution was then filtered. Filtered solution was further added into a 50 ml volumetric flask, and volume was made up to 50 ml.



2.2.2. Preparation of Granules of Drugs

2.2.2.1. Preparation of granules containing aceclofenac and serratiopeptidase: The granules containing aceclofenac and serratiopeptidase were prepared to fasten the drug release after lag time and to improve their physicochemical property. Starch paste was used as a binder to make granules.

2.2.2.2. Characterization of granules: Granules were evaluated for flow property. The flow properties of granules were evaluated in a laboratory by a fixed height funnel method. Granules were further characterized for angle of repose, bulk density, tapped density, Hausner's ratio, % car's index, content uniformity and drug release study.

2.2.3. Formulation of Pulsincap Drug Delivery System:

Granule equivalent to 100 mg of Aceclofenac and 10 mg serratiopeptidase were accurately weighted and added into the treated formaldehyde bodies by manual hand filling method. The bodies were then plugged with hydroxylpropylmethylcellulose K 15 M (Different Amount). Plugs were punched by rotary compression machine called as tablet plugs. Then capsule body and cap were sealed with a small amount of the ethanolic ethyl cellulose solution.

2.2.3.1. Plugging Materials Effects on Drug Release

Reports were not available on the use of HPMC K 15 M as plugging materials in pulsatile drug delivery systems of aceclofenac and serratiopeptidase. Hence this polymer was selected in the design of pulsincap dosage form of Aceclofenac and serratiopeptidase. Different concentrations of HPMC K15 M were used and their effect on drug release from pulsincap dosage form was investigated using the dissolution media mimicking the invivo conditions (without enzymes) of the GI tract. Plug was prepared by compressing different amount of HPMC K15 M and di calcium phosphate using rotary tablet press (Rimek Mini Press, Karnavati Engineering) keeping variation in thickness and amount of tablet plug. This plug was then fitted into the body of treated hard gelatin capsule (containing granules equivalent to 100 mg of Aceclofenac and 10 mg serratiopeptidase). Plug composition was optimized by using 3 level 2 factor factorial designs.

Table 1: Composition of 3 level and 2 factor factorial design

Independent Factor	**Level**		
Plug Amount	100 mg	150 mg	200 mg
% of Polymer in plug	10 %	40 %	70%
Dependent Factor	Y1= Lag time of rupture of PRTs		

Table 2: Formulation of Different Batches for 3^2 Full Factorial Designs

Batch No.	**Real Value**		**Transformed Value**	
	Plug Amount (mg) X1	**% of Polymer in plug (%) X2**	**Plug Amount X1**	**% of Polymer in plug X2**
F1	100	10	-1	-1
F2	100	40	-1	0
F3	100	70	-1	1
F4	150	10	0	-1
F5	150	40	0	0
F6	150	70	0	1
F7	200	10	1	-1
F8	200	40	1	0
F9	200	70	1	1

Table 3: Composition of Batches According to 3^2 Full Factorial Designs

Pulsincap Dosage Form	**Ingredients**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Granule	Aceclofenac	100	100	100	100	100	100	100	100	100
	Serratiopeptidase	10	10	10	10	10	10	10	10	10
	Starch Paste Binder	q.s.								
Plug	HPMC K 15 M	10	40	70	15	60	105	20	80	140
	Di Calcium Phosphate	90	60	30	135	90	45	180	120	60
	Plug Amount	100	100	100	150	150	150	200	200	200

2.2.3.2. Check point batch

Polynomial mathematical models including main effects, interaction effects and quadratic effects were generated for all the response variables using design expert software trial version 8.0.7.1. Validity of generated contour plot was confirmed by check point batch analysis. Subsequently, experimental trial was carried out for validation of contour plot and results of experimental trial was compare with predicted results.

Table 4: Independent Variables in Checkpoint Batch

Batch No.	**Real Value**	
	Plug Amount (mg)X1	**% of Polymer in plug (%) X2**
F10	144.2	62.72 % (90.44 mg)

Check point batch was prepared similar to the Factorial batches and evaluated for same parameters.

From the result of contour plot of Lag time of Rupture of PRTs of check point, the contour plot was validated.

2.2.3.3. Evaluation of Pulsincap formulation

Characterization of prepared plug : The prepared tablet plug evaluation was carried out for weight determination, hardness, friability, disintegration time. The prepared tablet plugs were plugged to capsule bodies containing formulated granules and the cap was closed. The lag time test was conducted using USP II dissolution testing apparatus using 6.8 pH for phosphate buffer for 6 hrs. The drug release was observed.

