

Response Surface Methodology for the Optimization OF Ethylcellulose Microspheres

Rahul Nair* , Sevukarajan.M , K. Vishnu Priya ,Arun Kumar K.S.

Department Of Pharmaceutics,Sree Vidyanikethan College Of Pharmacy, A.Rangampet,
Tirupathi,Andhrapradesh, India

**Corres. author email: rahulnair2476@gmail.com
Mobile no: 91-09052039996*

Abstract: One of the major challenges in developing a highly effective experimental plan to optimize the design space of a manufacturing process is the highly complex nature of the pharmaceutical manufacturing processes. Response surface methodology (RSM) techniques are widely used in pharmaceutical research and are the method of choice for demonstrating robust process which is as per regulatory requirements. Microspheres were prepared by using ethyl cellulose as a polymer by solvent evaporation method. In this investigation 3^2 full factorial design was used to investigate the joint influence of two variables: the stirring speed (X_1), concentration of ethylcellulose (X_2), on the time for % of drug release after 12 hours and entrapment efficiency. Potential variables such as stirring time, volume of volatile solvent were kept constant in the experimental design. A statistical model with significant interaction terms is derived to predict the % drug release. The results of F statistics revealed that for obtaining controlled drug release, the microspheres should be prepared using relatively high stirring speed and high concentration of ethylcellulose. The model F values are found to be significant in nature. Response surface plots are presented to show the effect of X_1, X_2 on the % of drug release and entrapment efficiency. Acceptable batches were identified with the help of experimental design.

Keywords: Response surface methodology, Ethylcellulose microspheres, entrapment efficiency.

1. Introduction:

The microparticulate delivery systems include mainly pellets, microcapsules, microspheres, lipospheres, emulsions, and multiple emulsions. Microcapsules are micrometric reservoir systems and microspheres are micrometric matrix system¹. Microencapsulation is one of the novel methods for retarding or controlling drug release from dosage forms therefore minimizing the adverse effects thereby increasing the patient compliance. Generally, the micro particulate delivery systems are intended for oral and topical use. Different types of coated particles can be obtained depending on the coating process used. The particles can be embedded within a polymeric or proteomic matrix network in either a solid aggregated state oral molecular dispersion, resulting in the formulation of microspheres.

Experimentation is needed for development of pharmaceutical manufacturing processes. The requirement of pharmaceutical development is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product. One of the major challenges in developing a highly effective experimental plan to optimize the design space of a manufacturing process is the highly complex nature of the pharmaceutical manufacturing processes. Response surface methodology (RSM) techniques are widely used in pharmaceutical research and are the method of choice for demonstrating robust process which is as per regulatory requirements². RSM is a collection of mathematical and statistical techniques useful for the modelling and critical analysis of problems in which a response of interest may be altered by several variables and the objective is to optimize this response³⁻⁶. The methodology

includes the use of various experimental designs⁷⁻⁸, generation of polynomial equations, and mapping of the response over the experimental domain to determine the optimum formulation(s).⁹⁻¹⁰ As the experimentation procedure and time requirement is very less, thus it is more effective and cost-effective than the other conventional methods of dosage form formulating methods. Factorial designs (FD, full or fractional), also known as experimental designs¹¹⁻¹⁴ for the first-degree models, are the most popular response surface designs. Paracetamol a nonsteroidal anti-inflammatory drug with well established nociceptive properties and antiarthritic effect. In treatment of rheumatic pain large doses of the drug needs to be administered to the patient which leads dose dumping related toxicity. Microspheres containing ethyl cellulose are well established delivery systems for controlled drug delivery to reduce the frequency of dosing. In this study paracetamol is chosen as a model drug for optimising process for preparation of ethyl cellulose microspheres using solvent evaporation technique.¹⁵ Ethylcellulose (EC) is used widely as an enteric coating for tablets and capsules. Several researchers have investigated the use of EC as a polymer with non aqueous manufacturing vehicles to microencapsulate a drug by different methods.¹⁶⁻¹⁷ A factorial design based on response surface method was adopted to optimize microspheres of model drug paracetamol. A 3² full factorial design was employed to evaluate the combined effect of selected independent variables on the % drug release and encapsulation efficiency.

