OPTIMISATION AND CHARACETRISATION OF CHITOSAN MICROSPHERES OF ACECLOFENAC

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Abstract :

Purpose: The purpose of this study was to optimize and characterize the controlled release microspheres of Aceclofenac using "Box-Behnken experimental design". The microspheres were prepared by using the natural polymer chitosan in different ratios with glutraldehyde as the cross linking agent. Method: The microspheres were prepared by ionic cross linking technique. The microspheres were evaluated for particle size, drug content, drug loading efficiency, in vitro drug release surface morphology. Results and Conclusions: The particle size of the prepared microspheres was between 3 to 800µm in diameter. In conclusion NSAID controlled release delivery system utilizing natural polymer i.e; chitosan was successfully developed. Further parameters for dosage form designing can be identified for optimum formulation in terms of desirable long term stability and to study the therapeutic effects of these particles in vivo.

Key Words : Aceclofenac, Chitosan, Box-Behnken experimental design, microspheres

Introduction

The basic concept in the design of oral controlled release drug delivery is that the availability of drug depends on the kinetics of the drug release rather than drug absorption. Aceclofenac is a non steroidal anti-inflammatory drug (NSAID) used to relieve pain and inflammation in rheumatoid arthritis, osteo arthritis and ankylosing spondilitis. The shorter biological half life (4hrs) and dosing frequency more than once in a day makes Aceclofenac an ideal candidate for sustained release. However, its adverse effects are seen in patients with active or suspected peptic or duodenal ulcer or history recurrent peptic ulcer or who have GI bleeding or bleeding disorders. These concerns led to the preparation of Aceclofenac loaded microspheres for the controlled release application using natural polymers.

In the present study, Aceclofenac microspheres are prepared and evaluated using "Box-Behnken experimental design". The study also aims to statistically optimize the formulation parameters of Aceclofenac loaded microspheres for maximum entrapment and controlled release. Variables selected were Aceclofenac concentration (X_1), glutraldehyde concentration (X_2) and chitosan concentration (X_3). The response variables were the mean diameter (Y_1) and the entrapment efficiency (Y_2) of the microspheres. The levels for these variables were determined from the preliminary trials.

Factorial design is nothing but a guideline for experimental plan. For a given number of factors, it gives number of experiments to be carried out as well as quantities of variable ingredients to be used in the experiments. The basic principle is to change level of one factor keeping the levels of other constant. Once the design is framed, products are made according to these formulas. The responses obtained from the products are analyzed for the main and interaction effects. The results are analyzed for the significance of effects too.

In factorial designs, the optimization procedure is facilitated by fitting of an empirical polynomial equation to the experimental results. The equation constructed from a 2n factorial experiment will have 2n terms.

The generalized expression is,

$$Y= B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{123}X_1X_2X_3.$$

Here, Y is the measured response and X_1 is the level of *i*th factor. B_i, B_{ij}, B_{ijk}, etc. represent coefficients of factors and interaction terms computed from the responses of the experimental formulation. From eight formulations, eight responses or eight values of Y are obtained. If the low levels of all zero, all the terms except B_0 become zero.

Materials

Aceclofenac was obtained as a gift sample from Micro Labs, Puducherry, India. Chitosan was purchased from High Media Laboratories Pvt. Ltd. , Mumbai. Glutraldehyde and acetic acid were purchased from Ranbaxy Fine Chemicals Ltd, New Delhi. Paraffin oil and n-Hexane were purchased from RFCL limited. Span-80 was purchased from Rolex Laboratory Reagent.

Method

Preparation of Aceclofenac Microspheres

Chitosan solution of varying concentration (1, 2 and 3%w/v) was prepared in 1%w/v acetic acid. Aceclofenac (5, 10 and 15mg) was dispersed in 5ml of this solution and mix well. This mixture was added drop wise using a 22-gauge hypodermic syringe to the oily phase (100ml of paraffin oil containing 2%w/v of sorbiton sesquioelate) kept in stirring. Stirring was continued for 5min after the complete addition of chitosan solution to oil. Later 2.5ml of glutaraldehyde solution was added to the mixture with continues stirring at 250rpm. Glutaraldehyde (2.5ml) was added twice to the mixture, once after 1hr and then 2hrs with continues stirring. Stirring was stopped after 1hr of the final addition of glutaraldehyde. All batches were prepared atleast three times. The microspheres were isolated by vacuum filtration (0.45µm, PFTE membrane filter), washed with equal volume of n-hexane and freeze dried (Heto power dry LL 3000 lyophilizer). The full factorial design and layout with coded variables are shown in table4 [Akbuja, J., 1996].