Rupture Test

Lag time of PRTs were visually evaluated in phosphate Buffer 6.8. Lag time means time at which formulation starts sudden drug release or formulation get break down under influence of dissolution media.

***In vitro* dissolution study**

The *in vitro* dissolution study was carried out using USP Type 2 dissolution apparatus. Modification was done in dissolution test apparatus to avoid dead volume in dissolution test procedure. This modification was in terms of addition of wire mesh screen above pulsincap formulation. 0.1 N HCl was keep as dissolution media for first 2 hours and then it was replaced with 900 mL of phosphate buffer (pH 6.8) from 2 to 7 h. Thermostatically controlled water bath was used to maintain temperature of 37 ± 0.5^0C. The pulsatile dosage form was then introduced into the dissolution jar below wire mesh screen. Agitation speed for paddle was kept at 50 rpm. At different time intervals, 10 ml sample was withdrawn and replaced with same volume of dissolution fluid in jar. The withdrawal sample was analyzed spectrophotometrically at 316 and 375 nm for the drug release as per validated specified method. Dissolution study of the optimized batch was carried out in both dissolution media phosphate buffer pH 6.8 and 0.1 N HCl to study the effect of pH on drug release from PRTs.

Drug Polymer Interaction

Infrared spectroscopy was used to predict possible drug excipients interaction using a FTIR spectrometer (8400S, Shimadzu, Japan) at 4000-400cm^{-1}. FT-IR spectra of physical mixture of Aceclofenac+ Serratiopeptidase+ HPMC K 15 M and drugs mixtures were carried out by using KBr pellet technique. Samples were scanned over the 400-4000cm^{-1} Spectral region at a resolution of 4cm^{-1}.

***In vivo* study of optimized formulation**

The Optimized dosage form was evaluated for an *in vivo* rabbit study to check the movement of the pulsincap in gastro intestinal tract. The basic aim of this research study was to assure the location of the pulsincap during its movement through gastro intestinal tract. In the present research work, barium sulfate powders were used in place of drug amount. The pulsincap was prepared in exactly same as optimized dosage form. An X-ray study was performed at the school of pharmacy, RK University using portable X ray Machine (Ram Krupa X Ray and Sonography Centre, Rajkot, Gujarat, India). The use of laboratory animal for research purpose was approved by IAEC committee of School of Pharmacy, RK University, Rajkot. (Approval number: RKCP/CT/RP/14/49)

RESULTS AND DISCUSSION

3.1. Results and Discussion

Formaldehyde exposure was given to decrease the solubility of HGC body. Formaldehyde exposure results in a dramatically decrease in solubility of gelatin. Decrease in solubility was probably due to cross-linkage of aldehyde groups of formalin with amino groups in the gelatin. The size of treated capsule bodies was slightly reduced and apart from there were no visual defects found in treated capsule shell. It was found that there is significant difference in solubility of treated capsule body and untreated shell. The results of study were concluded that untreated capsule shell dissolved in 15 minutes and treated capsule shells were not dissolving for period of 24 hrs. The above results confirm the suitability for the pulsatile delivery.

3.1.1. Dimension

Average capsule length

- Before formaldehyde treatment (untreated cap and body) : 23 mm
- After formaldehyde treatment (treated body and untreated cap) : 22.5 mm

Average diameter of capsule body
- Before formaldehyde treatment : 7 mm
- After formaldehyde treatment : 6.9 mm

Average length of capsule body
- Before formaldehyde treatment : 19 mm
- After formaldehyde treatment :19 mm

3.1.2. Solubility studies for the treated capsules
The following observation were made
- a) In all the case of normal capsules, both cap and body dissolved within fifteen minutes.
- b) In the case of treated formaldehyde capsules, only the cap dissolved within 15minutes, while the capsule remained intact for about 24 hours.

3.1.3. Disintegration studies for capsules: The results of disintegration study have followings conclusion. In treated formaldehyde capsules, cap was disintegrating within 15 minutes, while the capsule body remained intact for about 24 hours.

3.1.4. Identification attributes: They were lockable type, odorless, softy and sticky when treated with wet fingers. After formaldehyde treatment, there were no significant changes in the capsules. They were non-tacky when touched with wet fingers.