2. Materials and Methods:

Paracetamol was received as a gift sample from Fourrts India ltd. Ethyl cellulose, acetone were purchased from (Merck India ltd). All the other solvents and chemicals were of analytical grade and were used without further purification.

Experimental design of paracetamol loaded Ethyl cellulose microspheres

In the present investigation concentration of polymer ethyl cellulose & stirring speed were selected as independent variables and percentage drug release at the end of twelve hours and encapsulation efficiency were chosen as dependent variables. Amount of dispersion medium ie liquid paraffin and drug concentration were kept constant. Nine different batches formulations of microspheres were prepared by varying the stirring speed (300, 900, 1500 rpm) and polymer concentration(0.5g,1g,2g) and prepared microspheres were evaluated to determine the potential effect of those independent variables on dependent variables, ie ;drug release and encapsulation efficiency. The two independent formulation variables

evaluated Were X1: Ratio of drug: polymer and X2: Rate of stirring. The dependent variables investigated were Y1: Drug encapsulation and Y2. EC was dissolved in acetone and drug was dispersed in this solution with stirring for 20 minutes. The dispersion was poured into light mineral oil containing tween 80 (1.3%) and stirred for 5h at different stirring speed at room temperature to remove acetone completely by evaporation. The light mineral oil was decanted and the collected microcapsules were washed twice with n-hexane (100ml) at room temperature. The microcapsules were separated by filtration and air dried for 12h. The formulation and processing conditions were shown in table -1.

Evaluation of Microspheres¹⁹

Surface morphology: The microspheres were coated with gold vacuum at high voltage (800-1500V) using ion coater. Samples were examined with scanning electron microscope (LEICA S 440 i).

Micromeritic properties

The average particle size of the microspheres was determined by using an optical microscope. The flow properties and packing properties were investigated by measuring the angle of repose, tapped density and bulk density.

Drug entrapment:

Accurately weighed microspheres equivalent to 100mg of drug was dissolved in 25ml of 75% methanol and sonicated for 3 min. The solution was then filtered, diluted suitably and analyzed for drug content spectrophotometrically at 257 nm. The percentage drug entrapment was calculated as:

$$\% \text{ Drug Entrapment} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100.$$

Invitro Dissolution Studies

Dissolution test was performed in USP XXIII dissolution test apparatus by paddle method. 100 mg of paracetamol drug equivalent microspheres were placed in the dissolution apparatus and were evaluated for in vitro dissolution studies. The dissolution media used was 900ml of phosphate buffer pH 7.4 maintained at 37±0.5°C and rotated at 100 r/min. Aliquots samples were withdrawn at specified time intervals and replaced with same volume of fresh media, filtered and analyzed spectrophotometrically (Shimadzu 1600) at 257 nm for cumulative drug release

Statistical analysis: The effect of formulation variables on the response variables were statically evaluated by applying one-way ANOVA using Design-Expert® version 8 (Stat Ease, USA).

Stability Studies: The stability protocol was designed based on the ICH guidelines. The microspheres formulations chosen were stored at 30 ± 20 C and $65 \pm 5\%$ RH for a period of 12 months and at 40 ± 20 C and $75 \pm 5\%$ RH for a period of 6 months. The stored

samples were tested for their drug content and for any physical change. The testing was carried out at 0, 2, & 4 months for accelerated storage condition and at 3-month intervals for a period of 6 months for long-term storage condition²⁰.

Table1: Experimental design batches for Ethyl cellulose microspheres formulations with variable levels and response parameters.

Formulation code		F1	F2	F3	F4	F5	F6	F7	F8	F9
Variable level	X ₁	-1	-1	-1	0	0	0	+1	+1	+1
	X ₂	-1	0	+1	-1	0	+1	-1	0	+1
%DR (Q12)		95.06	69.97	64.18	86.25	65.79	62.93	68.65	63.59	59.41
EE		72.4	80.3	82.4	78.6	84.2	85.8	80.4	86.6	92.4
Coded values		Actual value								
		X1				X2				
-1		0.5				300				
0		1				900				
+1		2				1500				

X1-Polymer concentration (gm)

X2-Stirring speed (rpm)

