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Formulation and *In Vitro* Evaluation of Pulsatile Colon Drug Delivery System of Piroxicam using 3^2 Factorial design

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Abstract

The aim of the present investigation was to develop colon specific double cross linked alginate-chitosan blend gel beads for pulsatile release. A 3^2 factorial design was employed to study the influence of two independent variables amount of piroxicam (X1), amount of chitosan (X2) on dependent variables, encapsulation efficacy (Y_{EE}), amount of drug release at 5^{th} hour (Y_5) and amount of drug release at 6^{th} hour (Y_6). The prepared colon specific pulsatile beads were evaluated for particle size and invitro drug release studies. Drug polymer interaction studies were determined by fourier transform infrared (FTIR) spectroscopy. The surface characteristics and morphology was determined by scanning electron microscopy (SEM). The check point batch was prepared and the contour and 3D plots were also presented. It was concluded that the desired colon specific pulsatile release was obtained with alginate-chitosan blend gel double cross linked beads.

Key Words: Colon specific, Piroxicam, Factorial design, Sodium Alginate, Chitosan, Pulsatile release.

INTRODUCTION

Colon specific drug delivery systems have been tremendously developed over couple of decades for local and systemic delivery of drugs in crohn's disease, ulcerative colitis, irritable bowel syndrome, diurnal asthma and arthritis [1]. A pulsatile release profile after a defined lag time is advantageous for the drugs targeted to a specific site in the intestinal tract i.e., to the colon. Such site-specifc drug delivery systems are expected to provide majority of their drug load to colon without being released in stomach and small intestine. As a result, it is possible to provide an effective and safe therapy with a low dose of drugs.

Naturally occurring sodium alginate and chitosan have been received a great interest in drug delivery due to adequate biocompatibility. Chitosan is a weak cationic polysaccharide, consists of (1, 4) linked - 2 - amino - 2 -deoxy- β -glucan. Alginic acid is a linear copolymer of (1, 4)

linked - D-mannuronic and -1- guluronic acid residues arranged in a non regular block wise pattern. The pH sensitivity of chitosan and alginate molecules was due to amino groups and carboxyl groups respectively. The alginate beads for drug delivery were focused on single calcium crosslinking, such as chitosan-coating calcium alginate beads [2-4], calcium alginate beads contained chitosan powder [5-6] and recently dual crosslinked alginate–chitosan blend gel beads for oral site-specific drug delivery was reported [7].

Piroxicam a potent non steroidal anti-inflammatory drug with analgesic activity [8, 9] has been advocated in treatment of colonic inflammatory conditions and arthritis. Recent experimental and clinical studies suggest that piroxicam, may be useful for chemoprevention of colon cancer [10, 11]. This suggests that piroxicam may be an effective chemopreventive agent for the prevention of colon cancer. However, the gastrointestinal toxicity associated with the conventional NSAIDs may limit their long-term use [12]. In the light of this information, it was planned to develop double crosslinked alginate chitosan blend gel beads as colon specific drug delivery systems of piroxicam for pulsatile release.

MATERIALS AND METHODS

Materials

Piroxicam was a kind gift from Apex Health Care Pvt Ltd (Ankleshwar, India). Chitosan (85%deacylated) was kindly gifted by Central Institute of Fisheries Technology (Kochi., india), Sodium alginate purchased from SD fine chemicals (Mumbai, India), and Calcium chloride was purchased from Qualigens (Mumbai, India). And all other chemicals were of analytical grade.

Methods

Preparation of beads

Double cross linked chitosan-alginate beads were prepared by modified reported method [7]. The blend solution contained sodium alginate, chitosan and piroxicam was prepared. Firstly, the 2% w/v sodium alginate was prepared in 25 ml distilled water under mechanical stirring for 5 min and resulting alginate solution was kept for an over night, then chitosan powder was added in to the solution and mixed homogeneously. The added chitosan was dissolved by addition of 0.25 ml of acetic acid, the mixture was adjusted to pH 5.0 by NaOH (0.1 mol/l) solution; homogeneous solution of two polymers was formed under stirring for 20 min. Calculated amount of piroxicam was added to this homogeneous solution. The resulting blend solution was added manually dropwise in to calcium chloride (2% w/v) solution using needle size no. 16, spherical beads were formed under mechanical stirring for 15 min; and directly changed in to 2 % (w/v) sodium sulphate solution and kept for 15 min, then Ca^{+2} and So_4^{2-} double cross linked blend gel beads were obtained, washed with distilled water for three times and dried at room temperature.

Particle size determination

Particle size of double cross linked beads was measured using digital slide calipers (Digmatic, Mitutoyo Corp., Japan) and the mean particle size was calculated by measuring 50 particles.

