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APPLICATION OF CENTRAL COMPOSITE DESIGN BASED ON RESPONSE SURFACE METHODOLOGY FOR OPTIMIZATION OF EXTENDED RELEASE ACECLOFENAC MICROPARTICLES

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ABSTRACT

The purpose of the present study was to develop a central composite design based on response surface methodology to optimize the process parameters for the formulation of extended release aceclofenac microparticles. The micro particles were prepared by emulsion solvent evaporation technique using ethyl cellulose as the polymer. Response surface methodology with three factors for five level design of experiments was employed to study the effect of independent variable stirring speed (X_1), viscosity of oil phase (X_2) and emulsifying agents (X_3. The dependent variables includes particle size (Y_1), drug entrapment efficiency (Y_2) and percentage of drug release(Y_3). High speed stirring and viscosity of oil phase results in decrease particle size with increased entrapment efficiency and stirring speed. The viscosity of oil phase has a positive impact on aceclofenac release over the period of 24hours. Drug-loaded micro particles exhibited the size ranging between 220.56 µm to 575.83µm with entrapment efficient ranging between 62.18% to 81.22%. and cumulative percentage drug release were ranging between 85.09% to 99.87%. The present study helped in predicting the optimized formula with excellent extended drug release. The results obtained, indicated that response surface methodology can be successfully used to optimized the formulation thereby reducing the number of trial time and cost of formulation development.

KEYWORDS: Microparticle, Aceclofenac, Ethyl cellulose, Response surface methodology, Optimization, Extended Release.

INTRODUCTION

In recent years there is tremendous progress and innovation in the field of Pharmaceutical technology with an increasing effort to develop prolonged release dosage forms. Extended release drug formulations have been used since 1960[1] to optimize therapeutic effect and safety of a drug at the same time improving the patient convenience and compliance.[2]

Microencapsulation is one of the techniques used to prepare extended release product. Microparticles can also offer advantages like minimizing fluctuations of drug concentration within therapeutic range, maintaining steady state concentrations, reducing side effects, decreasing dosing frequency and improving patient compliance and thereby providing better and safer therapeutic management.[3,4,5]

Ethyl cellulose (EC) is a hydrophobic and pH independent polymer and has been widely used in the preparation of extended release dosage forms.[6] The substance encapsulated in the microparticles is released under the influence of a specific stimulus at a specified stage whereas interaction between dissolution media, polymer and drug is the primary factors in release control.[7,8] Various formulation variables influence the drug release rate to greater or lesser extent.[9] Thus, drug entrapment and drug particle size have been shown to affect drug release from EC matrices.[10]

Aceclofenac is well tolerated COX-2 inhibitor and is often the drug of choice in the treatment of osteoarthritis, rheumatic arthritis and other related conditions.[11,12] However, because of its short half life (2-4 hrs) it requires dosing of 100 mg twice daily. Missing of a dose, which is often common, would cause inconsistent drug level in the blood, which would in turn reflect therapeutic outcome.[13] It has been reported that more than 50% of patients fail to take medicine as advised. Extended release formulations are the tools useful in promoting medication adherence and improve therapeutic outcomes. Medication adherence in chronic conditions like arthritis improves the quality of life of the patients.[14]

Aceclofenac, a widely prescribed anti-inflammatory and analgesic drug, belongs to class II drug under BCS and exhibits low and variable oral bioavailability due to its poor aqueous solubility.[15] It is practically insoluble in water and aqueous fluids. As such its oral absorption is dissolution rate limited and requires enhancement in solubility and dissolution rate for increasing its oral bioavailability.[16] As a consequence many Physico-chemical properties such as solubility, dissolution rate, stability and bioavailability can be favourably altered.[17]

Response surface methodology (RSM) is extensively practiced approach for the development and optimization of drug delivery device.[18] Based on the principle of design of experiments (DOES), the methodology include the use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to determine the optimum formulation(s).[19] Optimization through trial and error approach of the formulation for retarded release becomes a challenging, time consuming and expensive affair. Hence the use of systemic experimental design along with mathematical optimization is time saving and cost effective and more assures the formulation very significantly.[20]

The present study was aimed to develop and optimize aceclofenac loaded extended release microparticles using RSM. Further, microparticles of the drug would involve relatively more economical and less complicated technology than many other drug delivery devices. Computer-aided optimization technique, using a full factorial design, was employed to investigate the effect of 5 independent variables (factors) on particle size, encapsulation efficiency and drug release.

MATERIALS AND METHODS

Aceclofenac was purchased from sigma Mumbai, India; Ethyl cellulose was obtained from Loba Chemie Pvt. Ltd, Mumbai, India; Aerosil 200 (colloidal silicon dioxide) was obtained from Cobot Sanmer Ltd, Mumbai, India. Dichloromethane AR & Acetonitrile HPLC grade, Tween 80 (AR grade) was obtained from Qualigens, Mumbai, India and Potassium dihydrogen orthophosphate AR, Sodium chloride & Sodium hydroxide AR was obtained from SD Fine Chemicals, Mumbai, India. All the reagents used were of analytical grade and used without any further purification.