Table No.2: Micromeritic properties of Ethyl cellulose Microspheres

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Particle size	126.1 ±04.6 µm	116.2 ±0.36 µm	118.4 ±0.16 µm	103.2±0 .12 µm	136.3 ±0.45 µm	108.1 ±0.85 µm	136.1 ±0.12 µm	129.1 ±0.45 µm	113.8 ±0.226 µm
Angle of repose	24.5 ⁰ ±0.35	28.2 ⁰ ±0.40	25.6 ⁰ ±0.25	30.4 ⁰ ±0.30	36.4 ⁰ ±0.20	32.15 ⁰ ±0.55	32.96 ⁰ ±0.15	38.4 ⁰ ±0.25	35.6 ⁰ ±0.20
Bulk density	0.363 ±0.12 g/cm ³	0.3 ±0.08 g/cm ³	0.375 ±0.06 g/cm ³	0.344 ±0.05 g/cm ³	0.421 ±0.03 g/cm ³	0.368 ±0.05 g/cm ³	0.456 ±0.02 g/cm ³	0.32 ±0.01 g/cm ³	0.34 ±0.05 g/cm ³
Tapped density	0.5 ±0.12 g/cm ³	0.8 ±0.06 g/cm ³	0.6 ±0.04 g/cm ³	1.25 ±0.02 g/cm ³	1.29 ±0.06 g/cm ³	1.5 ±0.08 g/cm ³	1.52 ±0.03 g/cm ³	1.7 ±0.04 g/cm ³	1.25±0.05 g/cm ³

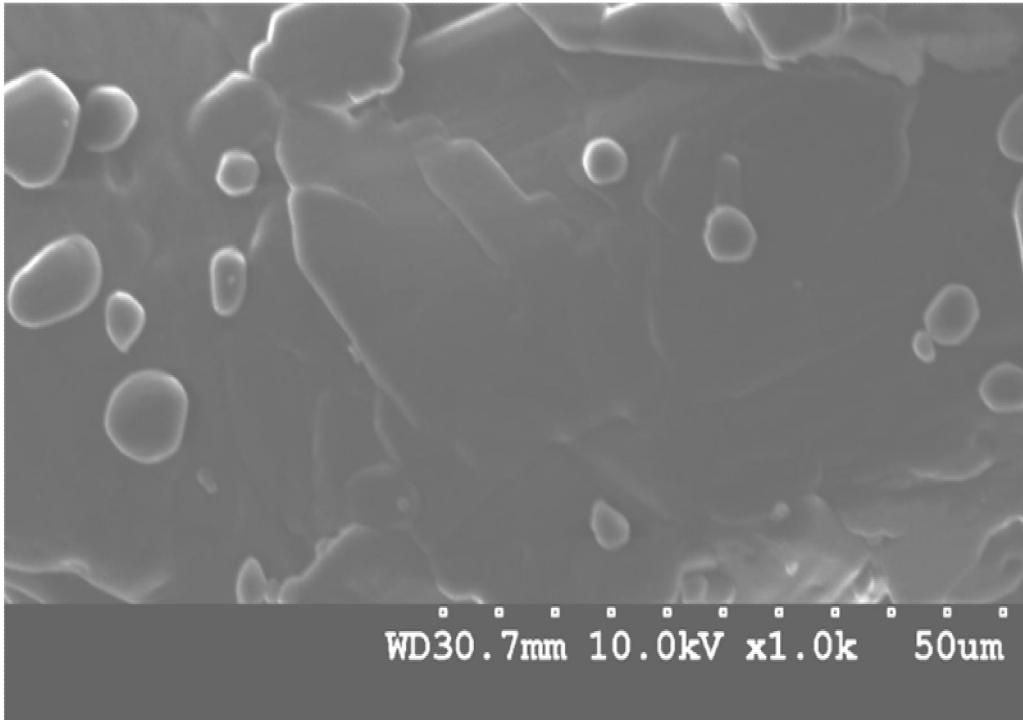


Fig no.1 SEM ANALYSIS : The sem analysis photographs of ethylcellulose Microspheres