Content estimation

Drug loaded beads (50 mg) were accurately weighed and transferred into conical flask containing 3 % sodium citrate solution and kept on a rotary shaker for 1 hour at room temperature. The resultant dispersion was centrifuged at 3000 rpm for 15 min and supernatant was diluted suitably and analyzed for concentration of piroxicam using UV spectrophotometer at 333 nm (Systronics PC Based, 2202, Ahmedabad, India). Each experiment was performed in triplicate.

In Vitro Drug Release Studies

In vitro drug release studies were performed in enzyme free simulated gastric fluid (SGF), enzyme free simulated intestinal fluid (SIF) using USP dissolution apparatus (type I) in 900 ml medium at 50 rpm with 37± 0.1^{o}C, SGF (pH 1.2) consisted of NaCl (2.0 g); HCl (7 ml) and pH was adjusted to1.2 ± 0.1. SIF (pH 7.4) consisted of KH_2PO_4 (6.8 g); 0.2 N NaOH (190 ml) and pH was adjusted to 7.4 ± 0.1. SIF (pH 4.5) was prepared by mixing SGF (pH 1.2) and SIF (pH 7.4) in a ratio of 39:61. The drug release studies were conducted in enzyme free SGF for the first 2 hours, in enzyme free SIF pH 4.5 for next 3 hours and continued for remaining 5 hours in SIF pH 7.4 (13,14). Aliquots of samples were withdrawn at predetermined time intervals and replaced with an equal amount of fresh dissolution medium and drug content was determined using UV spectrophotometer at 333 nm.

Factorial design

A 3^2 factorial design was employed in the present study. In this design experimental trials were performed for all 9 possible combinations (Table I). The amount of piroxicam (X_1) and amount of chitosan (X_2) were chosen as independent variables, while encapsulation efficacy (Y_{EE}) amount of drug release at 5th hour (Y_5), amount of drug release at 6th hour (Y_6) were selected as dependent variables in this factorial design. A statistical model incorporating interactive and polynomial terms was used to evaluate the response (equation 1).

$$Y=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_1X_1+b_{22}X_2X_2 \qquad (1)$$

Where Y is the dependent variable, b_0 is the arithmetic mean of the 9 trials, and b_i is the estimated coefficient for the factor X_i. The $X_1$and X_2 are main effects, represent the average results of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously varied. The polynomial terms (X_1X_1 and X_2X_2) are included to investigate nonlinearity [15, 16]**.** To assess the reliability of the model**,** a comparison between the experimental and predicted values of the responses is also presented in terms of % bias.

$$\%\ Bias = \frac{Predicted\ Value - Experimental\ Value}{Predicted\ Value} \qquad (2)$$

Surface Characteristics and Morphology of beads

The shape and surface characteristics of the beads was observed by scanning electron microscopy (Joel, Tokyo, Japan). The dried beads were coated with gold to a thickness of ~300 Å using sputter coater under argon atmosphere at room temperature, and photomicrographs were taken.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectra of the piroxicam, sodium alginate, pure chitosan and optimized formulation was recorded using Fourier Transform Infrared spectrophotometer (Jasco Model: 5300, Tokyo, japan). Samples were prepared using KBr disks by means of hydraulic pellet press at a pressure of 7-10 tons. The samples were scanned from 4000 to 400cm^{-1}.

RESULTS AND DISCUSSION

Factorial design

In the present study, the effect of amount of piroxicam and chitosan on EE, Y_5 and Y_6 studied using 3^2 factorial design, revealed wide variation (Table 1). The data clearly indicates that the

dependent variables are strongly dependent on the independent variables. The fitted equation relating the response Y_{EE}, Y_5 and Y_6 to the transformed factor are shown in equations 1, 2 and 3. The value of correlation coefficient (Table 2) indicates a good fit. The polynomial equation can be used to draw a conclusion after considering the magnitude of coefficient and the mathematical sign it carries (positive or negative).

Table 1: Observed responses from 3^2 factorial design along with yield and particle size of formulations.