Experimental design

The Experimental designs involve planning experiments systematically in order to extract the maximum efficient information with the least number of experiments possible. The basic idea is to change all relevant factors simultaneously over a set of planned experiments and then connect and interpret the results using mathematical models.[21,22] The various steps of the experimental procedure are (i) Objective screening; (ii) Selecting of factors; (iii) Selecting of responses; (iv) Selecting of an experimental design; (v) Planning and execution of the experimental set-up, measuring the responses and generating mathematical response models; (vi) Calculating and interpreting the results by graphical and/or statistical tools; (vii) Desirability conclusions i.e., selection of significant factors or prediction of optimal condition.

First, it is necessary to define the *objectives* of the experimental design. i.e., whether it is a (i) screening design, in which the significant factors that influence the responses are identified, or (ii) optimization design, in which two or more significant factors are simultaneously optimized in order to find optimal experimental conditions.

The second step concerns the selection of *factors* which are usually made based on the literature search, preliminary experiments and instrumental limitations.

The third step is to choose a response. Generally, responses measured may be both the effective and retarded responses can be used.

An *experimental design* is then used to evaluate the influence of the selected factors. The choice of an experimental design ultimately depends on the objectives of the experiment and the number of factors to be investigated.

The next step is the *calculation and interpretation* of results. In case of a two level screening design, the factor effects i.e., the influence of the factor *X* on the measured response *Y*, can be calculated as:

$$E_X = \frac{\sum Y(+)}{n} - \frac{\sum Y(-)}{n}$$

Where, ΣY (+) is the sum of responses, where *X* is at the extreme level (+), ΣY (−) is the sum of responses, where *X* is at the extreme level (−) and *n* is the number of times each factor is at the (+1) or (−1) level. The estimated factor effects are then usually *statistically and/or graphically* interpreted, to determine their significance.[23]

Preparation of Microparticles

Aceclofenac microparticles with varying proportion of the ethyl cellulose containing aerosil as an inert dispersing carrier to improve the dissolution rate were made by emulsion solvent evaporation technique. Aceclofenac (0.2 g) was dissolved with ethyl cellulose (0.2-0.6 g) in a mixed solvent of acetone (4-6 ml) and dichloromethane (bridging liquid, 8-12 ml). Then, Aerosil (0.1-0.3 g) dissolved in organic solvent was dispersed in milli Q water containing tween 80 (0.08 - 0.15%) with stirring at 700 - 1100 rpm [medium duty mechanical stirrer (ROL 124, Remi Motors Ltd, Mumbai)] to get fine emulsion. Stirring was continued for 1 hour. The microparticles were recovered by

filtration, washed with distilled water, air dried and stored in a desiccator containing fused calcium chloride as desiccant.

In the preparation process of these studies aerosil was introduced in the microparticles formulation as an inert solid dispersing carrier to improve the dissolution rate of aceclofenac. Due to its large surface area, high porosity, and unique adsorption properties, Aerosil has been successfully used as a dispersing agent to increase the dissolution rate of sparingly soluble drugs. At the same time, aerosil as an effective anti adhesive agent and it could accelerate the solidification of droplets and be packed in the microparticles well. These suggested that the higher recovery of microparticle could be obtained comparing with other conventional methods of microparticles.

The selection of factors for optimization was based on preliminary experiments and prior knowledge from literature, as well as certain instrumental limitations. From preliminary experiments the key factors selected for optimization process were stirring speed (rpm) (X_1), viscosity of oil phase (mpa.s) (X_2) and amount of emulsifying agent (%EA)(X_3). It shows the levels of each factors studied for finding out the optimum values and responses (Table 1). The ranges of each factors used were stirring speed between (700 & 1100 rpm), viscosity of oil phase between (0.4 & 0.6 mpa.s) [Drug: polymer ratio between (1:1, 1:2, 1:3)] and emulsifying agent between (0.08 & 0.15%). They were used as response variables, particle size and encapsulation efficiency and release behaviours.

Table-1:Coded central composite rotatable design for three factors in five levels (3^5CCD)

Factors	Symbols	Factor levels				
		- α	**-1**	**0**	**+1**	**+ α**
Stirring speed(RPM)	X_1	600	700	900	1100	1200
Viscosity of oil phase (mpas)	X_2	0.40	0.50	0.80	0.65	0.90
Emulsifying agents (%)	X_3	0.10	0.08	0.11	0.15	0.17

In the present investigation, the important formulation factors were selected and optimized by a central composite design experiment carried out twenty (F1 to F20) various optimum conditions.