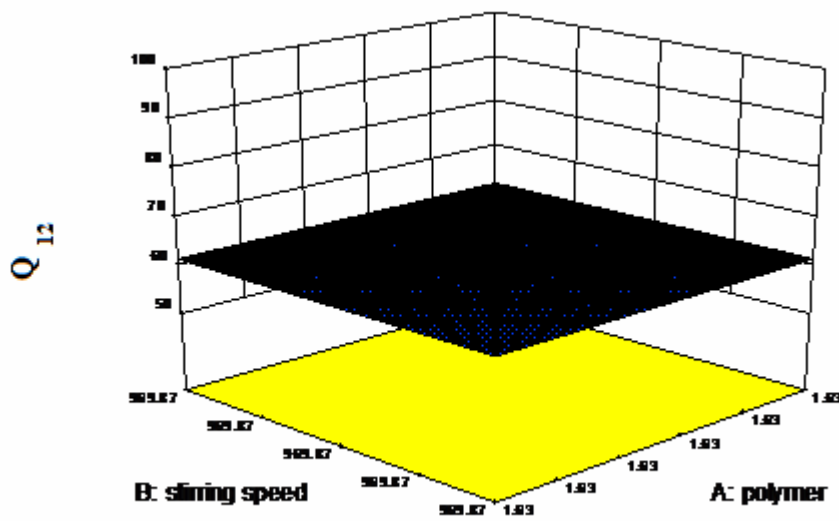


Fig:2 Response surface plot showing the effect of amount of Polymer (X1) and r/min (X2) on the response % Drug release in 12 hours (Y1).

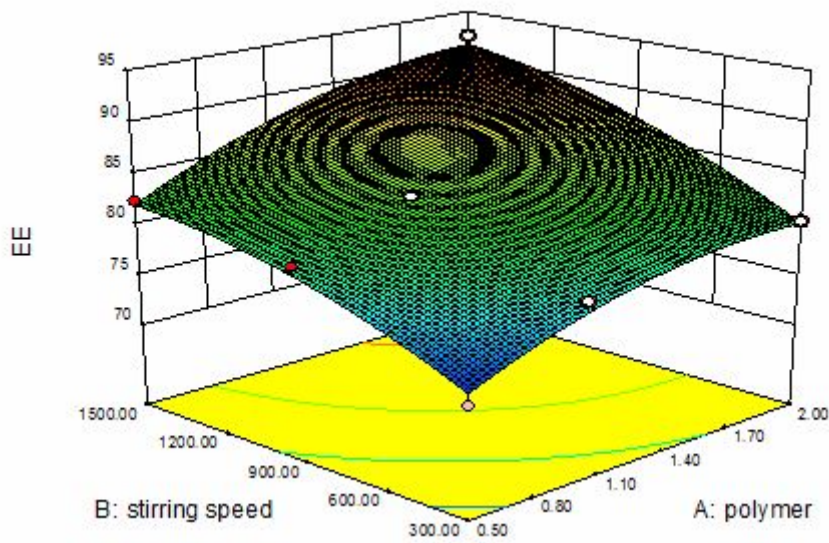


Fig:3 : Response surface plot showing the effect of amount of Polymer (X1) and r/min (X2) on the response Drug entrapment (Y1).

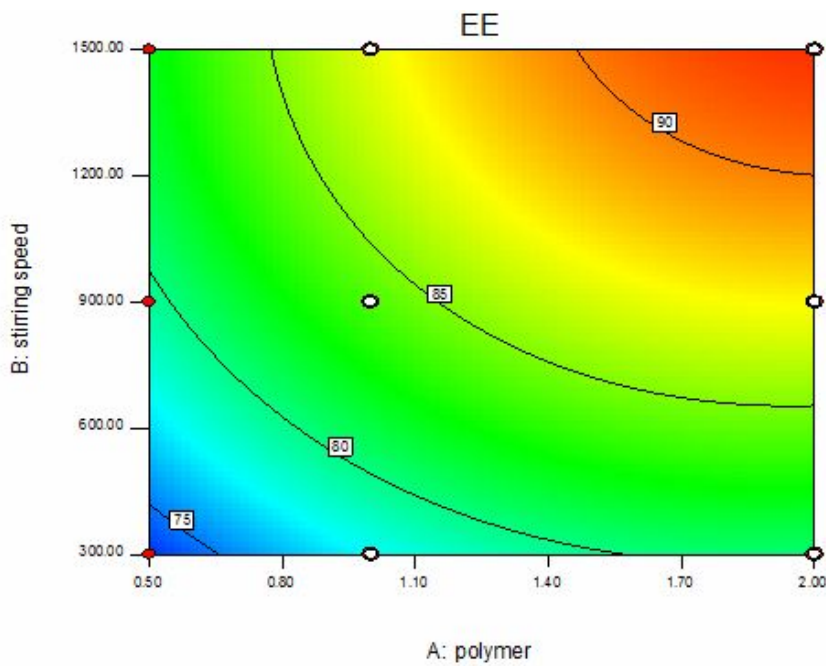


Fig4: Counter plots showing the effect of polymer concentration and stirring speed on the encapsulation efficiency