Experimental Design

A Box-Behnken experimental design was employed to statistically optimize the formulation parameters of ACE microsphere preparation for maximum entrapment, optimum diameter and controlled release. The Box-Behnken design was specifically selected science it requires fewer treatment combinations than other design in cases involving 3 or 4 factors. The box-Behnken design is also rotable and contains statistical “missing corners” which may be useful when the experimenter is trying to avoid combind factor extremes. This property prevents a potential loss of data in those cases. Generation and evaluation of the statistical experimental design eas perfomed with the STATEASE, design expert, 7.0.3. a design matrix comprising of 17 experimental runs was conducted. An interactive second order polynomial was utilized to evaluate both the response variables:

$$Y= B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_1X_1^2 + B_2X_2^2 + B_3X_3^2.$$

Where B_0-B_3 are regression coefficients, X_1-X_3 are the factors studied and Y is the measured response associated with each factor level combination.

Physical characterization of microspheres

Particle size analysis:

Samples of the microparticles were analyzed for particle size by optical microscopy method using a calibrated stage micrometer for randomly selected samples of all the formulations**.**

Determination of drug content:

For determination of drug content 100mg of aceclofenac microspheres was powdered 50mg of powder was transferred to 100ml volumetric flask, dissolved in water and made volume to 100ml. The solution was kept for 1hr with occasional shaking and filtered through the whatman filter paper. The filtrate was collected and diluted with to sufficient amount of distilled water maintaining concentration of the drug with in the standard plot range. The diluted solution was analyzed for the aceclofenac content by UV-spectrophotometer (UV-1700 Shimadzu Corporation) at 274nm.

Drug loading efficiency: (DLE)

DLE was studied by dissolving microspheres in distilled water for 24hrs. The amount of drug loaded was determined by spectrophotometrically at 274nm.

$$DLE = \frac{\text{Experiential drug content}}{\text{Theoretical drug content.}} \times 100$$

***In vitro* drug release**

In vitro drug release studies were carried out for prepared microspheres. Accurately weighed 100mg of microspheres was taken for dissolution studies. Dissolution was carried out using USP apparatus type-2 (Paddle method) maintained at 37°c, 100 rpm; in phosphate buffer P^{H} 7.4. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release for measuring the absorbance spectrophotometrically at 274nm.

SEM analysis

Scanning electron microscopy was used to examine the surface morphology of microspheres. Dried microspheres were mounted onto stubs by using double-sided adhesive tape and vacuum coated with gold film using sputter coater and observed under scanning electron microscope [Costa, P., 2001].

Results and discussion

In the present work controlled release microspheres of Aceclofenac were formulated using chitosan polymer by ionic cross-linking technique.

For response surface methodology involving Box-Behnken experimental design, a total number of 17 experiments were performed for three factors at three levels each. Table No. 1 summarizes the experimental runs, their factor combinations and the levels of experimental units used in the study as well as the entrapment and mean diameter obtainted for each factor combination. In order to determine the maximum entrapment mathematical relationships were generated. For estimation of coefficients the least square regression method was used.

A suitable polynomial equation was selected based on several statistical parameters. The resultant equations for both responses Y_1 & Y_2 are

$$MD\ (Y_1) = 17.66+0.36X_1-0.80X_2+1.92X_3-0.41X_1X_2+0.69X_1X_3-2.98X_2X_3+0.85X_1^2-2.92X_2^2-1.54X_3^2$$

$$EE(Y_2)=77.25+1.62X_1-5.10X_2+8.88X_3+0.035X_1X_2+1.30X_2X_3-0.087X_2X_3-3.98X_1^2-3.63X_2^2-1.78X_3^2.$$

PRESS is a measure of the fit the model to the points in the design. The smaller the PRESS statistic is, the better the model fits to the data points. From the p-values in table No.1 it was concluded that cross product contribution (2FI) was not significant indicating the absence of interaction effect.

EE and MD of STP microspheres showed R^2 values of above equations to be 0.9317 &0.9892 (Table No. 2) respectively; indicating good fit and it could be concluded that second order model adequately approximated the true surface. For estimation of significance of the model, the ANOVA was applied using 5% significance level.

All the formulations prepared with in the experimental design yielded smooth spherical microspheres with size in the range of **10.01- 21.11µm**. ACE at medium level (X_1,0), Glutaraldehyde at low level (X_2,-1) and

chitosan at high level (X_3,+1) yielded microspheres with highest drug entrapment **85.46 & 19.02µm** mean diameter.

In table No.3, factor effects of Box-Behnken model associated p-values and standardized mead effects (SME) values for both responses are also presented. A positive sign indicates synergistic effect and negative sign represents an antagonistic effect of the factor on the selected response. The large SME indicated that the CC was the main influential factor on the drug entrapment as well of size of microspheres. This was further investigated by the study of ANOVA. In three dimensional plot effects of independent variables were presented by keeping one factor at constant level.

The particle size of microspheres can also be increased by increase in the concentration of polymer by increasing the crosslinking and matrix density of microspheres.

ACE concentration (X_1) also exerted positive effect on both responses but it was not significant at higher concentration of the drug. Although further increase in drug concentration might be increasing the viscosity of the droplet, it does not result in significant change in MD of microspheres.