Evaluation of Aceclofenac Microparticles

Prepared aceclofenac loaded ethyl cellulose microparticles formulations were evaluated for % yield, entrapment efficiency, drug release and various physico-chemical properties.

Yield and Entrapment efficiency

Aceclofenac was extracted from micro particles with methanol after dissolving the micro particles in phosphate buffer pH 7.4. After suitable dilutions, the aceclofenac content was measured in a UV-VIS spectrophotometer (Lambda 25, Perkin Elmer and Germany) at 276 nm following the method.

Physical characterization of the microparticles

Surface morphology of the prepared micro particles was studied using scanning electron microscope[24] (JSM 5610 LV SEM, JEOL, Datam Ltd, Tokyo, Japan). Samples were prepared on 10 x 10 mm brass stub and coated with gold using sputter coater (Jeol auto fine coater, Japan) at accelerating voltage of 20 KV at high vacuum mode.

Particle size of aceclofenac micro particles were carried out using Malvern particle size analyzer.[25] (Malvern Instruments, UK). About 10 mg of microparticles was suspended in 5 ml of purified water and analysed with an obscuration index of about 10% (measure of amount of light lost due to introduction of sample against light path). Particle size distribution curves (Volume % vs. size) were recorded.

The angle of repose was determined by funnel method and was calculated using the following formula: $\theta = \tan^{-1}(h/r)$

Where, θ = angle of repose, h = height of heap and r = radius of base of the heap.

Drug release study

In vitro drug release from the aceclofenac microparticles was carried out in USP Auto Sampling Dissolution Test apparatus type I [Tablet Dissolution Tester, USP XVIII model, Electrolab, India] using 900 ml of phosphate buffer pH 7.4 maintained at 37 ± 0.5°C and 50 rpm. Microparticles, equivalent to 10 mg of aceclofenac were used for the study. 10 ml of the sample solution were withdrawn at predetermined time intervals to be set at Auto sampling monitor (viz. 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 hours). The samples were filtered through 0.45 µm membrane filter and analyzed in a UV-VIS spectrophotometer at 276 nm.[26] Auto Sampling Dissolution Test apparatus for using every time the test sample was withdrawn, it was replaced with an equal amount of fresh dissolution medium, maintained at 37°C.

RESULTS AND DISCUSSION

In this present investigation ethyl cellulose was used as a bonding and retarding agent in order to bind the Aerosil into micro particles and control the release rate. Since the polymer was easy to be precipitated as a fibrous aggregate or adhere to the equipment because of its high viscosity. In these studies slightly increase in the viscosity of oil phase by using Aerosil could be avoided

effectively due to the good adhesion property of Aerosil. And it was found that the micro particles having a good spherical shape were easy to form under strong agitation condition.

Particle size analysis

Particle size analysis was carried out as per the method above. All the analysis was carried out in triplicate and the average was taken for optimization study and ANOVA was performed. The results are shown in table 2.

Table 2: Evaluation of Aceclofenac loaded ethyl cellulose micro particles as per Central Composite rotatable design

Formulation Code	Factor levels					
	X_1 (RPM)	X_2 (VOP)	X_3 (EA %)	Particle Size	Drug entrapment efficiency	%Drug Release
F1	700.00	0.50	0.08	546.72	65.72	86.72
F2	1100.00	0.50	0.08	268.44	67.71	89.92
F3	700.00	0.80	0.08	539.19	65.81	85.72
F4	1100.00	0.80	0.08	249.44	79.61	91.55
F5	700.00	0.50	0.15	511.17	62.18	90.66
F6	1100.00	0.50	0.15	249.46	66.19	90.52
F7	700.00	0.80	0.15	575.83	64.65	85.85
F8	1100.00	0.80	0.15	228.32	76.33	95.62
F9	600.00	0.65	0.11	545.82	69.17	85.62
F 10	1200.00	0.65	0.11	220.52	81.22	99.87
F 11	900.00	0.40	0.11	291.96	69.59	89.05
F 12	900.00	0.90	0.11	268.33	79.31	91.96
F 13	900.00	0.65	0.10	302.88	73.62	92.85
F 14	900.00	0.65	0.17	267.32	74.22	87.18
F 15	900.00	0.65	0.11	262.48	79.80	92.80
F 16	900.00	0.65	0.11	262.48	79.80	92.80
F 17	900.00	0.65	0.11	262.48	79.80	92.80
F 18	900.00	0.65	0.11	262.48	79.80	92.80
F 19	900.00	0.65	0.11	262.48	79.80	92.80
F 20	900.00	0.65	0.11	262.48	79.80	92.80

Note: X_1, X_2, and X_3 represents the formulation variables stirring speed (RPM), viscosity of oil phase [VOP mpas], and the emulsifying agents [EA%], respectively.