Design Parameters						% yield	Particle size (µm)
Formula code	Independent variables		Dependent variables				
	X1	X2	Y_{EE}	Y_5	Y_6		
F1	-1	-1	80.61	25.29	56.95	99.39	1212.40 ±122
F2	-1	0	82.85	26.88	81.55	97.94	1229.00 ± 106
F3 *	-1	+1	86.93	27.34	89.16	99.73	1255.00 ± 116
F4	0	-1	83.67	28.11	72.62	96.19	1273.00 ±168
F5	0	0	86.85	30.90	83.94	98.16	1293.00 ± 153
F6	0	+1	90.93	31.75	89.48	90.37	1302.50 ± 099
F7	+1	-1	88.67	30.00	85.53	97.64	1422.00 ± 177
F8	+1	0	96.93	31.99	88.87	97.89	1444.50 ± 140
F9	+1	+1	100.90	33.39	91.63	91.23	1587.00 ± 163

Coded values	Actual values	
	X1	X2
-1	75	0
0	150	50
+1	225	100

*indicates optimized formulation; X_1 amount of piroxicam; X_2 amount of chitosan; Y_{EE} encapsulation efficacy; Y_5 percentage cumulative drug release at 5th hour; Y_6 percentage cumulative drug release at 6th hour

Table 2: Regression coefficients for the responses

Para meters	Coefficients of regression parameters							
	b_0	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2 -value	p-value
Y_{EE}	87.32	6.02	4.30	1.48	2.33	-0.26	0.9859	0.0056
Y_5	30.66	2.65	1.51	0.33	-1.11	-0.61	0.9927	0.0021
Y_6	84.61	6.39	9.19	-6.53	0.27	-3.89	0.9743	0.0137

Y_{EE}, encapsulation efficacy; Y_5, amount of the drug released at 5th hour, Y_6, amount of the drug released at 6th hour

Table 3: Results of analysis of variance for measured response.

Formula code	Observed Y_{EE}	Predicted Y_{EE}	Residuals	% Bias
F1	88.67	89.63	0.95	1.05
F2	86.85	87.32	0.47	0.53
F3	96.93	95.67	-1.26	-1.31
F4	83.67	82.76	-0.91	-1.09
F5	86.93	86.20	-0.73	-0.84
F6	90.93	91.37	0.44	0.48
F7	82.85	83.64	0.79	0.94
F8	80.61	80.55	-0.057	0.07
F9	100.90	101.19	0.29	0.28

Table 4: Observed and Predicted values with residuals of the response Y_{EE}

Parameters	Degree of Freedom	Sum Square	Mean Square	F-Value	P-Value
For Y_{EE}					
Regression	5	348.09	69.62	41.88	0.0056
Residual	3	4.49	1.66		
Total	8	358.07			
For Y_5					
Regression	5	59.35	11.87	81.67	0.0021
Residual	3	0.44	0.15		
Total	8	59.79			
For Y_6					
Regression	5	953.53	190.71	22.70	0.0137
Residual	3	25.20	8.40		
Total	8	978.73			

Results of ANOVA were depicted in table 3 and *in vitro* release profile of 9 runs was shown in figure 1**.** Low value (-1.31 to 1.05) of % bias for all batches showed good agreement between predicted and experimental values as shown in Table 4. To demonstrate the effect of the amount of piroxicam and chitosan, the response surface plots (Figure 2, 3 and 4) were generated for the dependent variables Y_{EE}, Y_5 and Y_6 using Design-Expert® 8.0.2.0 software (Stat-Ease Inc, Minneapolis).

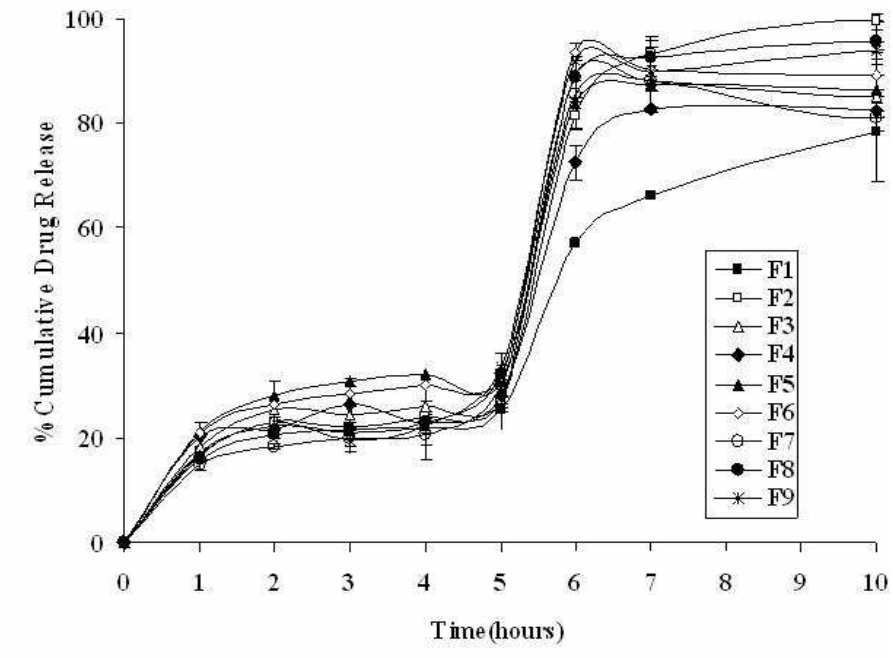

Fig. 1: Dissolution profile of piroxicam loaded double cross linked alginate-chitosan blend gel beads

Effect of Formulation Variables on Encapsulation Efficacy

The results of multiple linear regression analysis reveal that, on increasing the amount of piroxicam (X_1) and amount of chitosan (X_2) an increase in the release profile and an increase in the encapsulation efficacy (Y_{EE}) was observed.