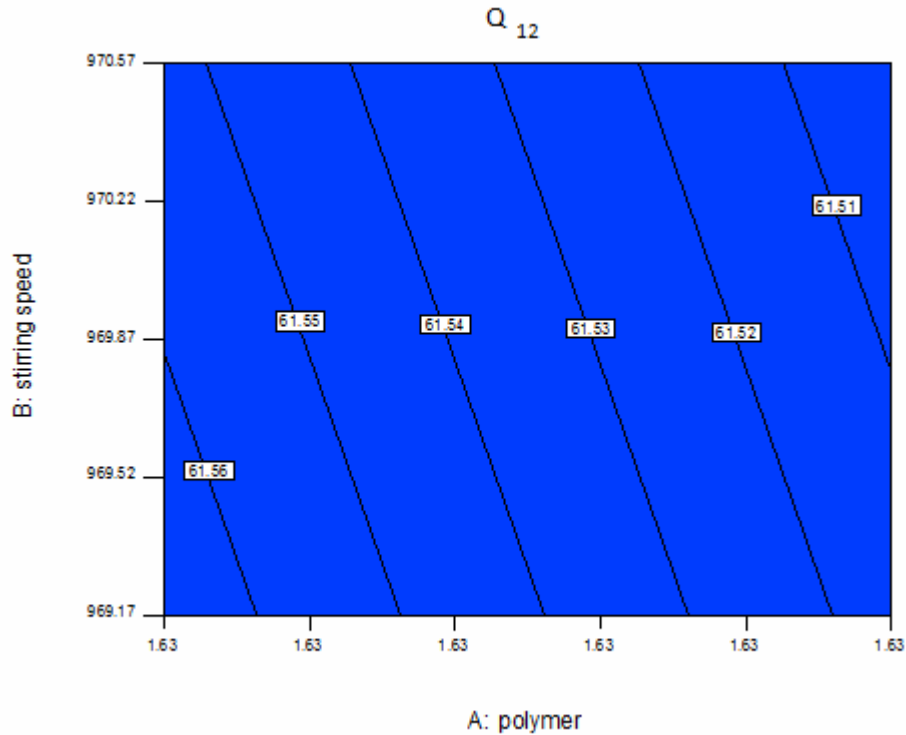


Fig5: Counter plots showing the effect of polymer concentration and stirring speed on the Drug release

Table – 3: Summary of ANOVA results for Surface linear model of drug entrapment and Quadratic model of drug release for the formulation batches

Response	Model	Sum of squares	F value	Prob>F
Drug Entrapment	Quadratic	255.04	46.26	<0.0001
Drug release	Quadratic	1144.93	50.88	<0.0001