The ANOVA result proved that the model for particle size was significant with the probability F value < 0.0001. The regression equation for entrapment efficiency was shown in Table 3. The positive interaction between the viscosity of oil phase and emulsifying agents shown in Figure 1. The predicted three dimensional response surface methodology graph was shown in Figure 2. The particle size distribution graph of selected (Factorial, centre point and Axial) formulated extended release aceclofenac micro particles as per experimental design shown in Figure 3. It was observed, the size of the micro particles was increased with an increase in the viscosity of oil phase the size of the micro particles was decreased with in increasing with stirring rate.

Table 3: Reduced response model for particle size and statistical parameters obtained from ANOVA (after backward elimination)

Response	Regression model	Adjusted R^2	Model P-value	Adequate precision
Particle size	$258.40-133.21X_1-1.62.02X_2 -15.67X_3-12.16X_1^1+ 5.15X_1^2X^2_2+8.79X_2^2X_3^{2+}70.42X_1^3+ 17.15X_2^3+31.16X_3^3$	0. 9489	<0.0001	14.276

Only significant coefficients with P < 0.05 are included. Factors are in coded levels.

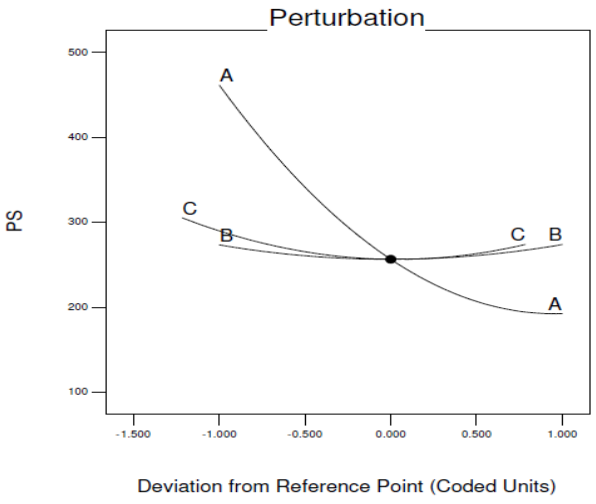


Figure 1: Perturbation plot- particle size (ps)

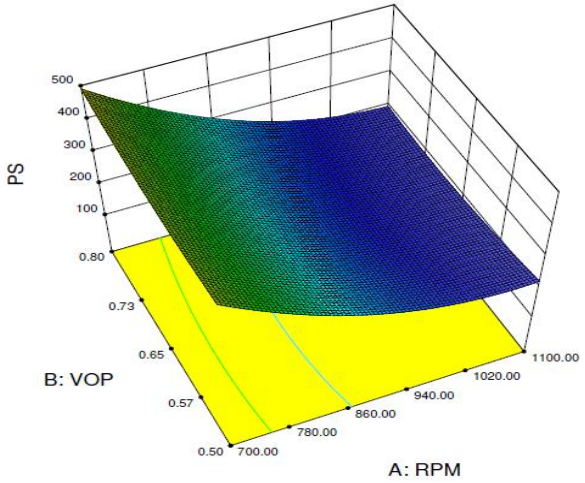
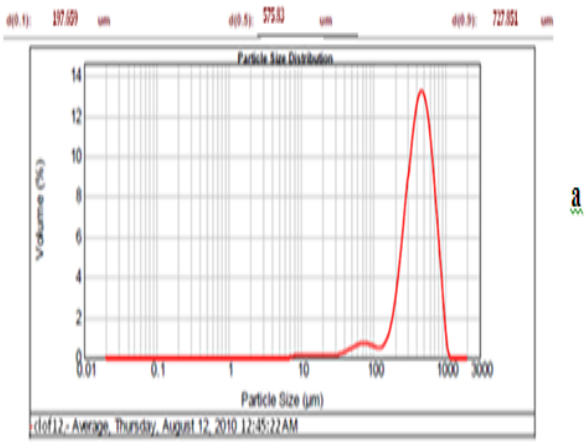


Figure 2: Predicted responses surface plot-Particle size (PS)