3.1.5. Visual defect: 5-6 capsule bodies were shrunk or distorted in about 100 capsule bodies treated with formaldehyde.

3.1.6. Quantity test for free formaldehyde: The results of free formaldehyde study concluded that the sample solution from treated capsule was not more intensely colored than the standard solution. Standard solution concentration is 20µg. So it is proved that treated capsule containing less than 20 µg free formaldehyde in 25 capsules.

2.3. Results and Discussion of Evaluation tests for granules:
The flow property of granule was evaluated in a laboratory by a fixed height funnel method. Following results suggests that granule having acceptable granule characteristic for performing filling procedure during production scale up. Study of content uniformity suggests that granule having uniform contents throughout granule batch. Drug release study was also carried out for formulated granule by filling granule in fast dissolving untreated capsule using modified dissolution test apparatus. Modification was done in terms of addition of wire mesh screen above capsule dosage form to avoid dead volume. Results of drug release study concluded that granule showing fast drug release of aceclofenac and serratiopeptidase (more than 90 %)

Table 5: Results of granule characterization

Material	Bulk Density (gm/cm3)	Tapped Density (gm/cm3)	Carr's index	Hausner's ratio	Angle of repose
Granule	0.52	0.61	14.75	1.17	27.8

within 20 minutes.

2.4. Results and Discussion of Evaluation tests for polymer plugs
Tablets prepared using direct compression technique was obtained in the range with uniform thickness and acceptable weight variation. (general consideration). The results are shown in table. Hardness was found in the range of 3.8-5 kg/cm^2 for all the batches of polymer plug formulation and friability for the same was found to be less than 1% indicating sufficient mechanical integrity and strength of the prepared tablets. All tablet plugs were not showing disintegrating characteristic for study duration (6 hr). Lag time of polymer plug ranges from 100-370 minutes. Study concluded that amount of HPMC K 15 M polymer in polymer plug and weight of polymer plug were significant factors for getting desired lag time.

Table 6: Results for characterization of polymer plugs

Polymer Plug Batch	Weight of plug * (mg)	Hardness (Kg/cm^2)*	Friability (%)	Disintegration time * (hr)	Lag time * (Minutes)
F1	102.6	3.9	0.104	All tablet plugs were not showing disintegrating characteristic for study duration.	100
F2	100.3	3.8	0.192		160
F3	103.8	4.0	0.217		220
F4	150.3	4.2	0.168		180
F5	151.8	4.3	0.121		290
F6	152.9	4.4	0.168		310
F7	200.6	4.4	0.244		220
F8	200.8	4.4	0.217		310
F9	205.8	4.2	0.256		370
** n= 03, **n= 10, results are mean value of all sample.*					

Rupture Test

Lag time of PRTs were visually evaluated in phosphate Buffer 6.8. The Results of lag time of prepared formulation was given in table

Lag time of polymer plug ranges from 100-370 minutes. Study concluded that amount of HPMC K 15 M polymer in polymer plug and weight of polymer plug were significant factors for getting desired lag time.

***In vitro* dissolution study**

Dissolution study was conducted to determine the drug release from the PRTs. The Results of Dissolution study were given in following figures:

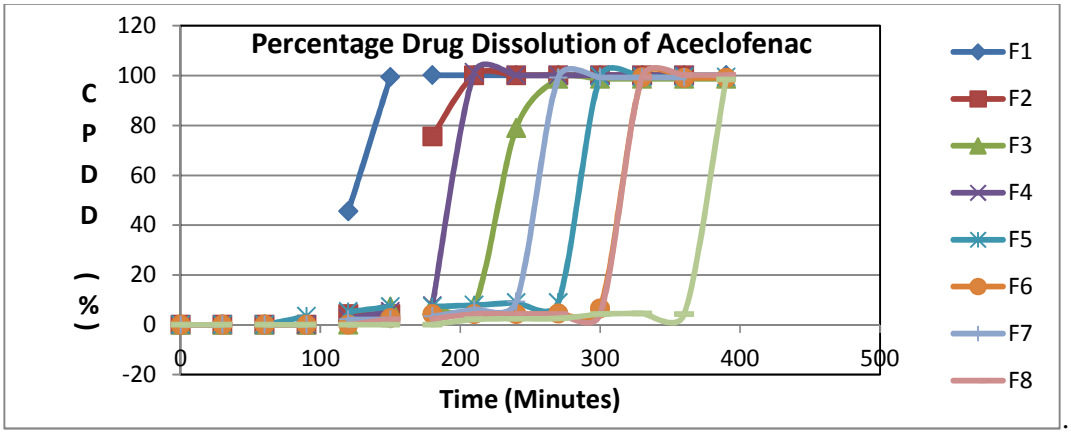


Figure 2: Graph showing invitro drug dissolution of aceclofenac (Batch F1 to F9)

