Studies in formulation development of chronotherapeutics dosage of model drug

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ABSTRACT

The objective of the present work is to develop hollow calcium pectinate beads for floating pulsatile release of aceclofenac intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release in alkaline medium. To overcome limitations of various approaches for peparation of buoyance, hollow and porous beads were simple process of acid-base reaction during ionotropic cross linking was adopted. Formulations where studied for swelling properties, in-vitro release characteristics and swelling–erosion properties. As increased in concentration of oil, increases floating time but decreases drug release after optimized concentration. The dissolution Tests were carried out in a USP paddle dissolution apparatus. The formulation was optimized by 3^2 factorial design. The optimized batch obtained had porous, hollow with a bulk density <1 and had a floating time of > 20 hrs. It also showed highest % of drug release with 99.805 % entrapment efficiency. It showed lowest swelling ratio in 0.1 N HCl and highest swelling ratio in pH 6.8 phosphate buffer. The floating beads showed a two-phase release pattern with initial lag phase during floating in an acidic medium followed by rapid pulse in phosphate buffer pH 6.8. The approach indicates the use of hollow calcium pectinate beads as a promising floating pulsatile drug delivery system for site- and time-specific release of drug acting as per chronotherapy.

Keywords: Chronopharmacotherapy, Pulsatile drug delivery, Aceclofenac, Oil-entrapped beads.

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INTRODUCTION

In the field of modified release, there has been a growing interest in time specific oral delivery, which generally refers to the pre-programmed release of drugs following administration to achieve improved therapeutic efficacy. These systems constitute a relatively new class of devices, the importance of which is especially connected with the recent advances in chronopharmacology [1]. Particular rhythms in the onset and extent of symptoms were observed in diseases such as, bronchial asthma, myocardial infarction, angina pectoris, rheumatic disease, ulcer, diabetes, attention deficit syndrome, hypercholesterolemia and hypertension [2]. Natural biodegradable polysaccharides like pectin, guar gum, chitosan, carrageenans, sodium alginate,

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gellan gum and agar have been used in controlled drug delivery [3-7]. A multiparticulate system obtained by ionotropic cross-linking of these polymers have been used to develop floating drug buoyancy in cross-linked beads, some of which include freeze drying entrapment of gas or gas forming agents and use of volatile oil or fixed oil have been used [8-10].

The polysaccharide pectin is an inexpensive, nontoxic product extracted from citrus peels or apple pomaces and has been used as a food additive, a thickening agent, and a gelling agent [11]. In addition, pectin can reduce interfacial tension between an oil phase and a water phase and is efficient for the preparation of emulsion [12]. Pectin has a very complex structure that depends on both its source and the extraction process. Basically, it is a polymer of α-D-galacturonic acid with 1→4 linkages [12]. This chain is regularly interrupted by some rhamnogalacturonan segments that combine galacturonic acid residues and α-L–rhamnopyranose by a 1→2 linkage [13]. The galacturonic acid of the backbone is partially methyl-esterified. Low-methoxy pectin with degree of esterification less than 50% can form rigid gels by the action of calcium ions or multivalent cations, which cross-link the galacturonic acid chains. Calcium pectinate hydrogels are stable in low pH solution and are being investigated as a carrier material for different controlled release systems. In recent years, gel beads of calcium pectinate have been developed as a unique vehicle for drug delivery.

Sodium alginate can be obtained from seaweed, mainly species of Laminaria. In pharmaceutical formulations, sodium alginate and calcium sodium alginate have been used as tablet disintegrants. sodium alginate is a polyuronide made up of a sequence of two hexuronic acid residues, namely D-mannuronic acid and L-guluronic acid. The two sugars form blocks of up to 20 units along the chain, with the proportion of the blocks dependent on the species of seaweed and also the part of the seaweed used.

Chronopharmacotherapy, the drug regimen based on circardian rhythm, is recently gaining much attention world wide. Various diseases like asthma, hypertension, acidity and arthritis show circardian variation, which demands time-scheduled drug release for effective action, e.g. inflammation associated with morning stiffness, asthma and heart attack in the early hours of the day. To follow this principle, one must have to design the dosage form such that it can be given at the convenient time, e.g. bed time for the above-mentioned diseases with the drug release in mornings compared with evenings and site-specific absorption from the small intestine., Drug pharmacokinetics show circardian variation for various anti-inflammatory drugs like indomethacin, ketoprofen, aceclofenac and diclofenac sodium, which have a greater absorption in the morning as compared with the evening and site-specific absorption from the small intestine. Therefore, to develop dosage forms for chronopharmacotherapy, the designed drug release should be time-specific as well as site-specific. Also, the purpose of the present study was to produce hollow/porous floating beads of polymer/s by a process of emulsification followed by ionic cross linking in an acidic environment. Aceclofenac, an acid-insoluble nonsteroidal anti-inflammatory drug (NSAID) was used as a model drug. Aceclofenac is COX-II inhibitor class, used for arthritis [14]. Aceclofenac cause gastric irritation due to large amount of drug delivered to stomach. It had no 0.1N HCl solubility but had pH 6.8 phosphate buffer solubility. It is generally absorbed highest amount from intestine in early morning time.

In the arthritis pain in the joints is severe in the early morning. So patients using conventional aceclofenac tablet must wake up in the morning and take tablet at mid night due to short biological half life.

To relief from discomfort to patient prepare floating dosage form which retained in stomach until stomach empting and afterward give drug release in intestine within half an hr.

The obtained beads were evaluated for drug content, size analysis, porosity, mechanical strength, in vitro floating properties and drug release properties. The final formulation was optimized using 3^2 full factorial design.