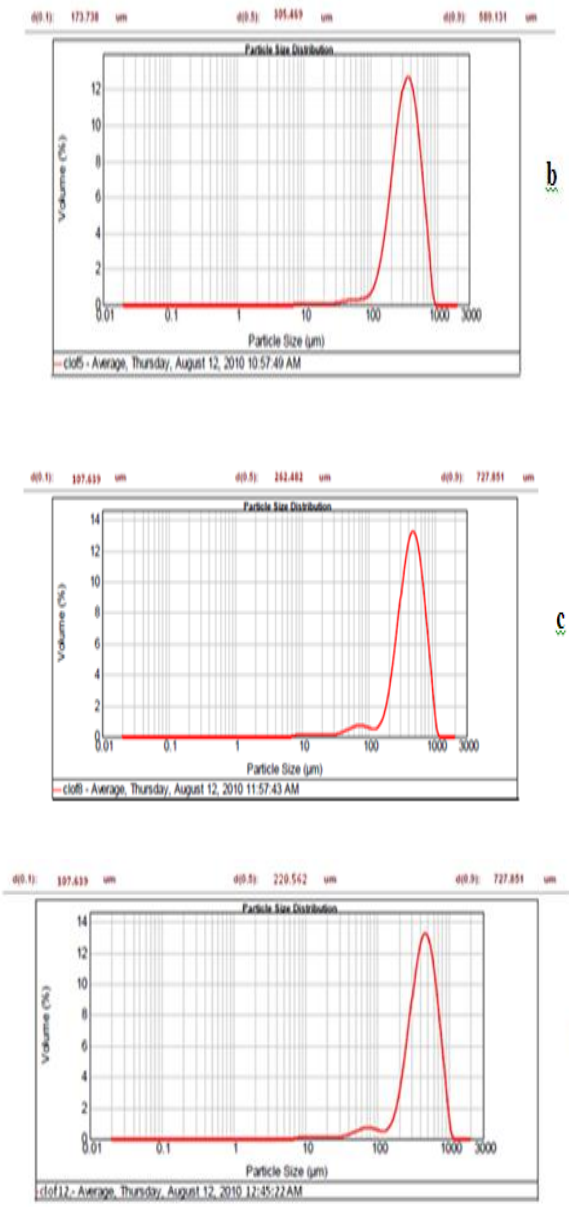


Figure 3: Particle size (µm) volume distribution curve of ethyl cellulose aceclofenac aerosil micro particles. (a) AECM(F7), (b) AECM (F15), (c) AECM (F10), (d) AECM(F13) Measured in a Malvern particle size

Drug entrapment efficiency

The percent drug entrapment efficiency for the prepared extended release aceclofenac microparticles was determined by the method as mentioned above. The microparticles of aceclofenac microparticles were analysed for the drug entrapment. The ANOVA result proved that the model for entrapment efficiency was significant with the probability F value < 0.0001. The regression equation for entrapment efficiency was shown in Table 2. The positive interaction between the RPM and drug polymer ratio shown in Figure 4. The predicted three dimensional response surface methodology graph was shown in Figure 5. The graphical representation of each formulated extended release aceclofenac microparticles entrapment efficiency shown in Figure 6.

Table 4: Reduced response model for particle size and statistical parameters obtained from ANOVA (after backward elimination)

Response	Regression model	Adjusted R^2	Model P-value	Adequate precision
Entrapment efficiency	$79.87+3.96X_1+3.00X_2-1.15X_3+2.44X_1X_2-2.67X_1^2-2.77X_2^2-3.88X_3^2$	0.8915	<0.0001	10.990

Only significant coefficients with P < 0.05 are included. Factors are in coded levels.

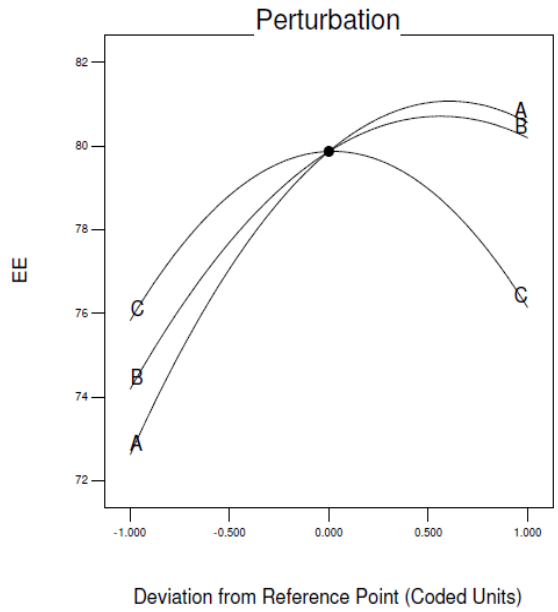


Figure 4: Perturbation plot - Entrapment efficiency (EE)