In the equation 3, b_1 bears positive sign that indicates when increasing the amount of piroxicam (X_1) inceasing in the encapsulation efficacy (Y_{EE}). And b2 also bears positive sign in the same equation indicating increase in encapsulation efficacy with increased amount of chitosan

$$Y_{EE} = 87.32 + 6.02X_1 + 4.3X_2 + 1.48X_1X_2 + 2.33X_1X_1 - 0.26X_2X_2 \quad (3)$$

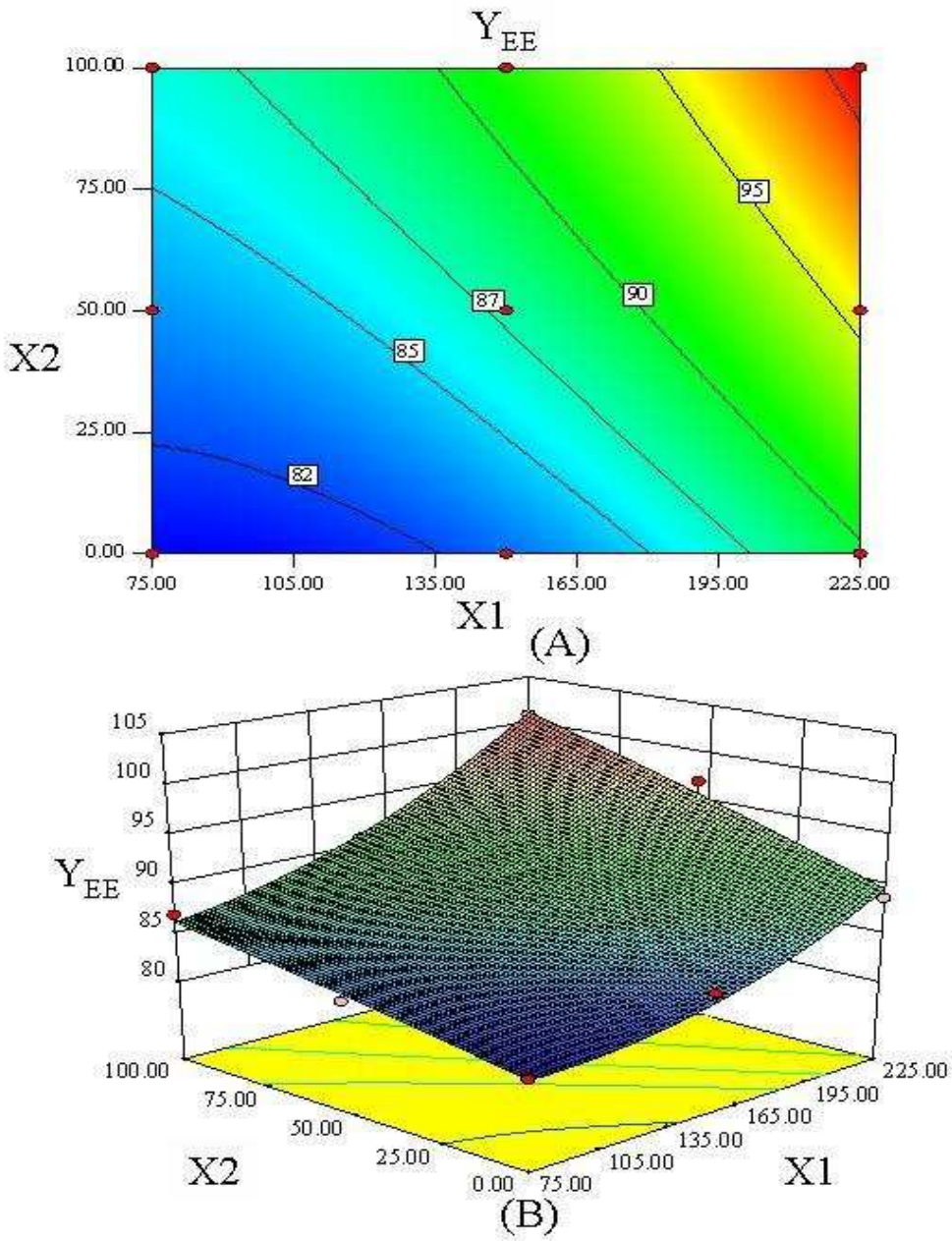

Fig. 2: Response surface plots showing effect of amount of piroxicam (X_1) and amount of chitosan(X_2) on encapsulation efficacy (Y_{EE}). (A) contour plot (B) 3D plot.