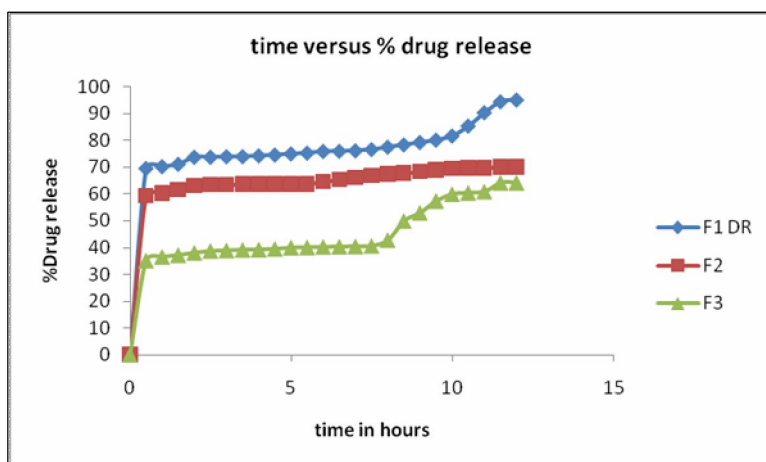


Fig 6 : Comparative dissolution profile of microspheres in 0.1N Hcl for F1- F3

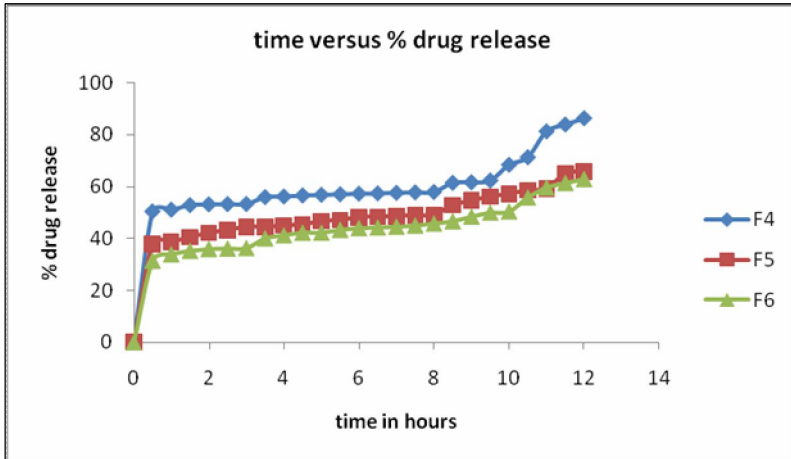


Fig 7 : Comparative dissolution profile of microspheres in 0.1N Hcl for F4- F6

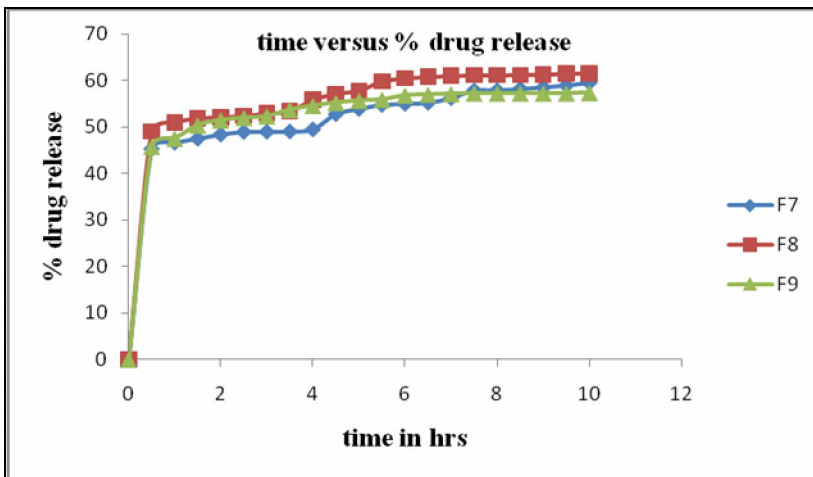


Fig 8 : Comparative dissolution profile of microspheres in 0.1N Hcl for F7- F9

Table-4 Regression coefficients and rate constants of the for release of optimised formulation:

Formulation code	Zero order		First order		Higuchi matrix		Peppas koresmeyer		Hixon Crowell	
	r	K1	r	K2	r	K3	r	K4	r	K5
F9	0.9705	3.1213	0.9605	6.09	0.895	0.4355	0.402	14.43	0.6366	4.7

3. Results and Discussion:

The process of optimisation by 3² full factorial design needs that experimentation should be completed so that mathematical model can be generated. The number of experiments required for the studies depend upon number of independent variables selected by formulator. The responses were measured for each trial and then linear, interactive or quadratic model if fitted by carrying out multiple linear regression analysis, and F-statistics to identify significant terms. A statistical model incorporating interactive terms and polynomials is eq (1)

$Y=b_0+b_1 X_1 + b_2 X_2+b_{12} X_1 X_2+b_{11} X_1^2 +b_{22} X_2^2$ (1)
 Where Y is dependent variable , b₀ is arithmetic mean response of nine runs and b_i (b₁, b₂, b₁₂ . b₁₁ b₂₂),is estimated for corresponding factor X_i(X₁, X₂, X₁₂, X₁₁) which represents the average results of changing one factor at a time from its low to high value. The interaction term(X₁ X₂) depicts the changes in response when two factors are simultaneously changed. To determine the nonlinearity polynomial terms (X₁², X₂²) are included. The data shown the Table no:1 clearly indicate that the % drug release and encapsulation efficiency are strongly depend upon Independent

variables. The fitted equations' relating the responses is clearly shown in the following equations.