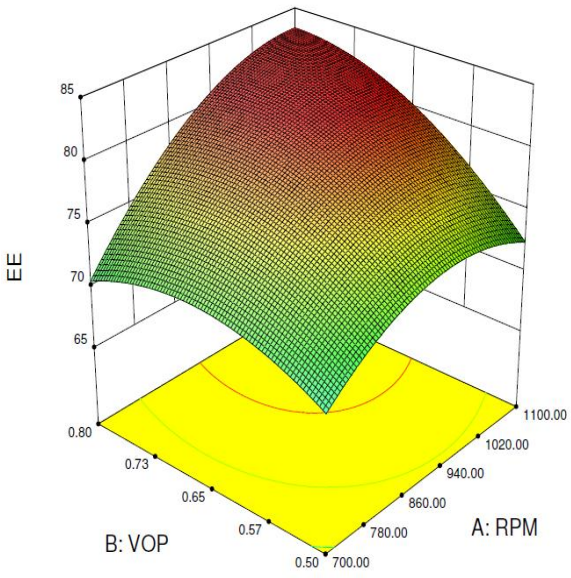
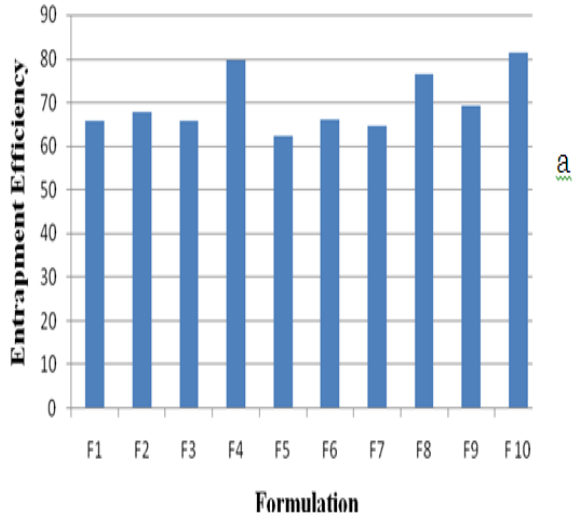


Figure 5: Predicted responses surface plot - Entrapment Efficiency



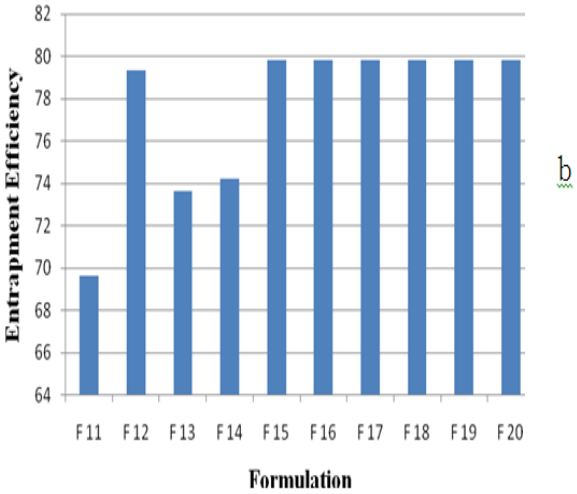


Figure 6: Graphical presentation of Entrapment efficiency (a) Formulation F1-F10 (b) Formulation F11-F20

***In vitro* drug release**

The *in vitro* drug release was studied as per the method mentioned above. The cumulative percentage drug release was calculated using the calibration curve in phosphate buffer pH 7.4 at 276 nm. The optimized microparticles of aceclofenac were filled in the capsule (size no: 0) and dissolution studies was carried out in phosphate buffer pH 7.4 for a period of 24 hours. The cumulative percentage drug release from the aceclofenac ethyl cellulose microparticles shown in Table 2. All the analysis was carried out in triplicate and the average was

taken for optimization study and ANOVA was performed. The results of ANOVA were presented in Table 5. The ANOVA result proved that the model for % drug release from microparticles was significant with the probability F value < 0.0001. The regression equation for drug release was shown in Table 5. The positive interaction between the RPM and viscosity of oil phase shown in Figure 7. The predicted three dimensional response surface methodology graph were shown in Figure 8. The graphical representation of each formulated extended release aceclofenac microparticles drug release shown in Figure 9.

Table 5: Reduced response model for drug release and statistical parameters obtained from ANOVA (after backward elimination)

Response	Regression model	Adjusted R^2	Model P-value	Adequate precision
Drug release	$92.96+3.22X_1+0.44X_2-0.66X_3+1.54X_1X_2+0.89X_1^2+1.98X_2^2-2.27X_3^2$	0.8554	<0.0001	13.290

Only significant coefficients with P < 0.05 are included. Factors are in coded levels.

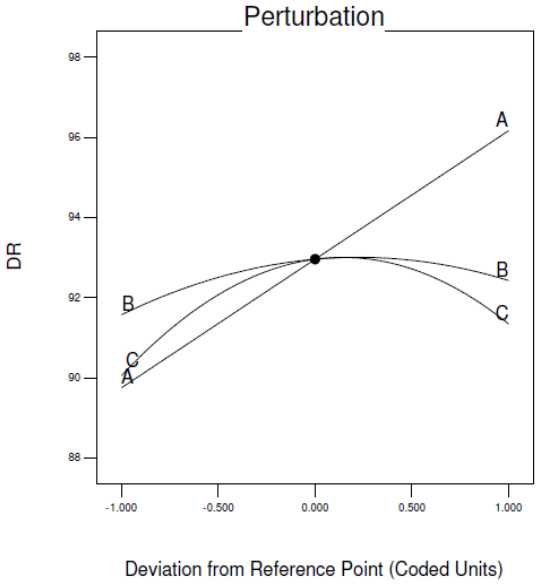


Figure 7: Perturbation plot- % Drug release (DR)