Table 1: Experimental Design: factors and responses

Factors (Independent variables)	Levels used			Responses (Dependent variables)
	-1	0	+1	
X_1= % of Oil concentration	15	22.5	30	Y_1= % of drug release in 0.1 N HCl
X_2 = % of polymer concentration	0	2	4	Y_2= % of drug release in pH 6.8 phospate buffer

Response surface methodology (RSM) is a collection of statistical and mathematical techniques, useful for developing, improving and optimizing processes [15]. The basic components of the methodology include various types of experimental designs, regression analysis and optimization algorithms which are used to investigate the empirical relationship between one or more measured responses and a number of independent variables in the form of polynomial equations and mapping of the response over the experimental domain, with the ultimate goal of obtaining an optimal problem solution and establishing the robustness of the process. The advantage of such methodology is in providing a rationale for simultaneous evaluation of several variables with minimum experimentation and time, thus proving to be far more efficient and cost effective than conventional methods of product development.

The current study illustrates development of a simple floating pulsatile drug delivery system of aceclofenac to provide early morning relief from stiffness, pain and swelling in arthritis. It was aimed to modulate the pulsatile release profile for time- and site-specific drug delivery of aceclofenac using pectin and sodium alginate polymers. Computer-aided optimization techniques using 3^2 FFD were employed to investigate the effect of two factors viz. percentage of oil concentration to percentage of polymer concentration on cumulative drug release with in 6 hrs in 0.1N HCl and cumulative drug release with in 2 hrs in pH 6.8 phosphate buffer.

Hence with the proposed delivery system, a new therapeutic dimension to an existing fallen-out-of-favor drug molecule can be achieved.

MATERIALS AND METHODS

2.1 Materials
Aceclofenac (2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid) was generously gifted by Alembic Pvt. Ltd, Vadodara, India. Pectin (Chemdyes corporation, Vadodara, India), Sodium alginate (Chemdyes corporation, Vadodara, India), Liquid paraffin oil (Chemdyes corporation, Vadodara, India) and Calcium chloride (Chemdyes corporation, Vadodara, India). All other ingredients and reagents were of analytical grade and were used as received.

2.2 Experimental design
A full factorial 3^2 design was used for optimization procedure. It is suitable for investigating the quadratic responses and for constructing a second-order polynomial model, thus enabling optimization of the time- and site- specific cumulative drug release process. Mathematical modeling, evaluation of the ability to fit to the model and response surface modeling were performed with employing Design-Expert®.

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Table 2: Composition of experimental formulations (runs).

Test run	Variable factors	
	X_1 (% of oil concentration)	X_2 (% of polymer concentration)
F1	-1 (15)	-1 (0)
F2	-1 (15)	0 (2)
F3	-1 (15)	+1 (4)
F4	0 (22.5)	-1 (0)
F5	0 (22.5)	0 (2)
F6	0 (22.5)	+1 (4)
F7	+1 (30)	-1 (0)
F8	+1 (30)	0 (2)
F9	+1 (30)	+1 (4)

A full factorial 3^2 design was used for optimization procedure. It is suitable for investigating the quadratic response and for constructing a second-order polynomial model, thus enabling optimization of the drug release process. Mathematical modeling, evaluation of the ability to fit the response surface modeling was performed with employing Design-Expert software (Version 7.12, Stat-Ease Inc.) The studied factors (Independent variables) were % of oil concentration (X_1) and % of polymer concentration (X_2). Preliminary studies provided a setting of the levels for each formulation variable. The responses (Dependent variables) studied were % cumulative drug release in 0.1 N HCl (pH 1.2) in 6 hrs (Y_1) and % cumulative drug release in phosphate buffer (pH 6.8) in 2 hrs. Table 1: Summarizes the independent and dependent variables along with their levels. The factors and responses for experimental design are listed in Table 1. The resulted formulations (testing runs) are listed in Table 2.

Table 3: Formulation of aceclofenacc for oil-entrapped floating beads (100 g)

Formulations	Aceclofenac concentration (g)	Oil concentration (%)	Sodium alginate concentration (g)	Pectin concentration (g)
F1	4	15	4	0
F2	4	22.5	4	0
F3	4	30	4	0
F4	4	15	2	2
F5	4	22.5	2	2
F6	4	30	2	2
F7	4	15	0	4
F8	4	22.5	0	4
F9	4	30	0	4

2.3 Formulation of oil-entrapped floating pulsatile released beads
The oil-entrapped sodium alginate and/or MP beads were prepared by emulsion gelation method. Different amounts (0-40%) of selected oils were added to the solution to make a 100 g mixture as shown in tables. Homogenized mixture was extruded using a nozzle number 23 into a calcium chloride solution with gentle agitation at room temperature. The distance from the nozzle to the calcium chloride solution was 5 cm. The oil-entrapped gel beads formed were allowed to stand in the solution for 20 minutes, and then were separated and washed with distilled water. The beads were dried at 40 ^{0}C for 24 hrs.

2.4. Swelling study[15]
The dried blend gel beads were dipped in SGF at 37±0.5 ^{0}C. The swollen beads were periodically removed and weighed. The wet weight of the swollen beads was determined by

blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing on an electronic balance. All experiments were done in triplicate. The swelling ratio of the beads was calculated from the eq. 1

$$SW = (Wt - Wo) / Wo \qquad\qquad ----- (1)$$

Where, Wt is the weight of beads at appropriate intervals in SGF and Wo is the absolutely dried weight of beads. Each Wo determination contained no less than 0.1 g beads.

