Hanan M. El-Nahas /J. Pharm. Sci. & Res. Vol.2 (10), 2010,663-671

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Optimization of Eudragit RS Microspheres for controlled release of Theophylline using Response Surface Methodology

Hanan M.El-Nahas
Department of Pharmaceutics ,Faculty of Pharmacy, Zagazig University of Pharmaceutical Sciences, Egypt

Abstract
The present study reports on the production of theophylline loaded Eudragit RS microspheres for controlled release. The microspheres were prepared by the emulsion solvent evaporation technique using Eudragit RS as the polymer. A three-factor, three-level design of experiment (DOE) with response surface methodology (RSM) was run to evaluate the main and interaction effect of several independent formulation variables that included theophylline concentration (X1), stirring rate (X2) span 80 concentration w/w (X3). The dependent variables included encapsulation efficiency (Y1) and cumulative percent release at 6hrs (Y2). A desirability function was used to maximize encapsulation efficiency and to obtain controlled release formula.The drug concentration had a positive effect on the encapsulation efficiency and a negative effect on cumulative recent release, stirring rate and span concentration had a positive effect on the cumulative recent release and a negative effect on encapsulation efficiency. Drug-loaded microspheres were spherical in shape and had a smooth surface with encapsulation efficiency ranging between 26.48 – 51.41%. Cumulative percent release were 39 - 69% for the 6hrs under most of the operating parameters studied.
Keywords: *Optimization; Eudragit RS ; Box-Behnken design; Microspheres; controlled release*

1-INTRODUCTION:

Theophylline is the cornerstone in the management of both the acute and chronic phases of reversible airway obstruction. However, it has a narrow therapeutic index. Fortunately, theophylline serum levels correlate well with both therapeutic and toxic effects. Concentrations of 10-20 mg/l are needed to produce bronchodilation with a minimum of side effects. Serum levels exceeding 20 mg/l are associated with an unacceptablele incidence of adverse reactions. Theophylline levels above 35 mg/l increase the incidence of seizures and cardiac arrythmias. theophylline has two distinct actions in the airways of patients with reversible obstruction; smooth muscle relaxation (i.e., bronchodilation) and suppression of the response of the airways to stimuli (i.e., non-bronchodilator prophylactic effects). It is rapidly absorbed and eliminated, so the conventional dosage forms of theophylline are administered 3–4 times a day[1]. These attributes make theophylline a good candidate for controlled release dosage forms, on the other hand, provide desirable serum concentrations for prolonged periods without frequent dosing thereby providing patient compliance Acrylic polymers are widely used as table coatings and as retardants of drug release in controlled released formulations [2]. The most interesting among acrylic polymers was low permeable Eudragit RS, which is neutral co-polymers of poly (ethylacrylate, methyl methacrylate) and trimethyl aminoethyl methacrylate chloride and are insoluble in water and digestive juices, but swell and are permeable, which means that the drugs can be released by diffusion [3]. Therefore, the permeability of drug through Eudragit RS and/or RL is independent of the pH of the digestive tract [4] .

There are some studies for theophylline controlled release dosage form such as theophylline coated with semipermeable membrane [5]. In this study, Eudragit RS was used as retardants to prepare the theophylline microspheres by a solvent evaporation method to attain controlled release dosage form of theophylline from Eudragit RS microspheres. Preparation methods of microspheres were primarily determined by the solubility of the drug and the polymer in various solvent systems, such as: single emulsion solvent evaporation [6], double emulsion solvent evaporation and or spray drying technique [7] and so on.

In the present work, Eudragit RS microspheres were prepared by emulsion solvent evaporation method. To achieve this, emulsification of aqueous Eudragit RS solution in the oil phase was carried out in the presence of Span 80 surfactant. The formed micro-droplets were crosslinked with n-hexan to obtain more or less solid spherical particles. To investigate the effect of process parameters, a series of experiments was carried out according to a working plan proposed by Box behken experimental design [8]. Three independent decision variables and

three levels for each factor. our purpose was to elucidate the effects of 3 process variables, namely drug concentration , the stirring rate used during emulsification and span concentration, on the entrapment efficiency of drug and cumulative percent released.

2-Materials and methods

2.1. Materials

Theophylline, n-hexan, acetone, magnesium stearate, liquid paraffin and span 80 were purchased from E. Merk (Germany). Eudragit RS were purchased from Rohm Pharma GMBH Weiter Stadt (Germany). All other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of microspheres

1.2 grams of Eudragit was dissolved completely in 25 ml of acetone in a glass vessel at 8 °C. The magnesium stearate (4%) and theophylline(0.6-1.8w/v) were dispersed into the Eudragit RS solution. The mixture was stirred at (400-1200) rpm in a water bath at 10 °C over 20 min, Emulsification was carried out by adding Eduragit solution dropwise to 100 ml liquid paraffin previously cooled to 10 °C containing various concentration of span 80 surfactant (0.1-1%) under continuous stirring at 35 °C. Magnesium stearate was used as the droplet stableilizer. The rotation speed of the paddle stirrer was varied in the range of 400 and 1200 min−1 with a constant temperature until the acetone was removed completely by evaporation. After stirring for 30 min, 30 ml of n-hexane (w/w) were added to the emulsion to solidify the droplets and then to harden the resultant particles, n-hexane was added as crosslinking agent to harden the microspheres. After addition of the n-hexane the mixture was further stirred for 10 min, then the formed microspheres were filtered out and washed with n-hexane and dried at room temperature in a desiccator under reduced pressure overnight. Morphological investigation of particles was carried out by electron microscope.