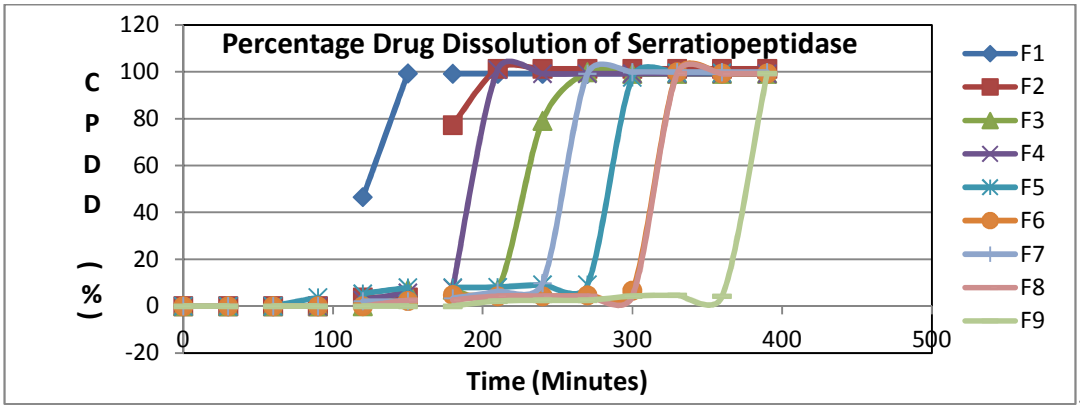


Figure 3: Graph showing invitro drug dissolution of serratiopeptidase (Batch F1 to F9)

The factorial design study was performed by design expert software trial version 8.0.7.1. Polynomial mathematical model was generated along with contour plot. Polynomial mathematical model was used to evaluate the response.

Polynomial Mathematical Model Equation:
Final Equation in Terms of Coded Factors:

$Y1 = 273.33 + 70.00\ X_1 + 66.67\ X_2 + 7.50\ X_{12} - 30.00 X_{11} - 20.00\ X_{22}$

Final Equation in Terms of Actual Factors:

$Y1 = -301.11 + 4.8\ X_1 + 3.25\ X_2 + 0.0005\ X_{12} - 0.012\ X_{11} - 0.020\ X_{22}$

Here, Y1 is the dependent variable, B_0 is the Average results of the nine runs; B_1 is the regression coefficient for the factor X_1. X_1 and X_2 stand for the main effects, X_1X_2 are the interaction terms between the factors. X_{11} and X_{22} are the quadratic terms included to investigate non linearity.

The data shown in table was applied to regression analysis with 95% confidence interval using design expert software trial version 8.0.7.1.

Table 7: Data of Regression Analysis of Lag time of rupture for Factorial Design

Batch no.	X1	X2	X1X2	X12	X22	Y1 (minutes)
F1	-1	-1	1	1	1	100
F2	-1	0	0	1	0	160
F3	-1	1	-1	1	1	220
F4	0	-1	0	0	1	180
F5	0	0	0	0	0	290
F6	0	1	0	0	1	310
F7	1	-1	-1	1	1	220
F8	1	0	0	1	0	310
F9	1	1	1	1	1	370

The P value for mathematical model is 0.0044 (less than 0.05) indicating that mathematical model is significant. P value for X1 and X2 factors were found to be 0.0015 and 0.0018 respectively (table) which is less than 0.05. Thus X1 and X2 has significant effect on dependent variable (Y1) while other terms X12, X11 and X22 were rendered insignificant having P value greater than 0.05 (0.4010, 0.0701 and 0.1629 respectively). Above Mathematical model can be used for prediction of response.

Optimized batch was selected from contour plot. (2 dimensional surface plot). Contour plot was generated from design expert software trial version 8.0.7.1. Polymer Plug amount and % of polymer in plug was plotted to generate contour plot against output variable lagtime of pulsatile release (Minutes).