2.5. Dissolution methodology[15]
The dissolution studies of the beads equivalent to 100 mgs of aceclofenac were performed using a USP XXIII type II dissolution test apparatus. The drug release study was carried out in 0.1N hydrochloric acid initially for 2 or 6 hrs depending on the floating characteristic of the beads followed by dissolution in phosphate buffer of pH 6.8. Each 900 ml maintained at $37\pm0.5\ ^0C$ and agitated at 50 rpm. Periodically, samples were withdrawn and filtered through a Whattman filter paper 41 and the concentration of aceclofenac was measured spectrophotometrically at 271 and 275 nm for acidic and basic media, respectively.

Dissolution parameter:
Medium: 0.1 N HCl, pH 6.8 phosphate buffer solution
Volume: 900 mL
Apparatus: USP-II (Paddle)
RPM: 50 RPM
Time point: 0, 5, 15, 30, 60, 120, 180, 240, 300, 360, 365, 375, 390, 420, 480 minutes.
Temperature: $37 \pm 0.5\ ^0C$

2.6. Statistical analysis of the data of the model
Response surface modeling and evaluation of the quality of fit of the model for the current study were performed employing Design Expert® software (Version 7.1.2, Stat-Ease Inc., Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. 3D response plots were constructed using Design-Expert® software. One final formulation corresponding to the predicted optimum oil concentration and optimum polymer concentration and zero additional random check points covering the entire range of experimental domain were carried out to determine the validity of the model generated. Substantially, the resultanrt experimental data of the response properties were quantitatively compared with those of the predicted values. Also, the linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel.

RESULTS AND DISCUSSION

Time- and site-controlled pulsatile releases are increasingly being considered as desirable modes of drug delivery because of the growing awareness of the correlation of circardian rhythms with respect to pathophysiological state. The aim of the present work was to design a novel floating pulsatile drug delivery device, for the better treatment of rheumatoid arthritis.

3.1. Preparation of oil-entrapped floating beads
Till date, mostly all floating single-unit systems were used. Floating drug delivery depends on 'all or none' phenomenon means either system gives full drug release or not. So, if single-unit system used is failed, the whole system failed. In multiple unit system each unit act as one

system. So using multiple unit system, one unit is failed to deliver drug other will deliver drug at right site and time. Also aceclofenac had shorter half life of 4-4.3 hrs. Also aceclofenac produce gastric irritation, if it directly delivered in stomach, with large amount. Hence, the major challenge to develope a system which was not affected by gastric juice and delivered drug into intestine in the early morning. Therefore, we have selected low density material liquid paraffin (0.86 g/cm^3) and pectin which allowed floating beads on the surface of gastric juice and also providing a burst release once in intestine.

Also pectin and sodium alginate had low swelling at gastric pH (1.2 to 5) and maximum swelling in phosphate buffer. So highest drug release occurs in intestine and lowest in stomach. Aceclofenac is insoluble at gastric pH and soluble in phosphate buffer. It forms salt in intestine with ions present in it.
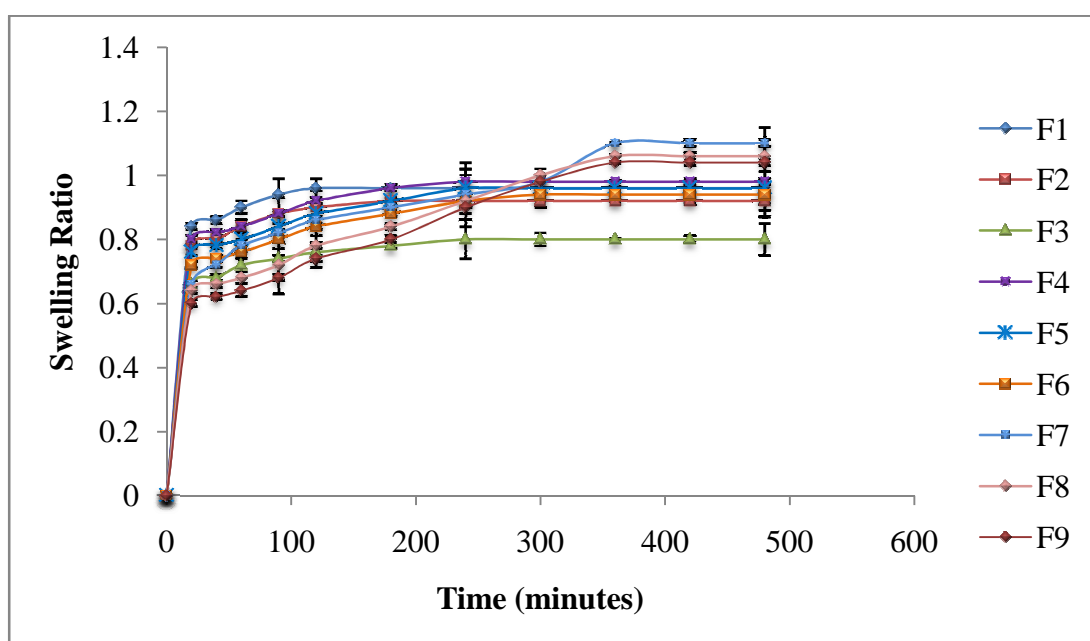
3.2. Swelling study result