Final equations in terms of coded factors:

$$EE = 85.57 + 4.05 X_1 + 4.94 X_2 + 0.69 X_1 X_2 - 2.02 X_1^2 - 1.70 X_2^2 \quad (2)$$

Final Equation in Terms of Actual Factors:

$$EE = 63.67 + 13.028 \text{ polymer concentration} + 0.014833 \text{ stirring speed} + 1.5238 \text{ polymer stirring speed} - 3.600 \text{ polymer}^2 - 4.722 \text{ stirring speed}^2 \quad (5)$$

(R-Squared:0.9698)

Final equations in terms of coded factors:

$$Q_{12} = 65.30 - 6.26 X_1 - 9.97 X_2 + 5.39 X_1 X_2 + 0.64 X_1^2 + 6.30 X_2^2 \quad (3)$$

Final Equation in Terms of Actual Factors:

$$Q_{12} = 120.1330y - 21.9961 \text{ polymer} - 0.063083 \text{ stirring speed} + 0.011981 \text{ polymer stirring speed} + 1.14667 \text{ polymer}^2 + 1.74907 \text{ stirring speed}^2 \quad (6)$$

(R-Squared:0.9698) (4)

The high values of correlation coefficient for % drug release and encapsulation efficiency as shown above indicates good fit. The polynomial equations can be used to draw the conclusions after considering the magnitude of coefficient and mathematical sign it carries.

The data of pure error and lack of fit are summarized in ANOVA Table 2. The coefficients X_1 , X_2 , $X_1 X_2$ were found to be significant at $P \leq 0.05$ and hence they were retained. Therefore conclusions can be drawn from considering the magnitude of the coefficient and mathematical sign (positive or negative) it carries. The results of multiple linear regression analysis shown that by increasing the concentration of polymer and stirring speed drug release has been decreased as the coefficient bears negative sign in the equation (4) and the encapsulation efficiency increased by increasing the stirring speed and polymer concentration as the coefficient bears positive sign in the equation (2). The response surface plots and counter plots for the polymer concentration and stirring speed versus % drug release at 12th hour (Q_{12}) and that versus encapsulation efficiency are shown in Figures (2- 5) respectively. The response plots showed that there is a significant effect of both factors on selected responses. The various standard and quality control tests carried out on microspheres demonstrated the following. A new microsphere formulation with the desired responses was formulated based on the desirability approach. All the optimized formulations were assessed for parameters angle of repose, bulk density, Carr's index and the values are indicated in Table 3. The particle size of the microspheres was found to be in range of 103-126 μm and the size of the

microspheres was found to increase with increased polymer loads which may be due to increase in viscosity of polymer solutions at higher concentration. The SEM photomicrographs indicated that the microspheres were discrete, spherical and uniform in shape (Fig.1). The results of the optimized formulation F9 showed that as the Concentration of polymer increases the drug entrapment increases significantly with the drug Release sustained over a period of 12 hours. The results demonstrated a good relationship between the predicted and experimental values, confirming the validity of the model. The bulk density for all nine formulations is less than 0.5 gm/cm^3 indicates good flow property. The angle of repose for F1-F9 ranges between 25 -35 $^\circ$ indicates good flow property.

The comparative dissolution profile of microspheres of paracetamol in Ph 7.4 for factorial design batches are shown in Figures 6-8. The drug release kinetics was studied values are indicated Table 4. It was observed that the R^2 value is 0.9617 that confirms the best fit model was found to be zero order release and the drug release may be by diffusion mechanism. The formulation showed minor changes in particle size only under long term stability study with no appreciable change in drug content proving good stability of the product conducted both in accelerated and long term stability studies

Conclusion:

Ethyl cellulose microspheres loaded with paracetamol using emulsion solvent evaporation technique were prepared. The microspheres show good drug entrapment and desirable release profile. Both the formulation variables i.e. increasing the concentration of polymer and stirring speed exerted a significant influence on the drug encapsulation and drug release. The results obtained indicated that response surface methodology can be successfully used to analyze the effect of formulation variables and develop an optimized formulation thereby reducing the number of trials, time and cost of formulation development.