Glutaraldehyde exerted a negative effect on the entrapment efficiency & negative effect on MD of microspheres as indicated by sign of coefficients in table No.3.

Using the model generated with both responses maximum entrapment of 85.46% and optimum diameter 7.29µm of formulation (ACE11) was identified.

In vitro release behavior of microspheres exhibiting maximum entrapment (ACE11) and pure drug (ACE) was investigated in phosphate buffer (P^H 7.4) for 24 hrs. An intial burst of 19.24% was observed in the first hour due to the drug located on or near the surface of microspheres. At the end of the 6^{th} hour the microspheres showed 40.23%drug release. The release rates were analysed by least square regression method. The release models such as First order model, Higuchi model and Ritger-peppas empirical model were applied to the released data.

Figure 1: SEM of ACE11 Chitosan microspheres

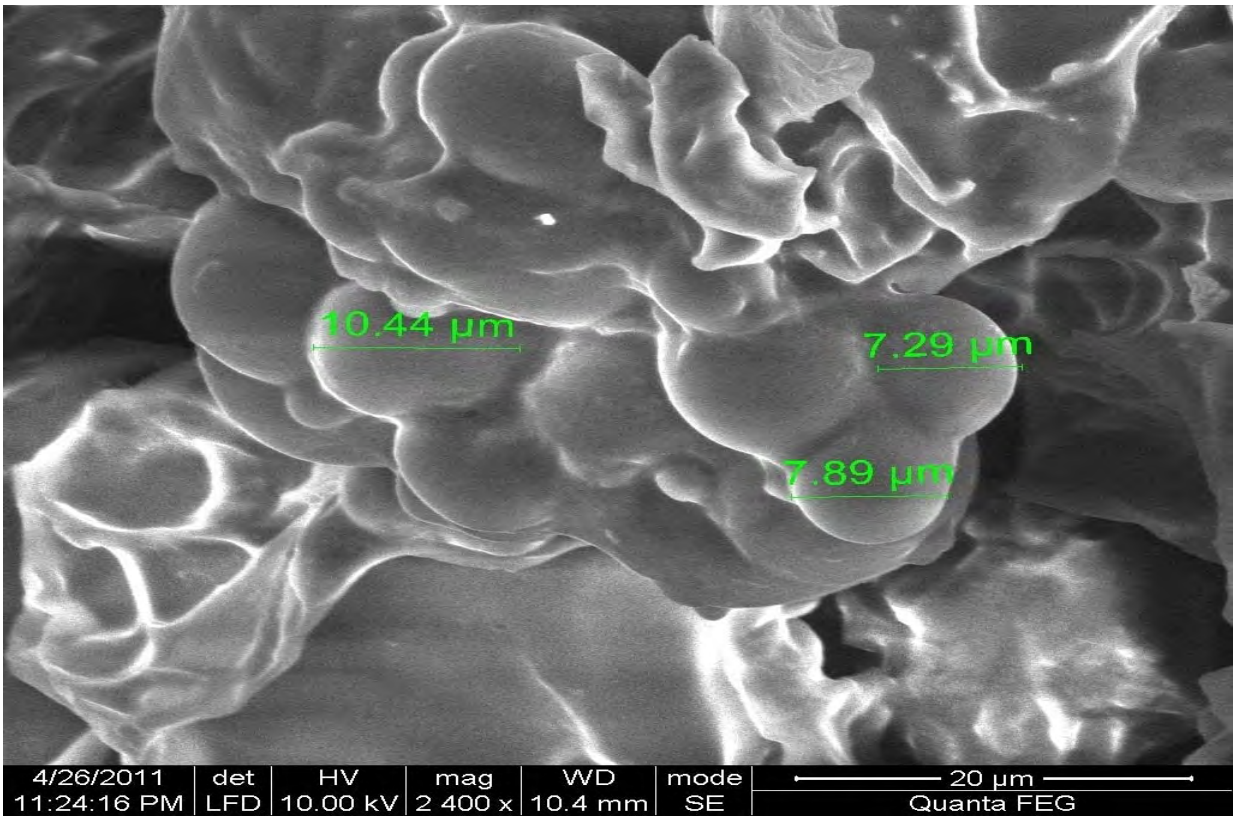


Fig.2 contour plot for the effect of selected variables (X_2, X_3) on mean diameter of microspheres