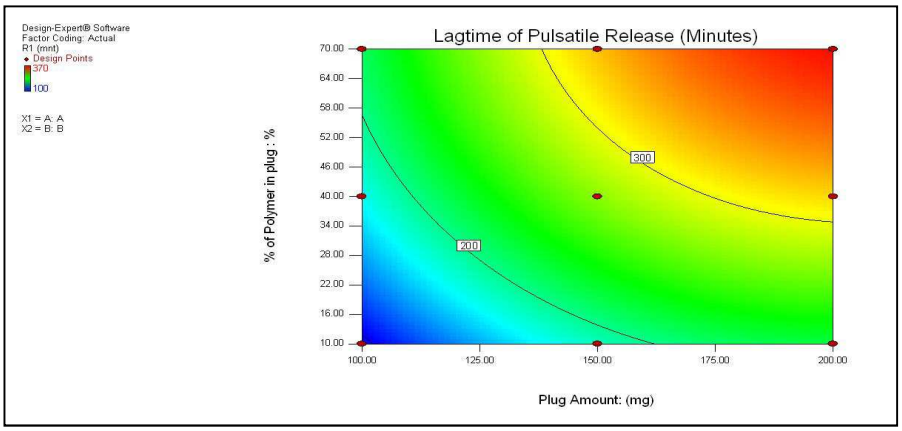


Figure 4: Contour Plot of effect of plugging amount and % of polymer in plug on lag time of pulsincap

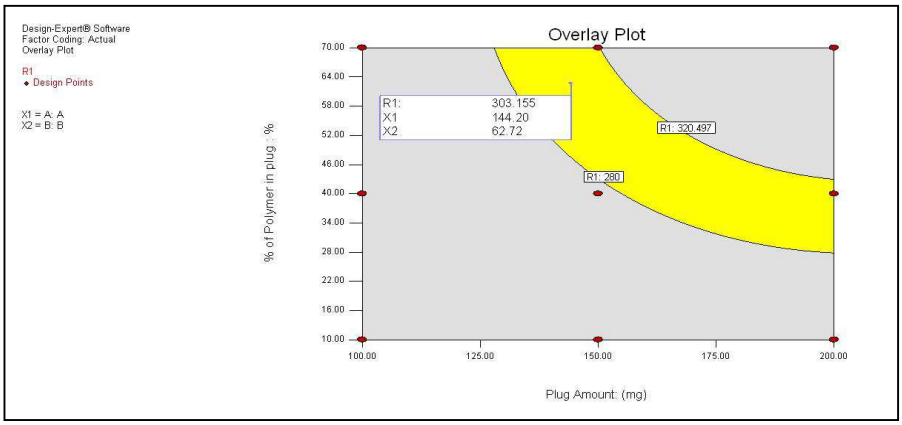


Figure 5: Overlay Contour Plot (Desirability Plot including flag chart as optimized batch)

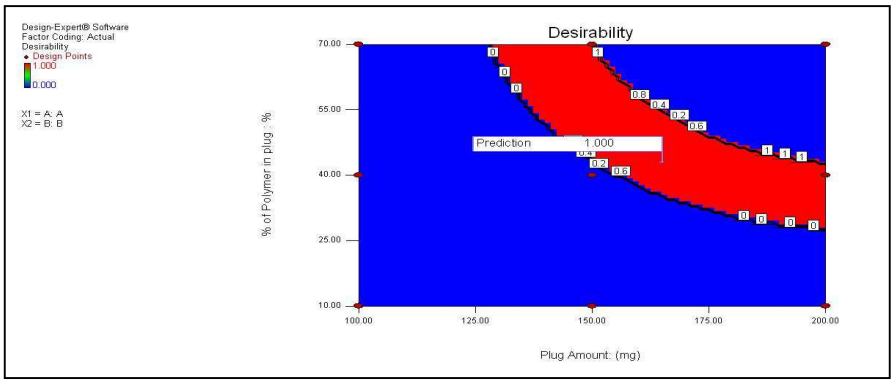


Figure 6: Overlay Contour Plot (Desirability Plot, Desirability 1 is ideal or optimized region)

Evaluation of check point batch F10:

In-vitro dissolution study

Dissolution study of Formulation F10 was carried out in both phosphate buffer pH6.8 and 0.1N HCl to observe the effect of pH on drug release from PRTs, given in table.