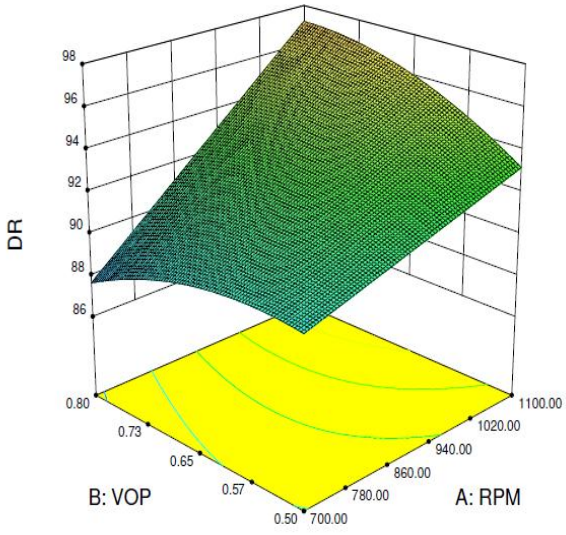
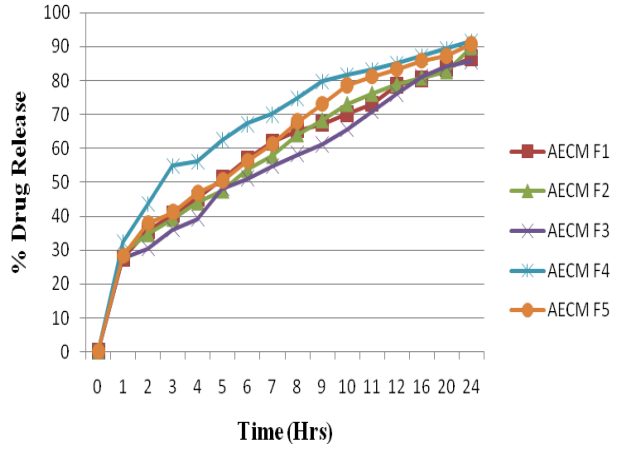
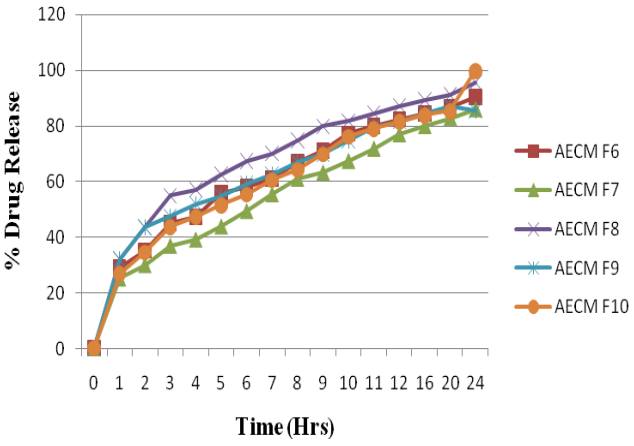
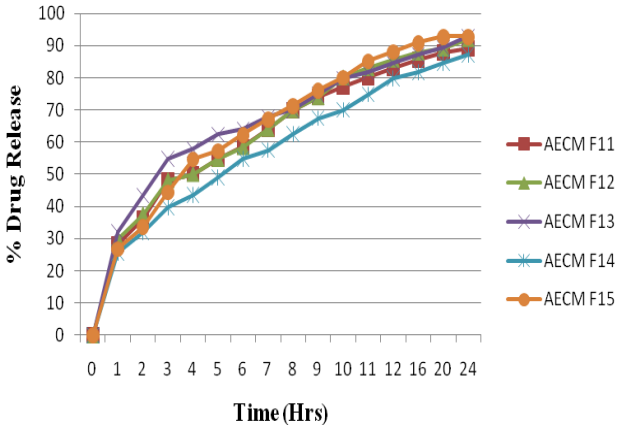


Figure 8: Predicted responses surface plot- % Drug release







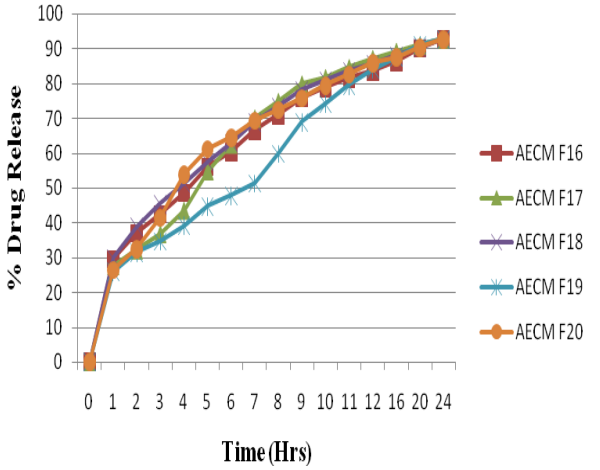


Figure 9: *In vitro* release profile of Aceclofenac Ethyl cellulose microparticle a) Formulation F1-F5, b) Formulation F6-F10, c) Formulation F11-F15, d) Formulation F16-F20.