Effect of Formulation Variables on Release Profile

The results of multiple linear regression analysis reveal that, on increasing the amount of piroxicam (X_1), and chitosan (X_2) an increase in the release profile Y_5, Y_6 was observed.

The pulsatile drug release of double cross linked alginate chitosan blend gel beads at Y_6 was attributed due to change in pH of the medium from 4.5 to pH 7.4 phosphate buffer. However this was in accordance with earlier reports which suggest fast disruption of calcium alginate matrix in presence of phosphate ions [17, 18].

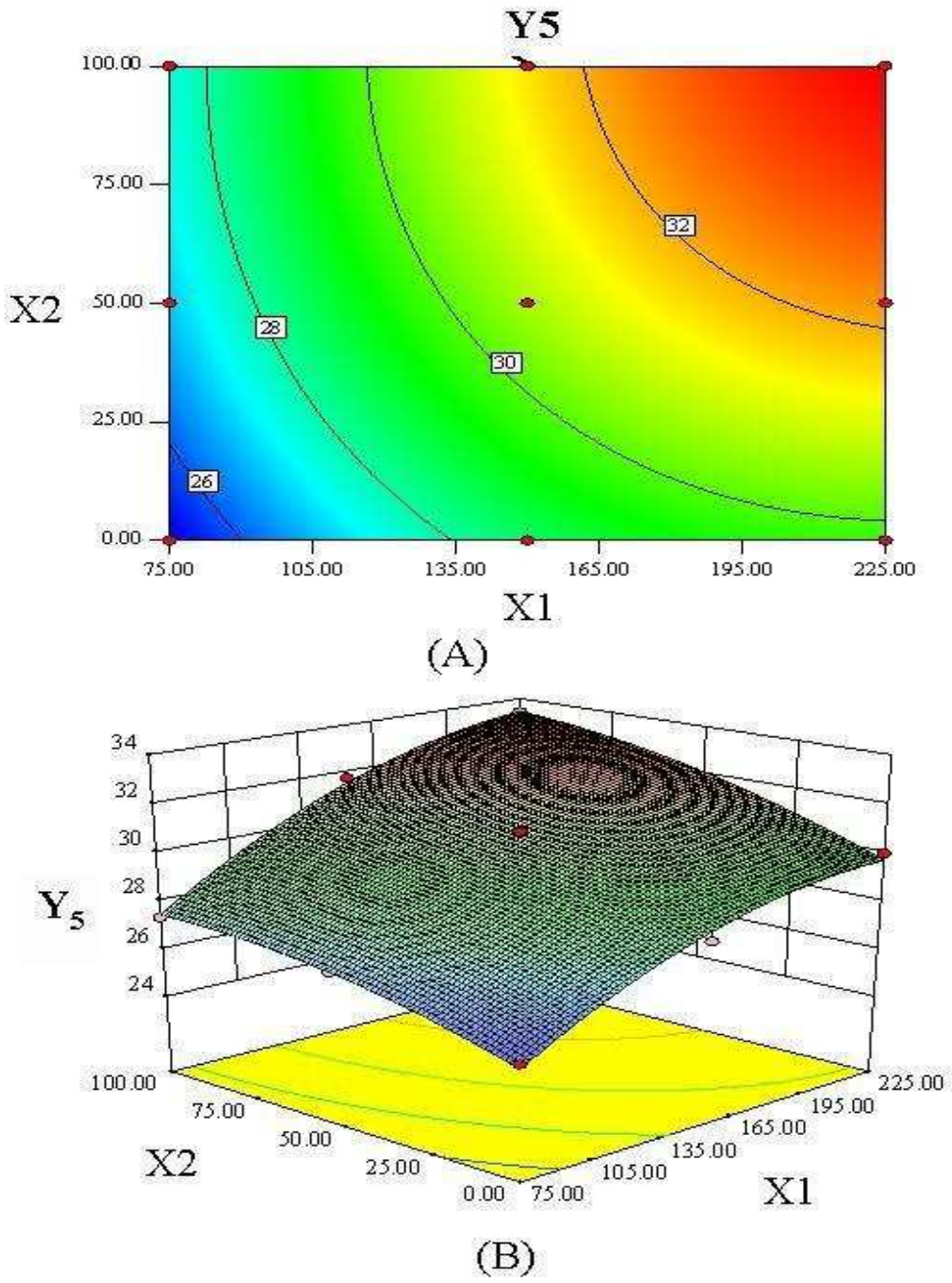

Fig. 3: Response surface plots showing effect of amount of piroxicam (X_1) and amount of chitosan (X_2) on piroxicam release at 5th hour (Y_5). (A) Contour plot (B) 3D plot