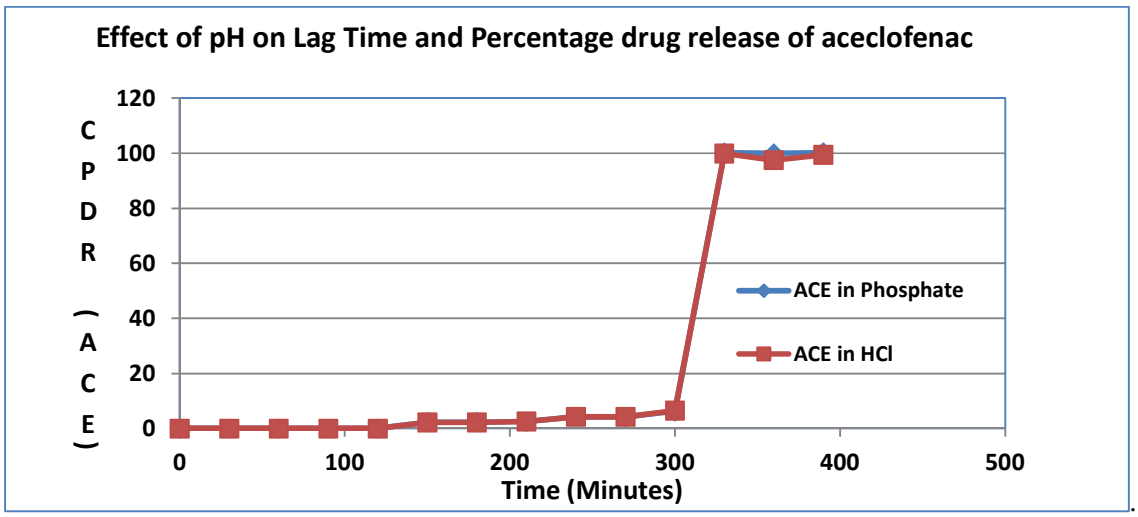


Figure 7 : Effect of pH on Lag Time and Percentage drug release of aceclofenac

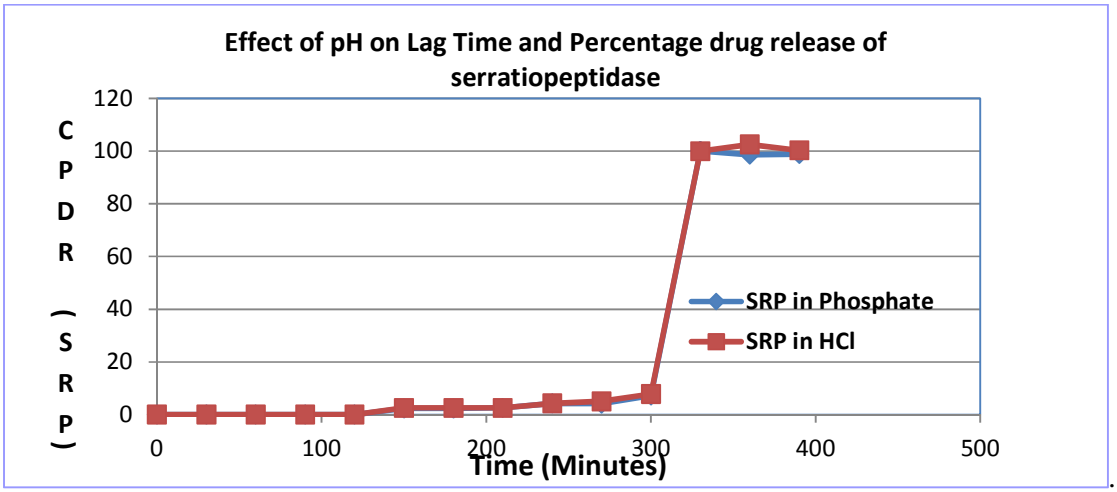
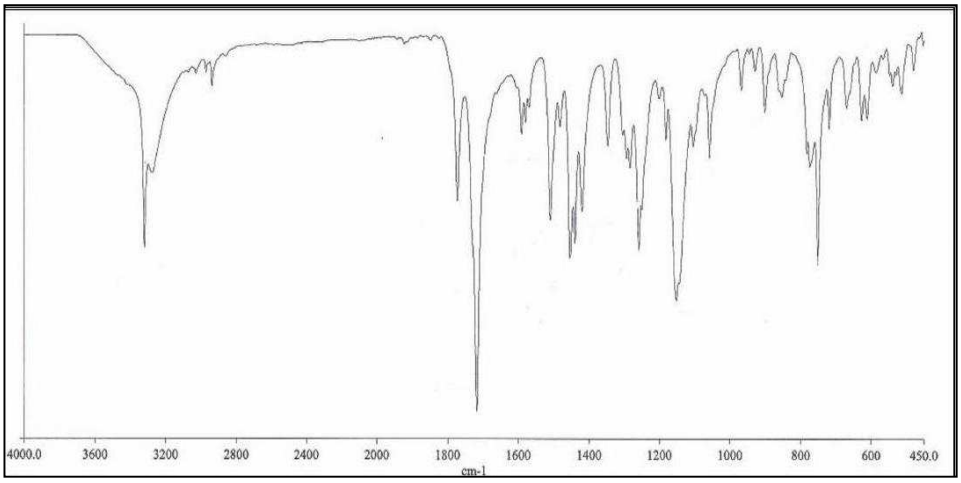