Following the conditions and restrictions above, the optimization procedure was carried out. The response surface obtained for the global desirability function is presented in (Figure 10). The coordinates producing the maximum desirability value (D= 0.930) were stirring speed (rpm) of 1100, viscosity of oil phase (D:P ratio) 0.80 amount of emulsifying agent 0.12%. The predicted response values corresponding to the latter value of D =225.813 were: particle size encapsulation efficiency (% w/w) = 83.25 and drug release at (24hrs) =97.25. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition. The agreement between experimental and predicted responses for the predicted optimum is shown in (Table.6 and 7).

Table 6: Predicted optimization of aceclofenac loaded ethyl cellulose microparticle

RPM	VOP	EA %	Particle size	Entrapment Efficiency	Drug release	desirability	Design solution
1100.00	0.80	0.12	225.813	83.25	97.25	0.930	Selected

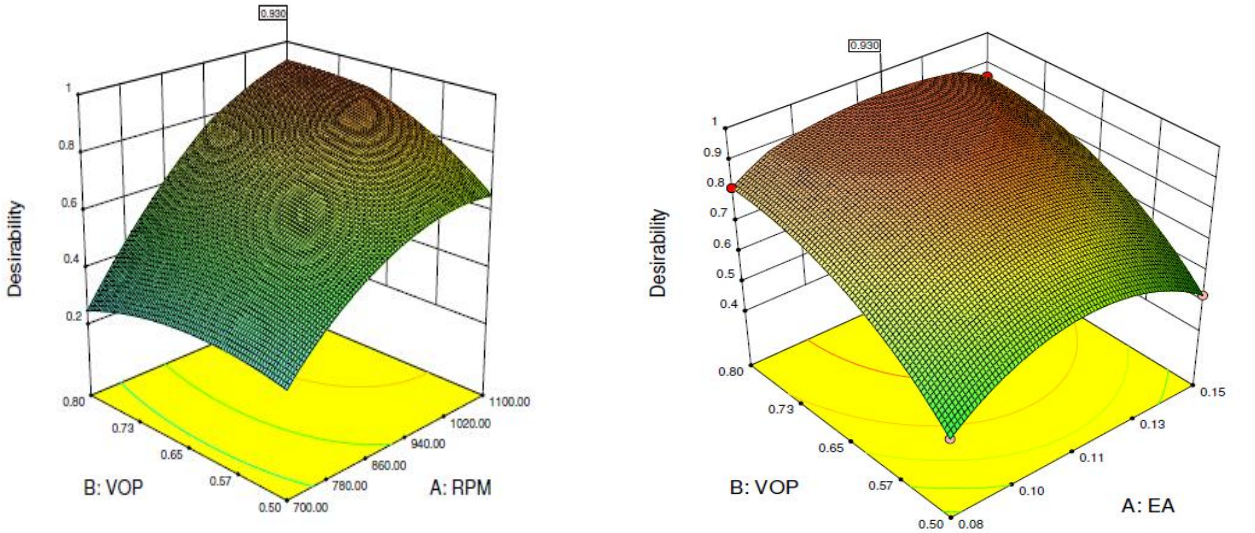


Figure 10: Graphical representation of the overall desirability function D. (a)RPM is plotted against VOP with factor of Emulsifying agent (EA) held constant at 0.12%(b).

Table 7: Composition of predicted optimized Aceclofenac ethyl cellulose micro particle (AECM) using aerosil as dispersing Agent

Formulation Ingredients	AECM
Aceclofenac (g)	0.200
Ethyl cellulose (g)	0.600
Aerosil (g)	0.100
Dichloromethane (ml)	14
Acetone (ml)	06
Aqueous Phase (ml) (mili Q water containing 0.12 % w/v Tween 80	150
viscosity of oil phase(VOP) mPas	0.8

Formulation of aceclofenac-loaded extended release microparticles was prepared as per optimum condition provided by experimental design methodology. Evaluation parameters of the prepared aceclofenac microparticle were studied.

CONCLUSION

Statistical validity of the polynominals was established on the basis of ANOVA provision in the Design Expert Software. Three-dimensional (3D) response surface plots and two dimensional counter plots were constructed based on the modal polynomial functions by using Design Expert software. These plots are very useful to see interaction effects on the factors on the responses. Twenty optimum checkpoint formulations for aceclofenac microparticles were selected by intensive grid search, performed over entire experimental domain, to validate the chosen experimental design and polynomial equation. To validate the model for particle size, Entrapment efficiency and drug release.

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