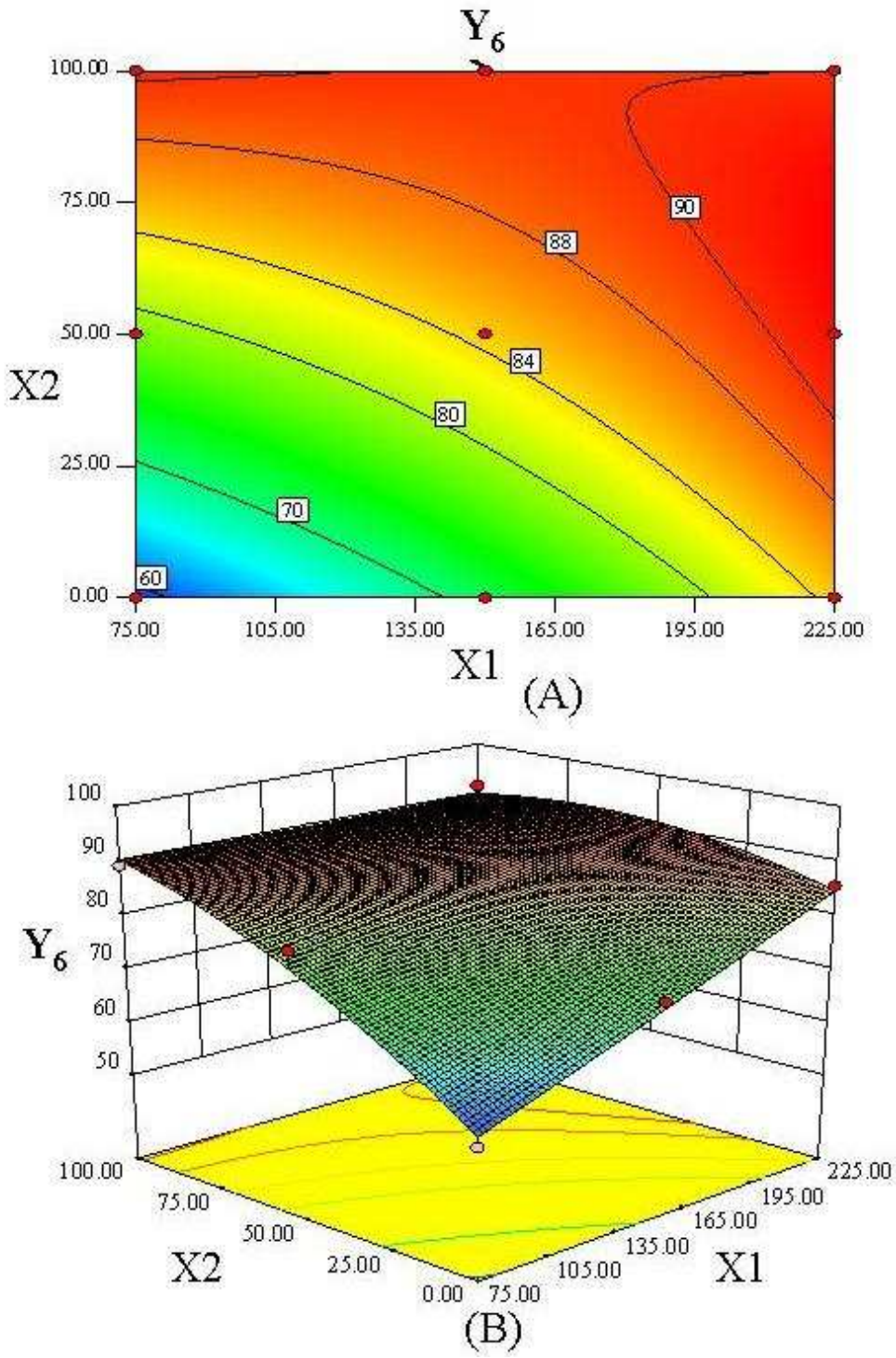

Fig. 4: Response surface plots showing effect of amount of piroxicam (X_1) and amount of chitosan (X_2) on piroxicam release at 6th hour (Y_6). (A) Contour plot (B) 3D plot.