Figure 1: Swelling Ratio in 0.1 N HCl

The swelling properties of beads were studied by measuring the water uptake at certain time intervals in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8). Being poly electrolyte, sodium alginate and pectin can exhibit swelling properties that are sensitive to the pH. The water uptake by the beads was shown in Figure I and II. In 0.1 N HCl, the ratio of water uptake was low and independent of time relative to that obtained at (pH 6.8). Maximum water uptake was obtained at 2 hrs in phosphate buffer (pH 6.8), after which erosion and breakdown of beads occurred. These results suggest that the dried gel particles will swell slightly in the stomach and, as they are subsequently transferred to upper intestine, the particles will begin to swell more and behaved as matrices for controlled release of incorporated drug. However, they are subject to erosion in the lower intestine.
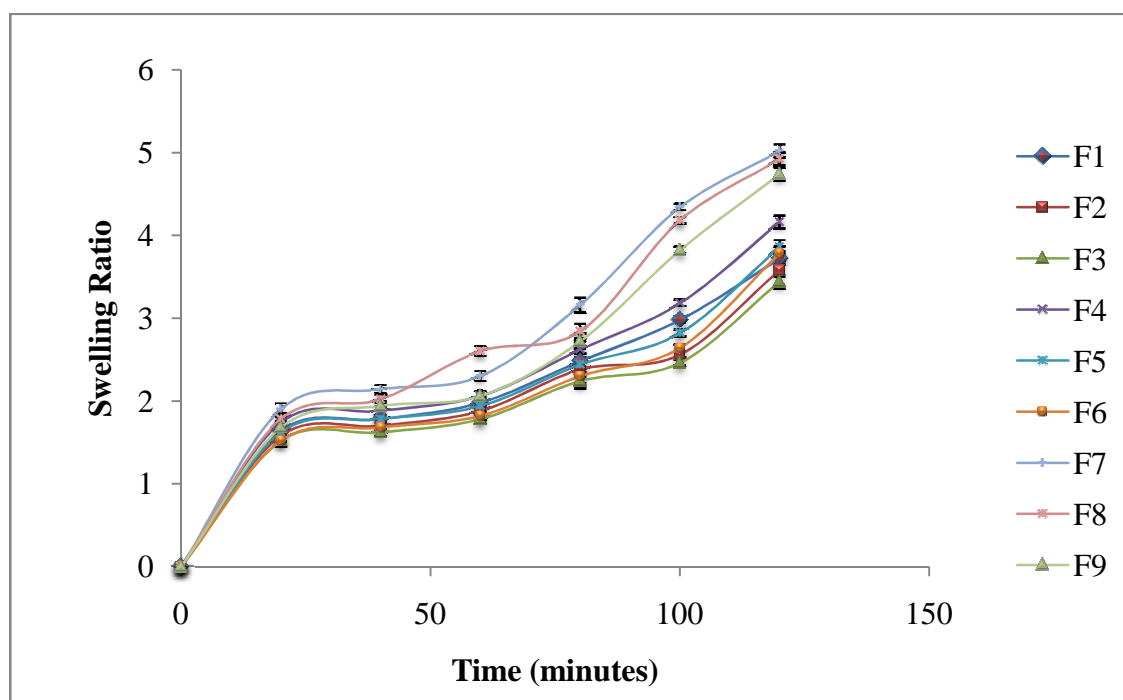
Figure 2: Swelling Ratio in phosphate buffer (pH 6.8)

The polymer concentration has significant effect on swelling ratios of beads. As the amount of polymer increased, the swelling ratio of beads decreased. This result may be because of maximum cross linking of polymers that yielded compact beads. The polymer mixture is also responsible for different swelling behavior of beads. As the proportion of alginate was reduced, the maximum swelling ratio as well as the time of rehydration increased. It proves that alginate makes compact structured beads in comparison to pectin. Oil has also a little effect on swelling behavior. In batches F1-F3, F4-F6 and F7-F9, the increased amount of oil in each pair of batches caused a slower swelling of beads. Thus the oil acted as a barrier for water absorption.

3.3. Dissolution study results
XXIV dissolution apparatus II filled with 900 ml of 0.1 N HCl (pH 1.2) at temperature $37 \pm 0.5 \,^{0}C$ with paddle rotation of 50 rpm. Results are shown in Figure 3.

3.4. Experiments of 3^2 FFD
To develop a system with time-dependent and site-targeted drug delivery with floating property in stomach, % of concentration of oil and % of concentration of polymer are important parameters affecting the drug release profiles. A multivariate optimization strategy was carried out with the aim of finding the optimum concentration of oil and optimum level of polymer to achieve a pulsatile release pattern from a oil-entrapped beads. Fig. 3 showed the release profiles of these 9 experimental runs performed in accordance with Table 2. Response data determined as per 3^2 full factorial experimental design: response Y_1 (% of cumulative drug release in 0.1 N HCl in 6 hrs) and response Y_2 (% cumulative drug release in 2 hrs) are presented in Table 4.

3.5. Multiple regression and mathematical model building
The targeted response parameters were statistically analyzed by applying one-way ANOVA (analysis of variance), at 7.5% significance level and the significance of the model was estimated using the statistical package Design-Expert. The individual parameters were evaluated using *F*-test and mathematical relationship was generated between the factors (dependent variables) and