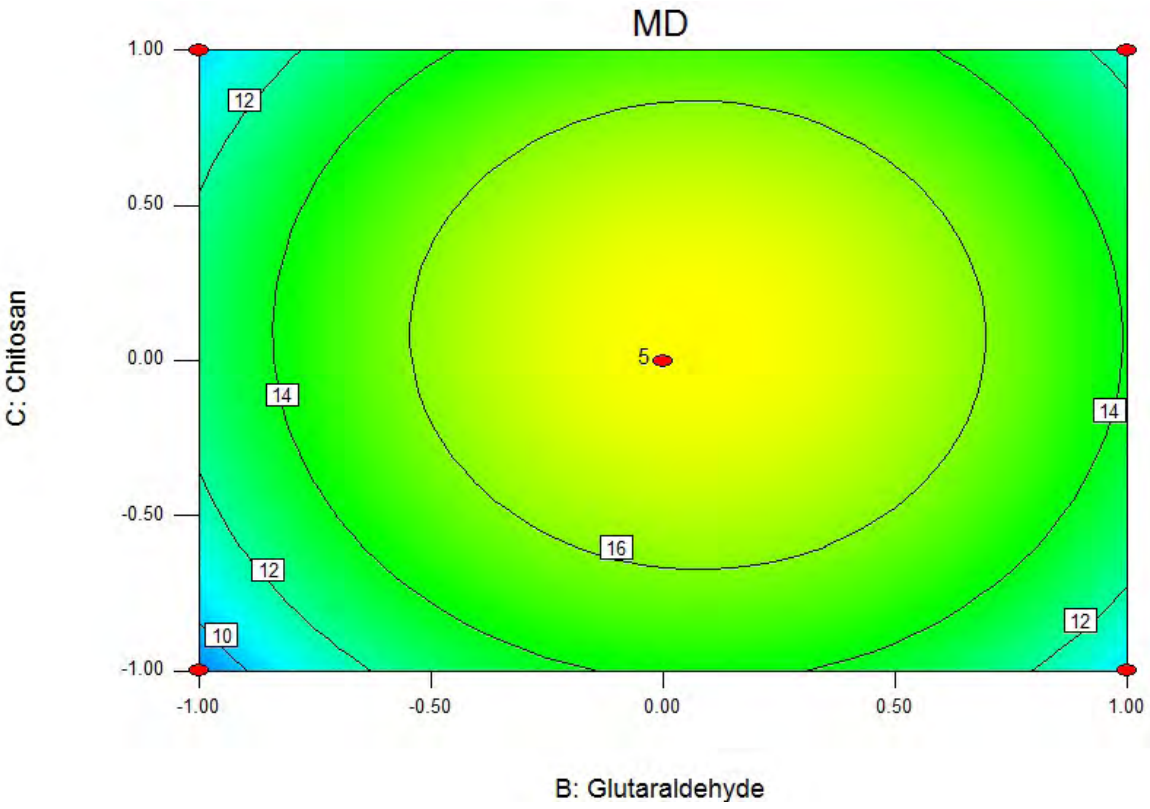


Fig.3 3D plot for the effect of selected variables (X_2, X_3) on mean diameterof microspheres

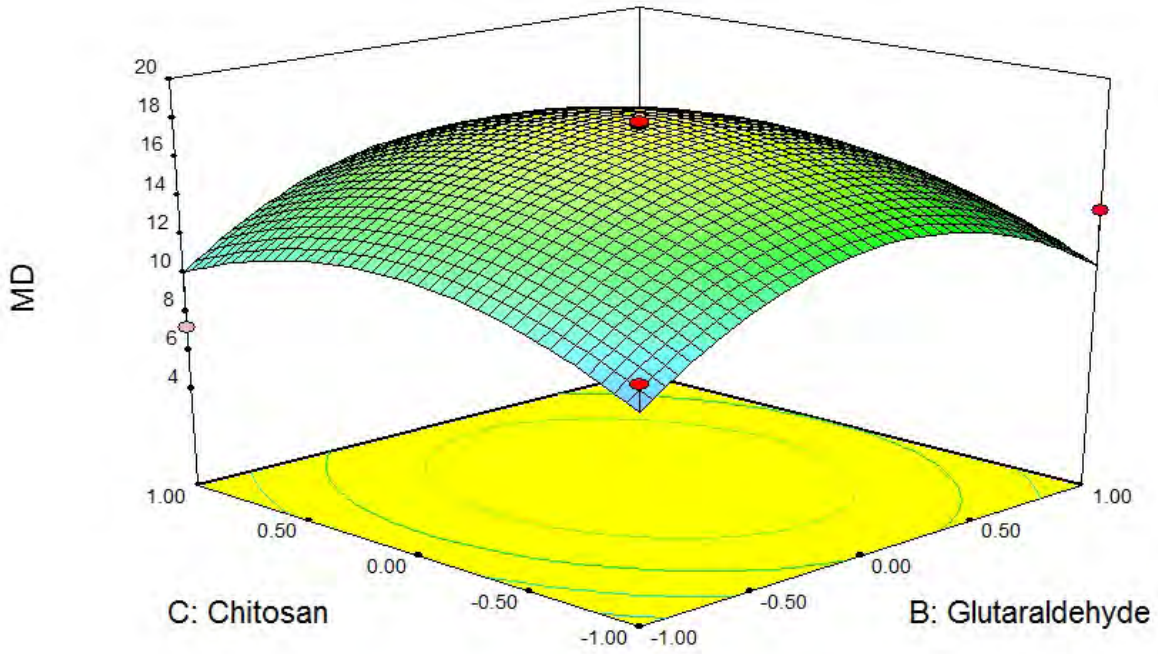


Fig.4 contour plot for the effect of selected variables (X_2, X_3) on entrapment efficiency of microspheres