Figure 8: Effect of pH on Lag Time and Percentage drug release of serratiopeptidase

From the Results of Dissolution Profile of Check Point Batch F10 (table), it was concluded that there was no significant difference in Experimental Lag time than that of Predictable one. Data of Dissolution of batch F10 at different pH of dissolution media reveals that pH had not significant effect on the lag time PRTs. Batch F10 release drug after lag time of 5:00 ± 0:02 hours, which was near to the said hypothesis of the study, 5 hours. Thus, batch F10 was the Optimized Batch of the present study.

FTIR spectroscopy:



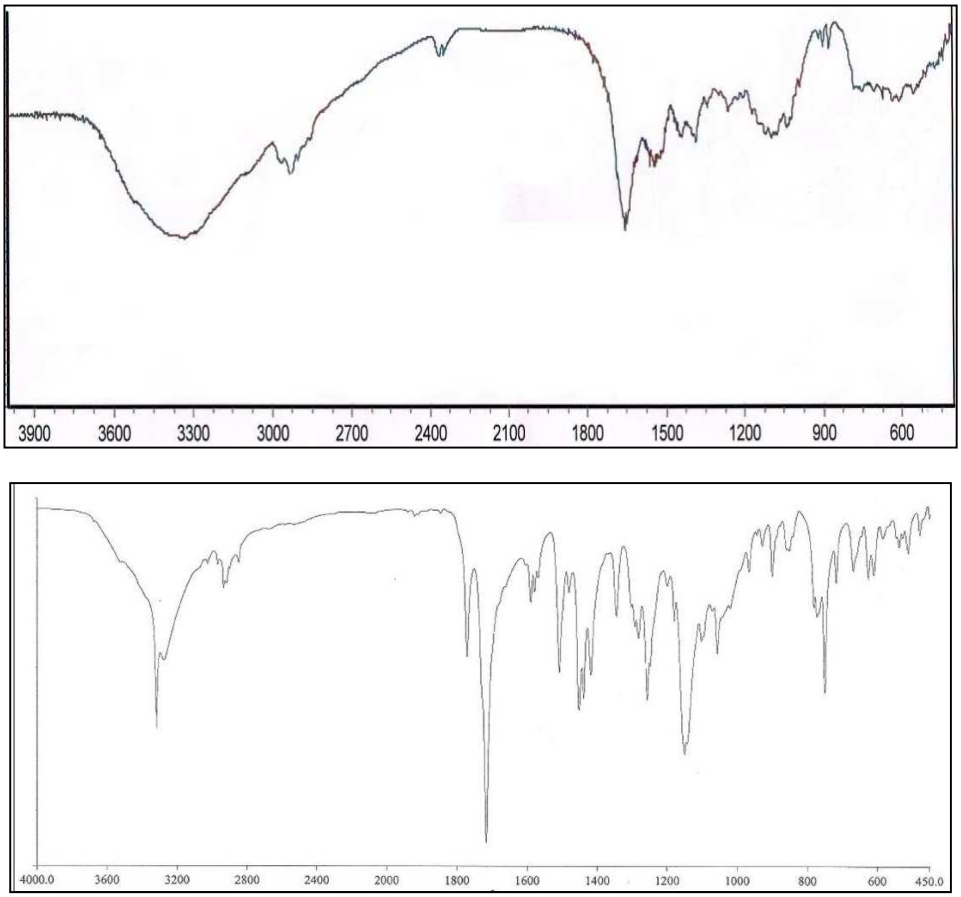


Figure 9: FTIR spectroscopy pure drugs (a, b) and physical mixtures (c)

- Infrared spectra of drugs and polymers were used to study the compatibility between them. No change in peak shows that there were no interaction between drugs and polymers.
- The FTIR spectrum of drugs shows characteristic bands at 1718.58 and 1770.55 Cm^{-1} of carbonyl group of ester, COOH (Carboxyl groups stretching at 3313.71 (Characteristic peak of aceclofenac(Figure a). The FTIR spectrum of drugs also shows characteristic bands 1648 (-CO-NH), 2927 (Aliphatic Chain) (Characteristic peak of Serratiopeptidase)(Figure b).
- The results of physical mixtures of drugs and various excipients revealed no considerable changes in the IR peaks of drugs thereby indicating the absence of any interaction.