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responses (independent variables) using multiple linear regression analysis, for determining the levels of factors which yield optimum dissolution responses.
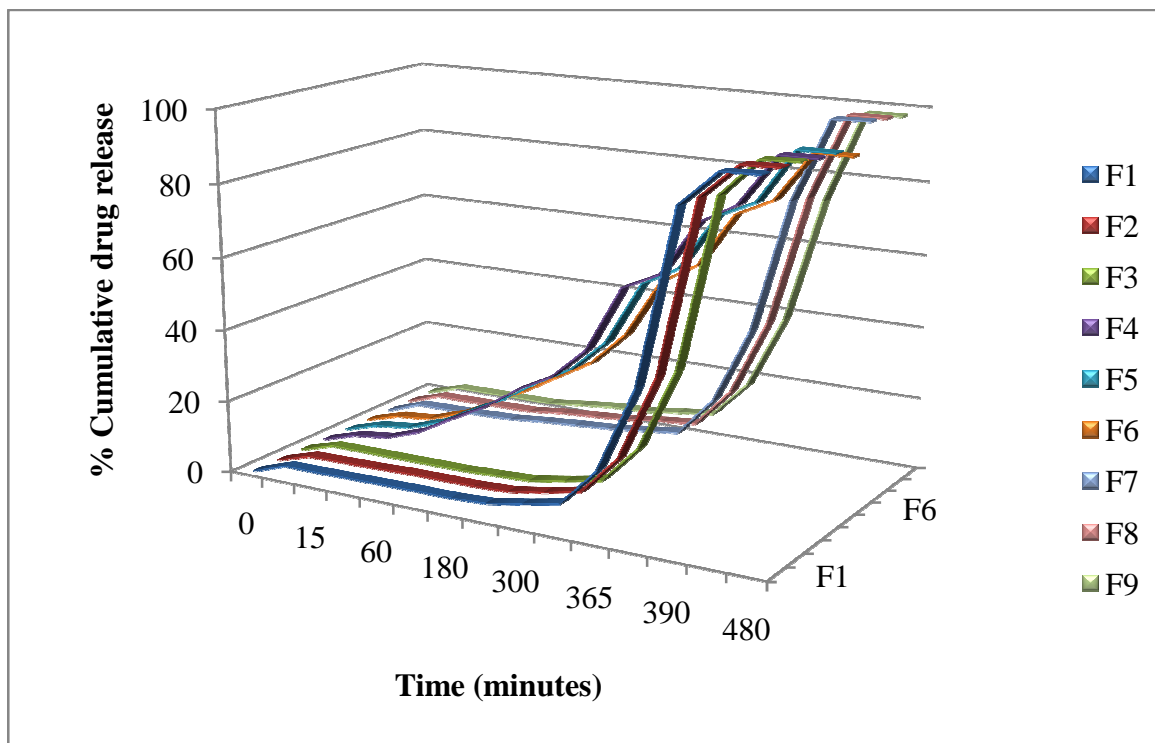

Figure 3: Drug release profile of all formulations (1-9)

A second-order polynomial regression equation that fitted to the data is as follows:
$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1{}^2 + b_{22} X_2{}^2$$ ----- (2)

Where, b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; b_1, b_2, b_{12}, b_{11} and b_{22} are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 stand for the main effects. The terms $X_1 X_2$ and $X_i{}^2$ ($i = 1$ and 2) represent the interaction and quadratic terms, respectively used to simulate the curvature of the designed sample space. In Table 4, factor effects of 3^2 FFD model and associated *p*-values for the responses Y_1 and Y_2, are presented. A factor is considered to influence the response if the effects significantly differ from zero and the *p*-value is less than 0.05. Data are in Table 5.

Table 4: Dissolution studies as per 3^2 full factorial experimental design

Batch	% cumulative drug release in 0.1 N HCl in 6 hrs (Y_1)	% cumulative drug release in 2 hrs (Y_2)
F1	7.816±0.848	97.015±1.673
F2	7.686±1.320	96.88±0.531
F3	7.455±1.753	96.417±1.506
F4	56.747±0.567	95.388±1.268
F5	55.475±1.459	95.004±0.240
F6	53.816±0.455	92.499±0.546
F7	8.554±0.245	99.805±0.334
F8	8.167±1.450	98.994±1.560
F9	8.086±1.455	97.969±1.456

A backward elimination procedure was adopted to fit the data into different predictor equations. The final equations of the responses are given below:

$$Y_1 = +55.36 - 0.52\ X_1 + 0.29\ X_2 - 0.048\ X_1 X_2 + 0.16\ X_1{}^2 - 47.49\ X_2{}^2 \qquad ----- (3)$$

$$Y_2 = +94.70 - 0.99\ X_1 + 1.21\ X_2 - 0.077\ X_1 X_2 - 0.29\ X_1{}^2 + 3.21\ X_2{}^2 \qquad ----- (4)$$

To confirm the omission of non-significant terms, an *F* statistic was calculated after applying analysis of variance for the full model. The equations represent the quantitative effect of factors (X_1 and X_2) upon the responses (Y_1 and Y_2). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors.

In above equation 3, coefficient of X_1 shows negative sign, so increase in concentration of oil decreased drug release in 0.1 N HCl. Coefficient of X_2 show positive sign, so increase in concentration of polymer increases drug release 0.1 N HCl.

In equation 4, coefficient of X_1 shows negative sign, so increase in concentration of oil decreased drug release in phosphate buffer pH 6.8. Coefficient of X_2 show positive sign, so increase in concentration of polymer increase drug release in phosphate buffer pH 6.8.

3.5. Response surface analysis

The quadratic models generated by regression analysis were used to construct 3D response surface plots in which response parameter *Y* was represented by a curvature surface as a function of *X*. Figure 4 and 5 showed the effect of two factors in the % cumulative drug release in 0.1 N HCl in 6 hrs and cumulative percentage drug release in phosphate buffer within 2 hrs, respectively.

Table 5: Summary of each factor effect and its *p*-values, for responses Y_1 and Y_2

Factor	Y_1		Y_2	
	Factor effect	p-value	Factor effect	p-value
X_1	-0.52	0.0002	-0.99	0.0002
X_2	+0.29	0.0050	+1.21	0.0004
X_{12}	-0.048	0.3716	-0.077	0.0592
$X_1{}^2$	+0.16	0.8948	-0.29	0.3347
$X_2{}^2$	-47.49	0.7600	+3.21	0.0556

Figure 4 showed antagonistic relationship between the two independent variables (factors) on response Y_1 (% cumulative drug release in 0.1 N HCl within 6 hrs) as was also evident from the *p*-values listed in Table 5.