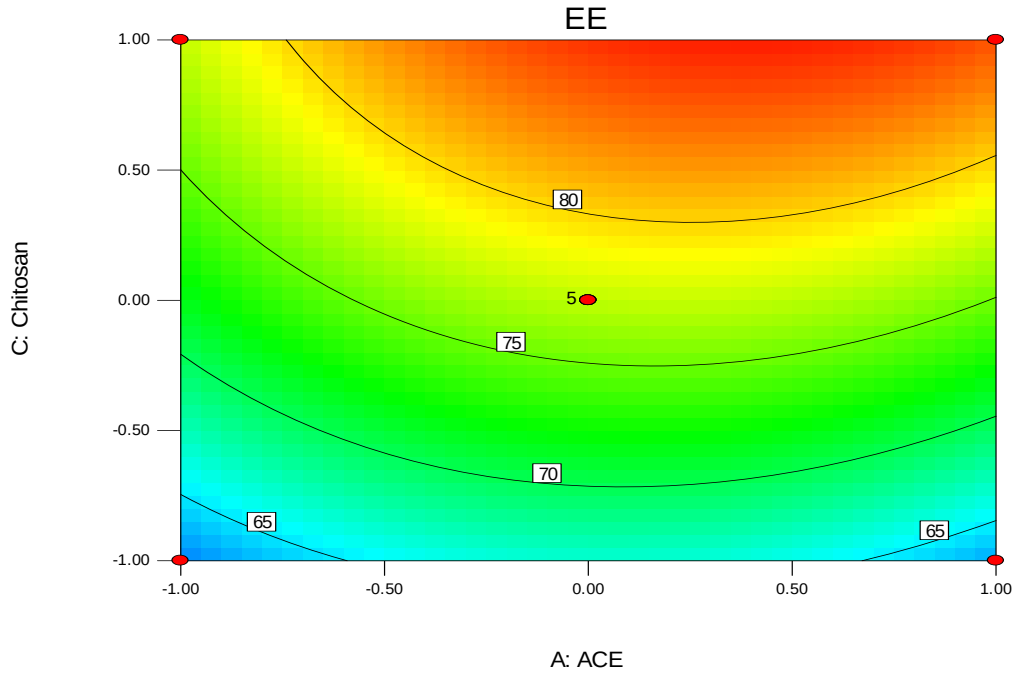


Fig.5 Intraction plot for the effect of selected variables (X_1, X_3) on entrapment efficiency of microspheres