Acknowledgment:

The authors wish to thank the management of Sree Vidyanikethan College of Pharmacy for providing the necessary facilities for carrying out the research work and Indian Institute of chemical technology, Hyderabad for providing SEM facility.

References:

- 1) Robinson J.R, Lee V.H.L., Controlled Drug Delivery: Fundamental and Applications, N.Y, Maecel Dekker Inc 1987.
- 2) Box G.E.P, Draper N.R., Empirical model building and response surfaces. Wiley New York., 1987, 221-247.
- 3) European Federation of Pharmaceutical Industries and Associations. Draft pharmaceutical development document. 2006.
- 4) Anderson M.J, Whitcomb P.J., DOE Simplified – Practical Tools for Effective Experimentation (2nd Edition), Productivity I New York, 2007.
- 5) Anderson, M.J., Whitcomb P.J. 2005., RSM Simplified – Optimizing Processes Using Response Surface Methods for Design of Experiments, Productivity. New York, 2005.
- 6) Cornell J.A., Experiments with Mixtures (3rd edition) John Wiley and Sons, New York, 2002.
- 7) Anthony Armstrong N., Pharmaceutical Experimental Design and interpretation (2nd ed). Taylor & Francis Group. 2006.
- 8) Singh B, Kumar R, Ahuja N., Optimizing drug delivery systems using systematic “design of experiments”. Part I: Fundamental aspects., Crit Rev Ther Drug Carrier Syst., 2005, 22:1, 27-105.
- 9) Singh B, Dahiya M, Saharan V, Ahuja N., Optimizing drug delivery systems using systematic “design of experiments”. Part II: Retrospect and prospects., Crit Rev Ther Drug Carrier Syst., 2005, 22:3, 215-294.
- 10) Singh B, Mehta G, Kumar R, Bhatia A, Ahuja N, Katare O.P., Design, development and optimization of nimesulide-loaded liposomal systems for topical application. Curr Drug Deliv., 2005, 2, 143-153.
- 11) Montgomery, Douglas C., Design and Analysis of Experiments: Response surface method and designs ., John Wiley and Sons, Inc . New Jersey., 2005, 210-56.
- 12) Boza A, De la Cruz Y, Jordan G, Jauregui-Haza U, Aleman A, Caraballo I., Statistical optimization of a sustained-release matrix tablet of lobenzarit disodium. Drug Dev Ind Pharm., 2000, 26, 1303-1307.
- 13) Box G.E.P, Wilson K.B., On the experimental attainment of optimum multifactorial conditions. Royal Statistics Society., 1951, 13, 1-12.
- 14) Singh S.K, Dodge J, Durrani M.J, Khan M.A., Optimization and characterization of controlled release pellets coated with experimental latex I. Anionic drug. Int J Pharm., 1995, 125., 243-255.
- 15) Jones D.S, Pearce K.J., Contribution of process variables to the entrapment efficiency of propranolol hydrochloride with in ethylcellulose microspheres prepared by solvent evaporation method as evaluated using a factorial design., Int.J.Pharm., 1996, 131:25-31.
- 16) Sanchez-Lafuente C, Furlanetto S, Fernandez-Arevalo M., Didanosine extended-release matrix tablets: optimization of formulation variables using statistical experimental design. Int J Pharm, 2007, 237, 107- 118.
- 17) Davies W.L, Gloor W.T., Batch production of pharmaceutical granulation in a fluid bed I: effects of process variables on physical properties of final granulation. J Pharm Sci., 1971, 60, 1869–1874.
- 18) Gohel M.C, Amin A.F.,Formulation optimization of controlled release diclofenac sodium microspheres using factorial design, Journal of Controlled Release., 1988, 51, 115-122.
- 19) Meral Yuce, Canefe., Indomethacin-loaded microspheres : preparation, characterization and in-vitro evaluation regarding ethylcellulose matrix material ,Turk J. Pharm. Sci., 2008, 5 (3), 129-142.
- 20) ICH guidelines, Q1A(R2), 21 November 2003, Vol, 68, No. 225, 65717-18.