This decreased in drug release in 0.1 N HCl is due to increase in oil concentration, because oil forms barrier to an additional barrier for the release of drug resulting slow release of drug. Also oil retard swelling of polymer. Also increase in concentration of pectin increase in drug release, calcium pectinate had less compact structure then calcium alginate. So increase in concentration of pectin, increase in drug release. Also combination of alginate an showed higher drug released in 0.1 N HCl due to decreased in alginate concentration.
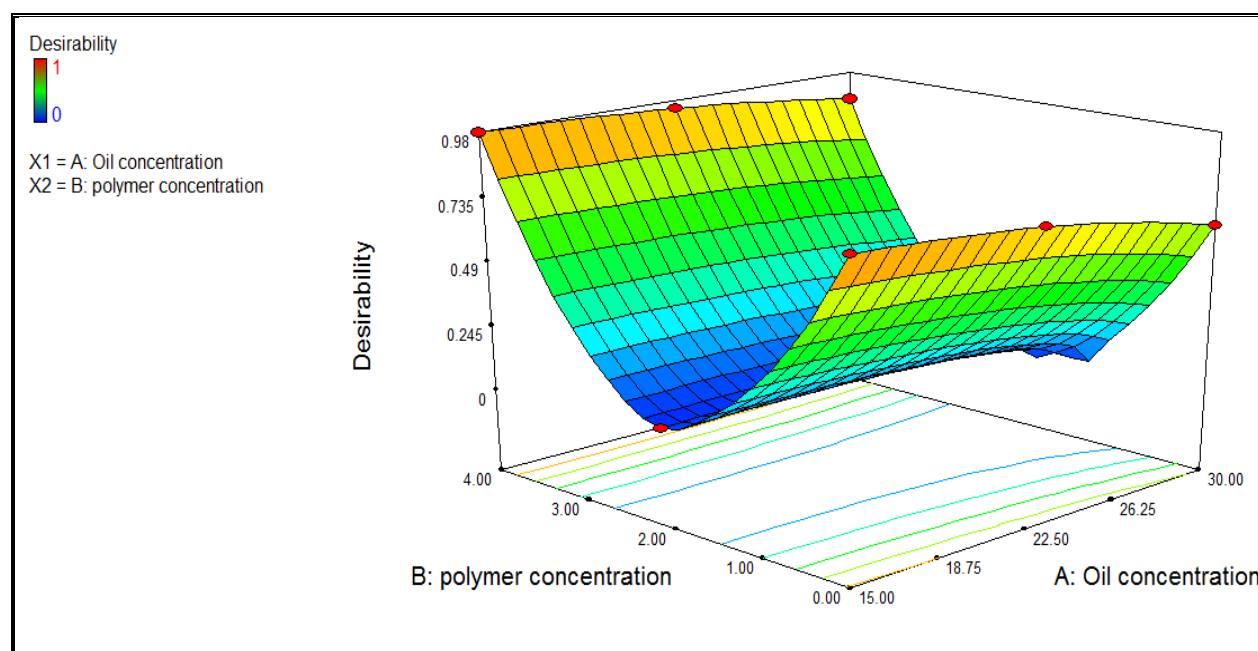
Figure 4: Response surface plot showing the influence of % of oil concentration (X_1) and % of polymer concentration (X_2) on response Y_1 (% cumulative drug release in 0.1 N HCl within 6 hrs)
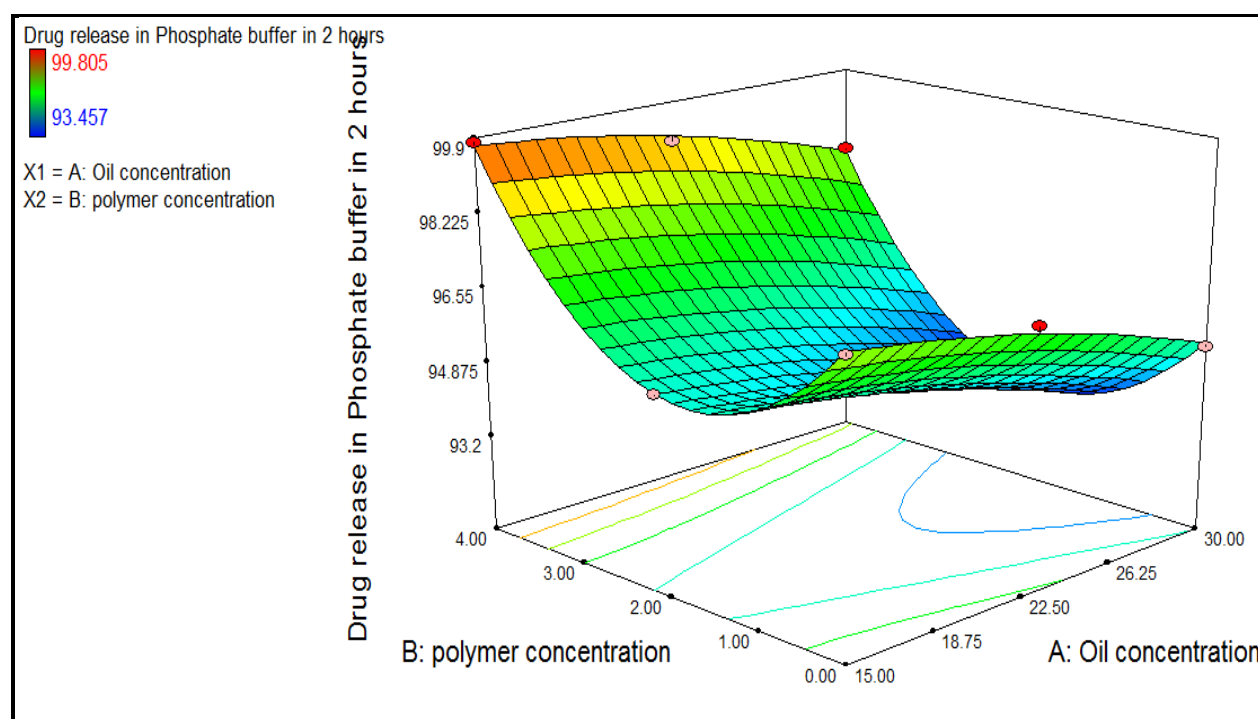

Figure 5: Response surface plot showing the influence of , % of oil concentration (X_1) and % of polymer concentration (X_2) on response Y_2 (% cumulative drug release in phosphate buffer within 2 hrs)

Figure 5 showed antagonistic relationship between the two independent variables (factors) on response Y_2 (% cumulative drug release in phosphate buffer within 2 hrs) as was also evident from the *p*-values listed in Table 5.