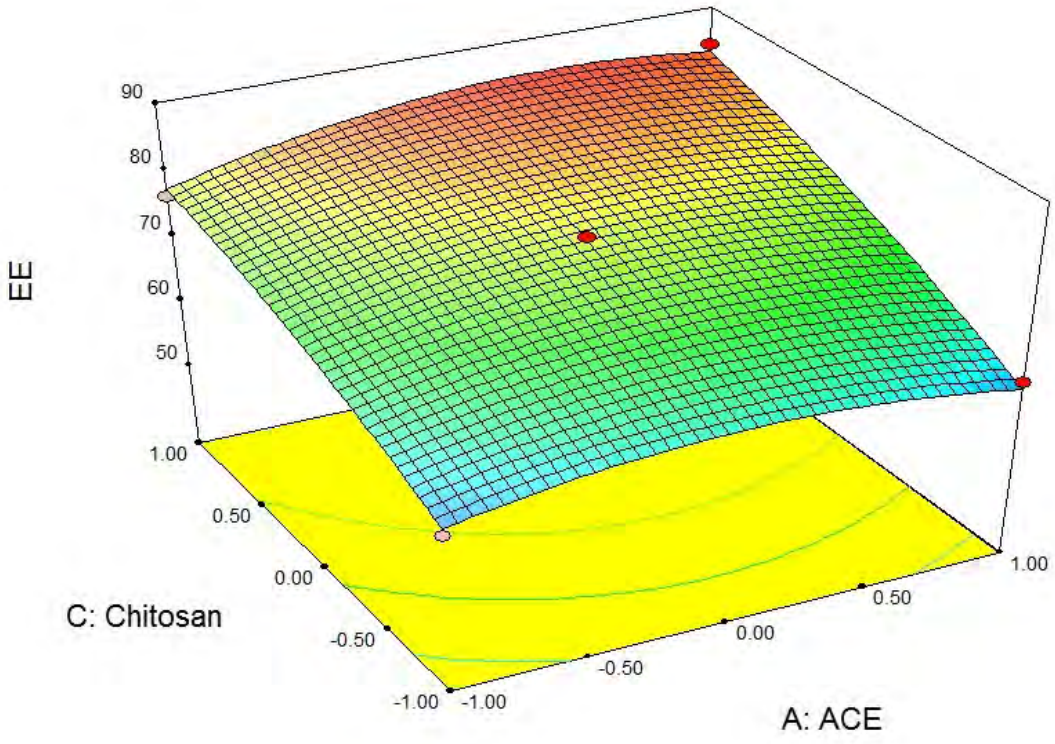


Fig.6 Contour plot for the effect of selected variables (X_1, X_2) on entrapment efficiency of microspheres

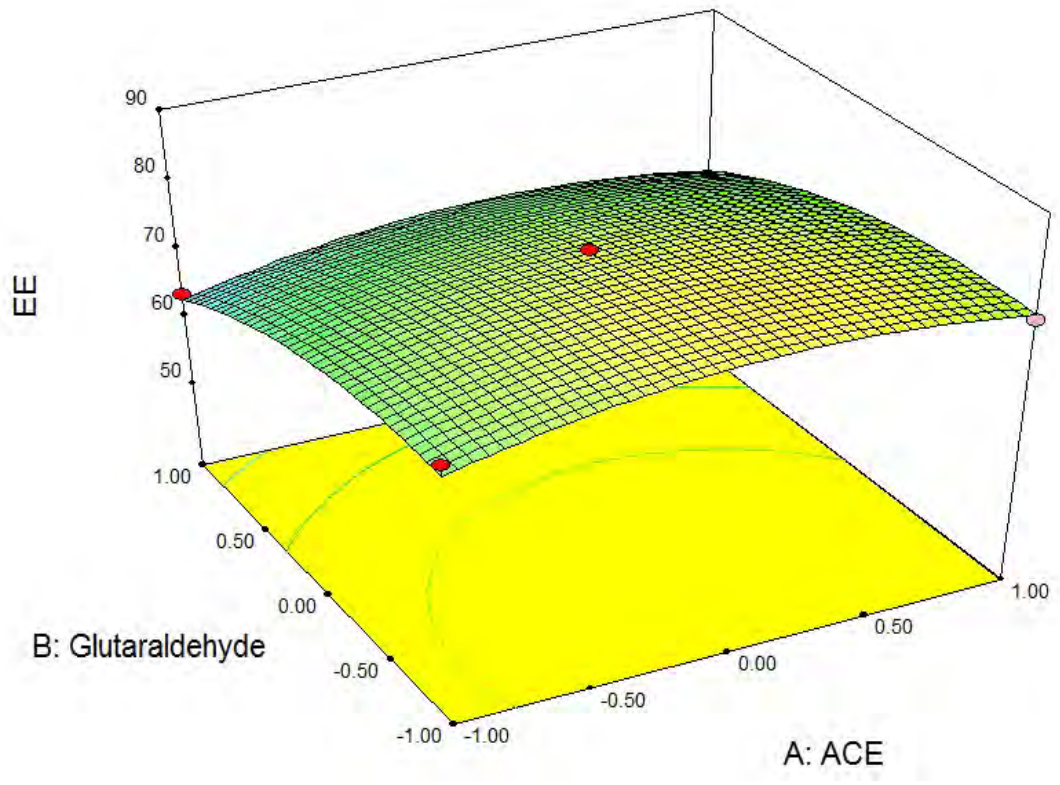
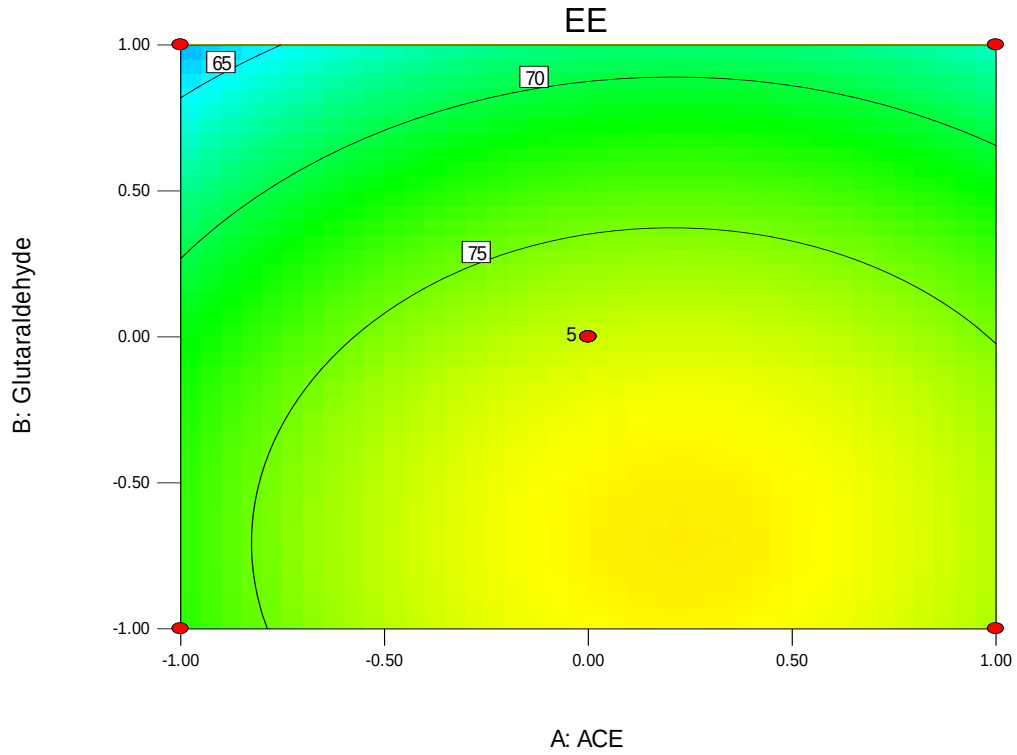