***Invivo* Study:**

Radiographic study in animal for research purpose was approved by IAEC committee of School of Pharmacy, RK University, Rajkot. (Approval number: RKCP/CT/RP/14/49). From the following radiographic images, it was proved that pulsincap dosage form remains intact in its structural integrity and shape in invivo system of rabbit upto 4.30 hrs of dosage form administration. The changes in position of pulsincap in figure provide the evidence of pulsatile nature of formulation in rabbit's gastro intestinal tract. After 4.30 hr of pulsincap administration capsule was observed very clearly with presence of granule mass of barium sulphate and a small swelling layer around it. So, it provides the swelling property of polymer plug. As the swelling continues, outer polymer plug was removed from capsule body. Radiographic images after 5.30 hrs of pulsincap dosage form administration indicating that polymer plug completely removes at that duration and provide evidence of complete drug release.

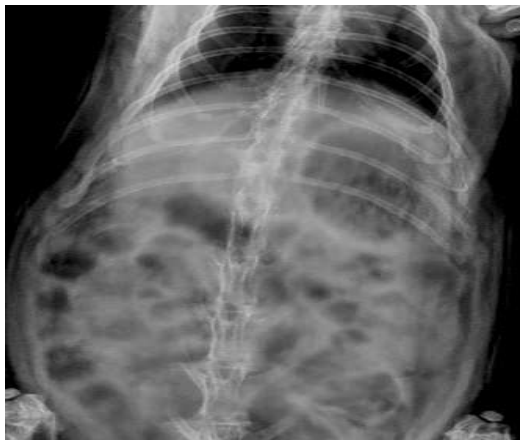


Figure 10: X ray of Rabbit Before Capsule Administration

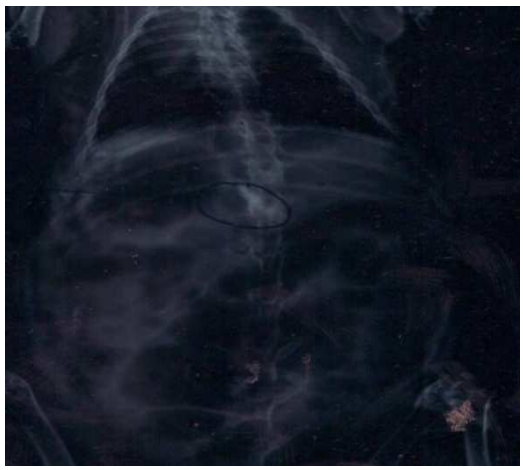


Figure 11: X ray of Rabbit after 4.30 hr of pulsincap capsule administration

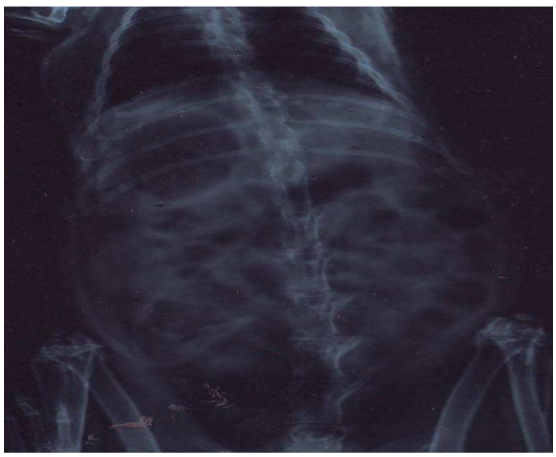


Figure 12: X ray of Rabbit after 5.30 hr of pulsincap capsule administration

CONCLUSION

The present study was carried out to develop pulsatile release of aceclofenac and serratiopeptidase drugs by pulsincap approach. Formaldehyde treatment has been given to decrease solubility of hard gelatine capsules. Through this research work, it was proved that there are unpredictable decreases in solubility of gelatine because of exposure of formalin vapours. It is concluded that amount of plug and percentage of polymer in plug were significant factors affecting lag time of drugs release.

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