This decreased in drug release in phosphate buffer was due to increase in oil concentration, because oil forms barrier to an additional barrier for the release of drug resulting slow release of drug. Also oil retard swelling of polymer in phosphate buffer. Also increased in concentration of

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pectin increased in drug release, calcium pectinate had less compact structure then calcium alginate. So increased in concentration of pectin, increased in drug release in phosphate buffer. Also combination of sodium alginate and pectin showed lower drug release in phosphate buffer than sodium alginate and pectin beads alone due to formation of two protective layer of calcium alginate and calcium pectinate retarded drug release in phosphate buffer.

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. In this study optimization was performed with constrain for Y_1 (7.455 < Y_1 < 56.747 %) and Y_2 (92.499 < Y_2 < 99.805 %) set as goals to locate the optimum setting of the independent variables in the new formulation.

The optimal calculated parameters were:
- Percentage of oil concentration X_1 = 15 (equivalent to 15%)
- Percentage of polymer (pectin) concentration X_2 = 4 (equivalent to 4 %).

3.7. Validity of response surface methodology

In order to assess the reliability of the developed mathematical model, dissolution test of the formulation corresponding to the predicted optimum oil and polymer concentration with three additional random chek points covering the entire range of experimental domain was performed[16] For each of these test runs, responses were estimated by use of the generated mathematical model and by the experiment procedure. Table 6 lists the test conditions of the optimum and the random check points, their experimental and predicted values for both the response variables, along with the calculated percentage prediction error (percentage bias). Fig. 6A and B shows linear correlation plots between the observed and predicted response variables. The graphs demonstrate high values of correlation coefficient, r^2 (>0.9) indicating excellent goodness of fit. Thus, the lower magnitude of percentage prediction error (-1.679 to -0.199 for Y_1 and -0.195 to +0.051 for Y_2) as well as significant values of r^2 in the current study indicate the robustness of the mathematical model and high prognostic ability of RSM. As desired, the beads prepared according to optimum formulation achieved a % cumulative drug release in 0.1 N HCl was 8.554, followed by complete drug release within next 2 hrs. The release profile of optimized beads formulation combined with immediate release dose is as shown in Fig. 7.

Table 6: The experimental and predicted values for response Y_1 (cumulative percentage drug released in 6 hrs in 0.1 N HCl, %)) and for response Y_2 (cumulative percentage drug released in 2 hrs in phosphate buffer, %) along with percentage prediction error observed for the optimum test condition (A) and random check points (B–D).

Sr. No.	Test Condition [a] (Coded) X1/X2	Response	Experimental Value	Predictable Value	Percent Prediction Error [b]
A	15.00/4.00	Y_1	8.554	8.698	-1.683
		Y_2	99.805	99.754	+0.051
B	15.90/4.00	Y_1	8.695	8.841	-1.679
		Y_2	99.734	99.928	-0.195
C	20.24/4.00	Y_1	8.373	8.483	-1.313
		Y_2	99.412	99.537	-0.126
D	18.06/0.00	Y_1	8.001	8.017	-0.199
		Y_2	97.106	97.251	-0.149

[a] *X1, percentage of oil concentration (coded) and X2, Polymer concentration (coded).*
[b] *Percent error was calculated using the formula (experimental value–predicted value)/experimental value×100.*