Fig.7 3D plot for the effect of selected variables (X_1, X_2) on entrapment efficiency of microspheres



ENTRAPMENT EFFICIENCY AND MEAN DIAMETER

Table No. 1 Box-Behnken experimental design layout with coded levels and actual values of variables

Formulation code	**X_1 ACE(mg)**	**X_2 Glutaraldehyde (%w/v)**	**X_3 Chitosan(%w/v)**	**Y_1 MD(µm)**	**Y_2 EE (%)**
ACE1	1.00	0	1.00	21.10	84.45
ACE 2	0.00	-1.00	-1.00	10.42	67.66
ACE 3	0.00	1.00	1.00	10	75.84
ACE 4	-1.00	0.00	-1.00	14.31	61.12
ACE 5	0.00	1.00	-1.00	13.35	58.39
ACE 6	1.00	1.00	0.00	15	64.72
ACE 7	1.00	0.00	-1.00	14.55	63.94
ACE 8	0.00	0.00	0.00	17.84	76.98
ACE 9	-1.00	0.00	1.00	18	76.43
ACE 10	1.00	-1.00	0.00	15.98	75.61
ACE 11	0.00	-1.00	1.00	7.29	85.46
ACE 12	0.00	0.00	0.00	17.79	77.58
ACE 13	-1.00	-1.00	0.00	15.36	74.62
ACE 14	0.00	0.00	0.00	17.46	77.13
ACE 15	0.00	0.00	0.00	17.74	77.61
ACE 16	-1.00	1.00	0.00	16.26	63.59
ACE 17	0.00	0.00	0.00	17.45	76.94

Table No. 2 Summary of results of a) model analysis b) lack of fit c) R-square analysis for measured responses

Source	**MD (Y_1)**		**EE (Y_2)**	
	Sum of sqares	**P>F**	**Sum of squares**	**P>F**
a)Model analysis				
Mean vs. total	4328.03	-	90165.72	-
Linear vs. mwan	35.69	0.2373	860.55	<0.0001
2FI vs. meam	38.14	0.1544	6.80	0.9331
Quadratic vs. 2FI	49.62	0.0030	149.60	0.0002
Cubic vs. quadratic	8.76	0.0005	10.69	0.0027
Residual	0.14	-	0.42	-
Total	4460.38	-	91193.78	-
b) Lack of fit				
Linear	96.52	<0.0001	167.09	<0.0001
2FI	58.38	<0.0001	160.29	<0.0001
Quadratic	8.76	0.0005	10.69	0.0027
Cubic	0.00	-	0.42	-
Pure error	0.14			
c) R-Square analysis	**Adjusted R^2**	**PRESS**	**Adjusted R^2**	**PRESS**
Linear	0.1011	188.26	0.7995	256.02
2FI	0.2926	242.15	0.7499	432.38
Quadratic	0.8464	140.31	0.9753	171.75
Cubic	0.9958	-	0.9984	-

Table No. 3 Regression analysis data and ANOVA for measured responses with SME of the factors on the responses and associated p-values