In the equation 4 and 5**,** b_1 and b_2 bears positive sign indicating an increase in the amount of piroxicam (X_1) and amount of chitosan (X_2) an increase in release profile Y_5, Y_6 was observed

$$Y_5 = 30.66 + 2.65X_1 + 1.51X_2 + 0.33X_1X_2 - 1.11X_1X_1 - 0.61X_2X_2 \quad (4)$$

$$Y_6 = 84.61 + 6.39X_1 + 9.19X_2 - 6.53X_1X_2 + 0.27X_1X_1 - 3.89X_2X_2 \quad (5)$$

The relationship between dependent and independent variables was further elucidated using contour plots and 3D plots. The effect of X_1 and X_2 and their interaction on $Y_{EE,}$ Y_5 and Y_6 is given in Figures 2, 3 and 4. It could be seen that increasing the amount of drug and chitosan had a positive effect on Y_{EE} and $Y_{5,}$ Y_6.

Check point batch was also considered at the following levels and prepared at X_1 = -1 level and X_2 = -0.15, it is predicted that Y_{EE}, Y_5 and Y_6 value should be 66.20, 27.47 and 90.31 respectively (Table 5). The measured values were compared with predicted values by using student t-test and the differences were found to be insignificant ($p>0.05$). It clearly indicates that the statistical model is mathematically valid.

Table 5: Check point batch with predicted and measured values of Y_{EE}, Y_5, and Y_6.

Independent variables	Coded values	Dependent variables	Measured values	Predicted values
X_1	-1.00	Y_{EE}	64.88	66.20
X_2	-0.15	Y_5	28.22	27.47
-	-	Y_6	89.00	90.31

The particle size of the double crosslinked blend gel beads were in the range of 1212.40±122 to 1587.00±163 µm. The size of beads increased with increase in the amount of piroxicam and chitosan in the formulations. The % yield of beads was (90.37 to 99.39) shown for all 9 runs (Table 1). Batch F3 (X_1= 75 mg; X_2 = 100 mg) exhibited low Y_5 (27.34 %) and high Y_6 (89.16 %). Therefore, batch F3 was considered as promising formulation for targeting and pulsatile delivery of piroxicam in the colon. Thus the batch F3 was evaluated for physicochemical characterization viz SEM and FTIR.

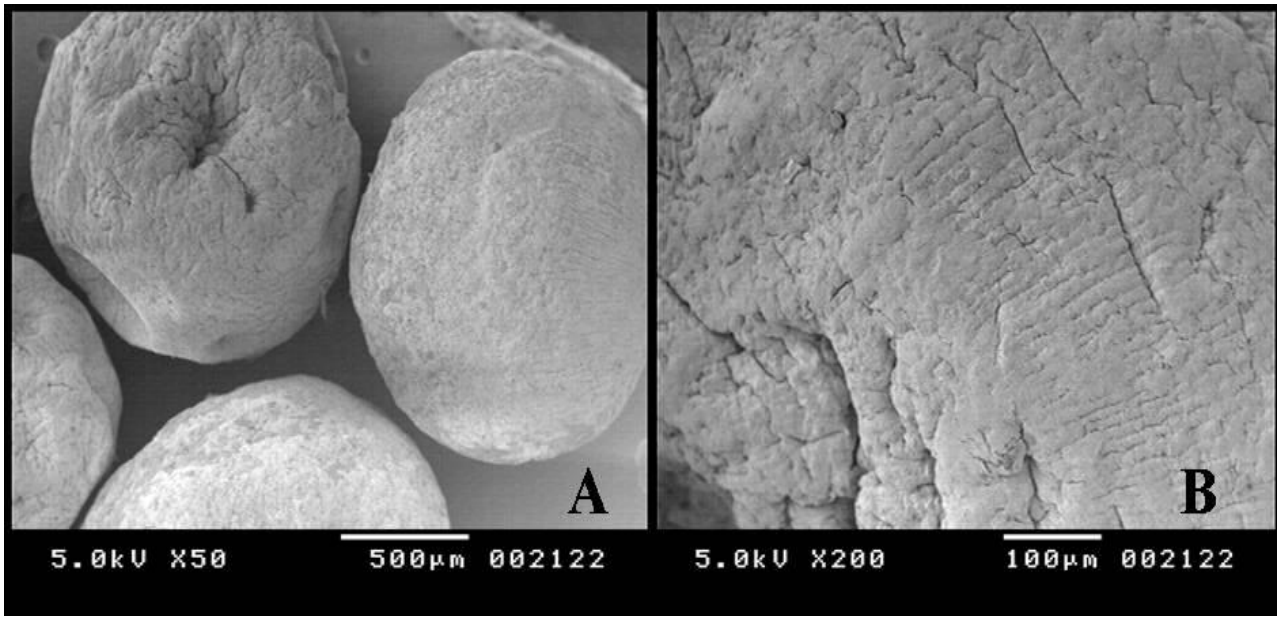

Fig. 5: Scanning electron microscopy photographs of alginate- chitosan double cross linked beads A) whole image B) surface photograph.