$$y = 0.964x + 0.254$$
$$R^2 = 0.988$$
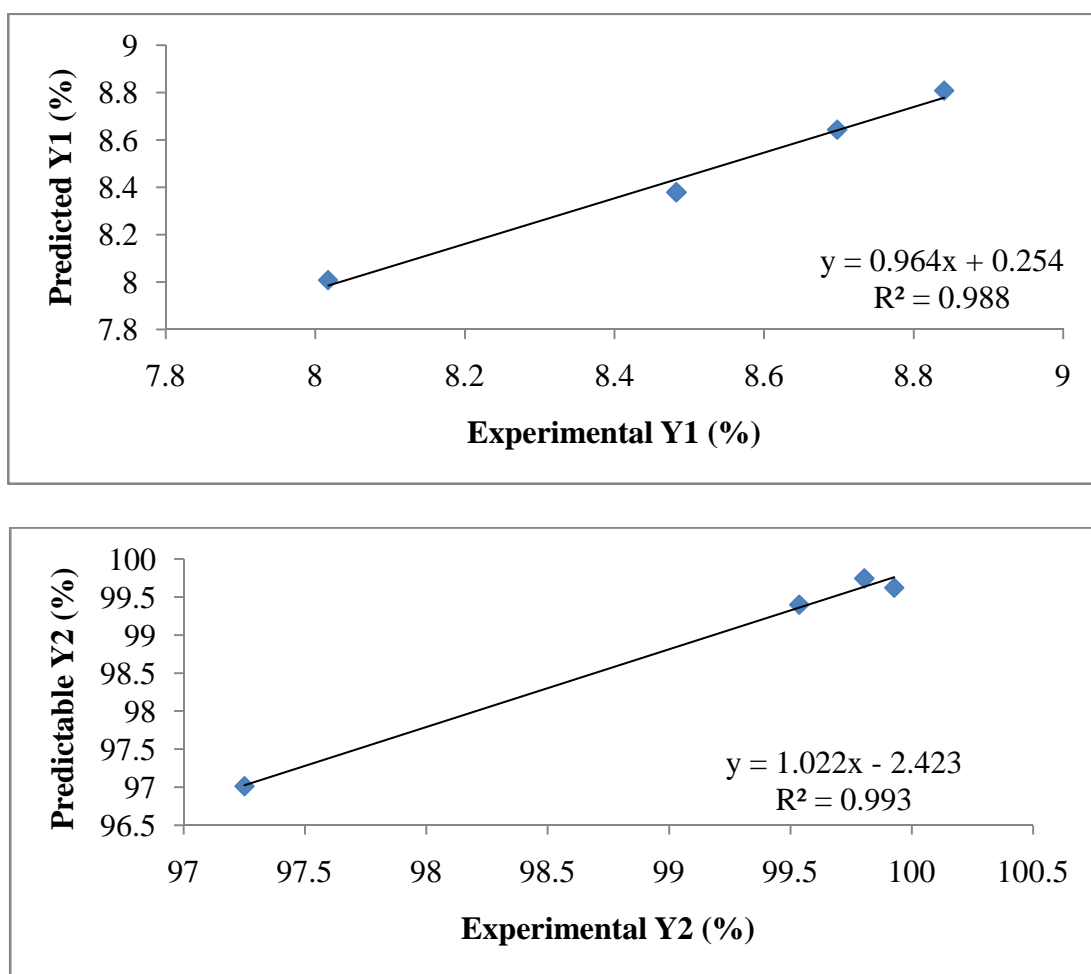

$$y = 1.022x - 2.423$$
$$R^2 = 0.993$$

Figure 6: Linear correlation plots (A and B) between observed and predicted values for response Y_1 (% Cumulative drug release in 0.1 N HCl within 6 hrs) and response Y_2 (% Cumulative drug release in pH 6.8 phosphate buffer within 2 hrs)
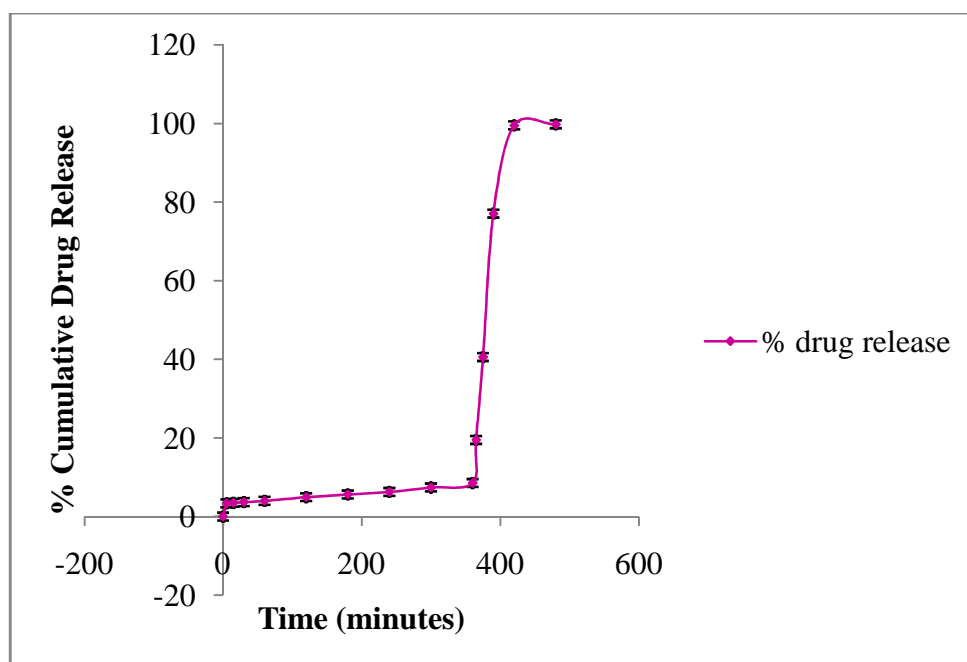

Figure 7: Drug release profile of optimized batch.

CONCLUSION

The present study demonstrates that acecclofenac could be successfully delivered to provide early morning relief of arthritic pain and stiffness by design of a floating pulsatile chronopharmaceutical formulation. The formulation is to be taken after 2 hrs of meal; where immediate release dose will provide relief from pain and stiffness in response to the COX-II, while timed pulsatile release floating beads with delayed "burst" release will attenuate early morning pain and stiffness. This will provide an ideal therapeutic regimen with enhanced patient compliance. Concerning statistical analysis, it was shown that appropriate factorial design and optimization technique can be successfully used in the development of time- and site- depend drug released formulations based on floating (liquid paraffin) and swelling of polymer (pectin) to achieve the desired pulsed release profile after a programmed lag time. Response surface methodology is an important tool for understanding the change of responses and locating the area of interest. The optimized formulation containing oil concentration and polymer concentration percentage ratio of 15.00/4.00 and has the potential for time-controlled pulsatile delivery of aceclofenac. The optimized formulation exhibited release profiles which were close to the predicted responses. High degree of prognosis obtained for 3^2 full factorial design corroborates that RSM is an efficient tool in optimization experiments. This work can be extended for time scheduled release of drugs having less solubility, and highest absorption in the colon. Thus the designed device can be considered as one of the promising formulation technique for preparing floating pulsatile drug delivery systems and hence in chronotherapeutic management of rheumatoid arthritis by opening a "new therapeutic dimension" to an existing drug molecule.

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