	Y_1 (MD)				Y_2 (EE)			
Coefficients	**Full Model**	**Reduced Model**	**p-value**	**SME**	**Full Model**	**Reduced Model**	**p-value**	**SME**
b_o	17.66	18.02	0.0026	0.51	77.25	77.25	<0.0001	0.56
b_1	0.36	-	0.4074	0.40	1.62	1.62	0.0083	0.45
b_2	-0.80	-0.80	0.0875	0.40	-5.10	-5.10	<0.0001	0.45
b_3	1.95	1.95	0.0019	0.40	8.88	8.88	<0.0001	0.45
b_1b_2	-0.41	-	0.5016	0.57	0.035	-	0.9573	0.63
b_2b_3	0.69	-	0.2682	0.57	1.30	-	0.0780	0.63
b_1b_3	-2.98	-2.98	0.0012	0.57	-0.087	-	0.8935	0.63
b_1^2	0.88	-	0.1600	0.56	-3.98	-3.38	0.0003	0.61
b_2^2	-2.95	-2.90	0.0011	0.56	-3.63	-3.36	0.0006	0.61
b_3^2	-1.51	-1.46	0.0303	0.56	-1.78	-1.78	0.0230	0.61
R^2	0.9317	0.8810	-	-	0.9892	0.9826		
Significance	0.0004	0.0006	-	-	0.0027	0.0001	-	-
F	10.61	16.29	-	-	71.16	94.0	-	-

In vitro dissolution profile

Table No.4 In vitro release profiles of ACE from ACE11 microspheres

S.No	**TIME(hrs)**	**%CDR**
1	0	0
2	1	19.24
3	2	22.79
4	3	28.62
5	4	36.04
6	5	38.67
7	6	40.23

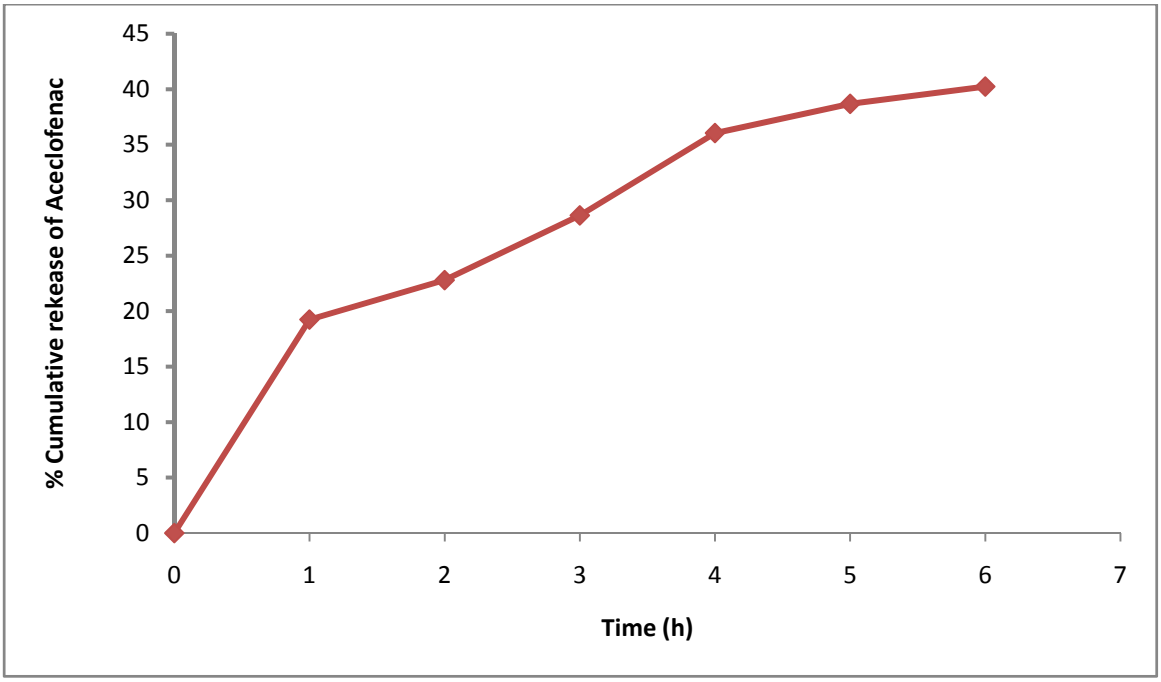


Fig8 *In vitro* release profiles of ACE from ACE11 microspheres

Table No. 6 Release behavior of ACE from ACE11 in phosphate buffer (P^H 7.4)

Release Kinetics	**Correlation Coefficient (R^2)**
First order equation	0.920
Higuchi (diffusion) co-efficient	0.989
Korsmeyer-peppas equation	0.961

CONCLUSION AND SUMMARY

In present study, "Box-Behnken experimental design" was employed to statistically optimize the formulation parameters (by changing the drug, polymer, cross linking agent concentration) of Aceclofenac loaded chitosan microspheres for maximum entrapment and controlled release.

The optimized formulation for Aceclofenac was obtained with 2%ACE concentration, 2%w/v Glutaraldehydre concentration and 3%w/v Chitosan concentration using response surface methodology based on Box-Behnken design. It was found that the observed responses were close to the predicted values for the optimized formulation. In conclusion NSAID controlled release delivery system utilizing natural polymer i.e. chitosan for Aceclofenac was successfully developed. Further parameters for dosage form designing can be identified for optimum formulation in terms of desirable long-term stability and to study the therapeutic effects of these particles *In vivo.*

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