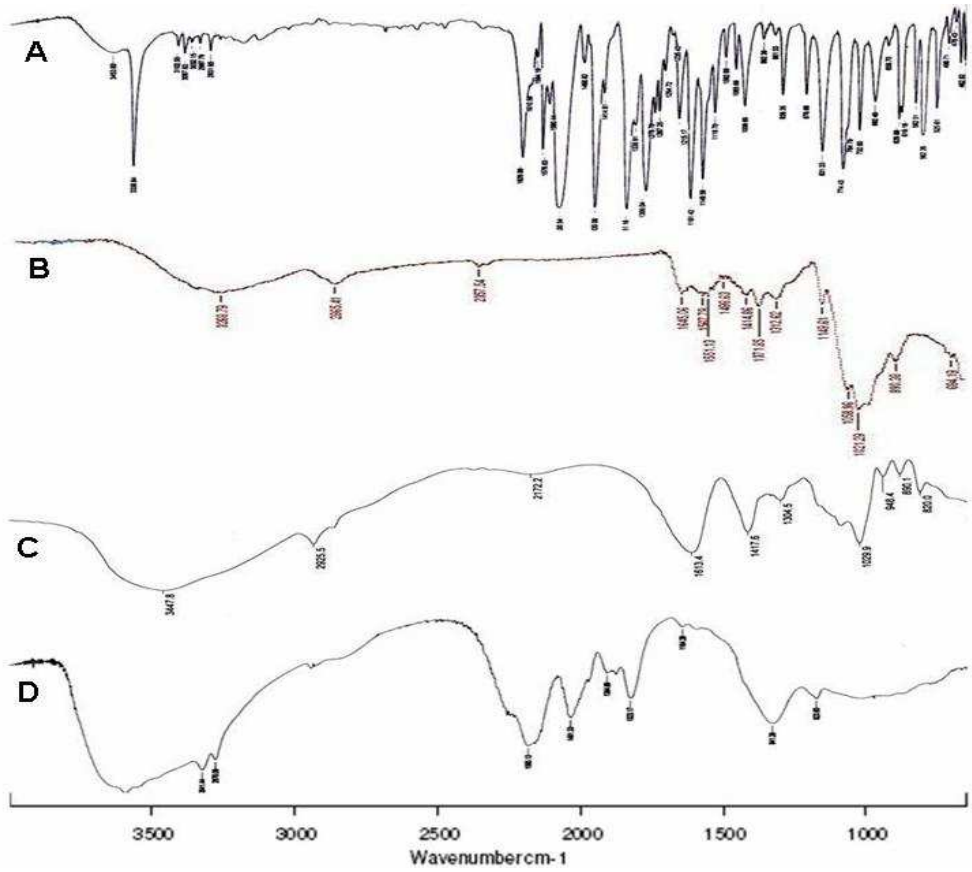

Fig. 6: FTIR spectra of (A) piroxicam (B) chitosan (C) sodium alginate (D) optimized formulation.

Scanning electron microscopy photographs of alginate chitosan beads are shown in figure 5. The photograph reveals that beads were nearly spherical with rough surface. FTIR analysis is commonly used to study the interaction or disappearance of peaks or shifting in their position gives an indication about the type of interaction such as hydrogen bonding [19]. FTIR spectrum of pure piroxicam showed strong absorption band at 3453 cm^{-1} (OH stretching) and 3338.4 cm^{-1} (-C=ONH stretching), 2931 cm^{-1}(–CH stretching), 1595 (-S=O stretching) and 1481 cm^{-1}(-C-N-S vibration). The spectra of sodium alginate and chitosan showed absorption peaks at 3447$cm^{-1}$and 3268 cm^{-1}respectively for –OH stretching. In final formulation, the absorption band due to –OH stretching of piroxicam completely lost which can be attributed to the formation of hydrogen bonding between carbonyl group of piroxicam with the hydrogen group of alginic acid or chitosan (figure 6)

CONCLUSION

This article discussed an application of optimization technique for the development of double crosslinked alginate-chitosan blend gel beads for colon specific drug delivery, in which amount of piroxicam and chitosan affected encapsulation efficacy (Y_{EE}), release profile (Y_5, Y_6). The resultant beads showed desired pulsatile release of piroxicam in colon by dripping an alginate – chitosan blend solution into calcium, transferring the calcium crosslinked gel beads into sodium sulfate.

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