2.3. Scanning electron microscopy

The surface characteristics were examined by means of a scanning electron microscope. The microspheres were coated with platinum/palladium alloy using an ion coater (Eiko Engineering counter) under vacuum, and then samples were examined with a scanning electron microscope (Hitachi S-450).

2.4. Drug content determination

Microspheres (10mg) were dissolved in a 20-ml chloroform in a separating funnel to dissolve the wall of microspheres. After shaking for 10 min, 50 ml of deionized water was added and was continually shaken for 30 min. The deionized water layer was diluted and determined by spectrophotometry at 278 nm,

The loading capacity was calculated using the equation:

Loading capacity=actual theophylline content/theoretical theophylline content×100

2.5. In vitro dissolution test

Dissolution tests were performed in 500 ml deionized water using the basket method with a rotation speed of 100 rpm at 37±0.5 °C. At fixed time intervals (15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min), 2-ml samples were withdrawn and replaced with the same volume of dissolution medium. The theophylline contents in the dissolution samples were measured spectrophotometry at 278 nm .The dissolved amount of drug at each time was expressed as a percentage of the dose.

2.6. Data analysis

Three kinetic models including the zero-order release equation Eq. (1), Higuchi equation Eq. (2) and first-order equation Eq. (3) were applied to process the in vitro data to find the equation with the best fit [9-10].

$$Q=k_1 t \quad (1)$$

$$Q=k_2(t)^{0.5} \quad (2)$$

$$Q=100(1-e^{-k3t}) \quad (3)$$

where Q is the release percentage at time (t). k_1, k_2 and k_3 are the rate constant of zero-order, Higuchi and first order model, respectively.

2.7. Design of experiments

The design of experiments (DOE) technique was used to provide an efficient means to optimize the emulsion solvent evaporation process. DOE is an approach for effectively and efficiently exploring the cause and effect relationship between numerous processes

Table 1 Factors and levels in the Box-Behnken design

Independent variables	**Levels**		
	Low	Middle	High
*X*1: Drug conc. (mg)	0.6	1.2	1.8
*X*2: Stirring rate (rpm)	400	800	1200
*X*3: Span 80 conc. (%)	0.1	0.6	1
Dependent variables	**Goal**		
*Y*1: Entrapment efficiency (%)	Maximize		
*Y*2: Cumulative percent release % (6hrs)	Minimize		

variables and the output. A sequence of experiments were performed that would yield the most information about the factors and their interactions in as few experiments as possible. A 3-factor 3-level factorial Box-Behnken experimental design technique was employed to investigate the variables using the statistical software package Statvision (Statgraphics, Manugistics Inc., Rockville, MD). This technique was applied to quantify the influence of operating parameters on the production of microspheres during the emulsion solvent evaporation operation. The factorial Box-Behnken design created constituted 15 of the experiments in this study. The independant variables were drug concentration, stirring rate and span 80 concentrations. Preliminary experiments were performed to confirm the operational phase range that would successfully yield microspheres and to verify that the runs could be conducted at the operational units dictated by the factorial design. The software was also used to construct mathematical models for making response predictions for further experiments.

Graphs showing the magnitude of effects for each variable and interactions were generated for analysis. The experimental design matrix is shown in Table 1. A design matrix comprising of 15 experimental runs was constructed. An interactive second order polynomial model was utilized to evaluate both the response variables: where b1 –b9 are the regression coefficients, X1 –X3 are the factors studied and Y1-Y2 is the measured responses associated with each factor level combination

3. Results and discussion:

3-1- Scanning electron microscopy:

The particles obtained by emulsion solvent evaporation were generally nearly spherical with smooth surface as shown on the picture in Figure. 1 It should be emphasized that microscopy was used only to check the morphology of particles.

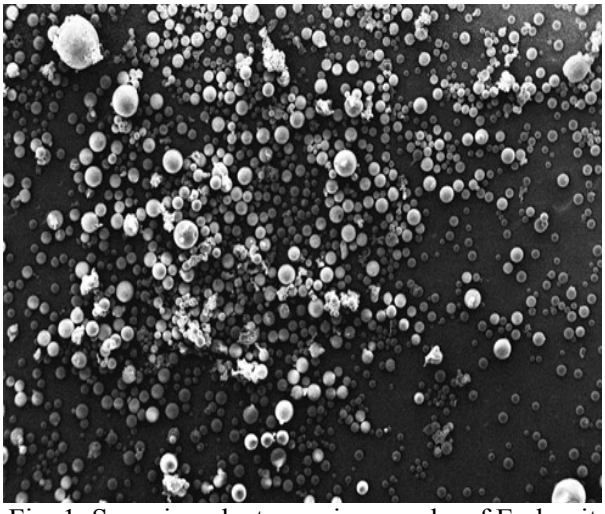


Fig. 1. Scanning electron micrographs of Eudragit RS microspheres containing Theophylline.

3.2. In vitro study

The result can be explain the incorporation efficiency of theophylline was good in all loadings and was increased with the drug concentration[11] . The high entrapment efficiency of the drug was believed to be due to its poor solubility in liquid paraffin with a

companied by increase the viscosity of continuous phase [12]

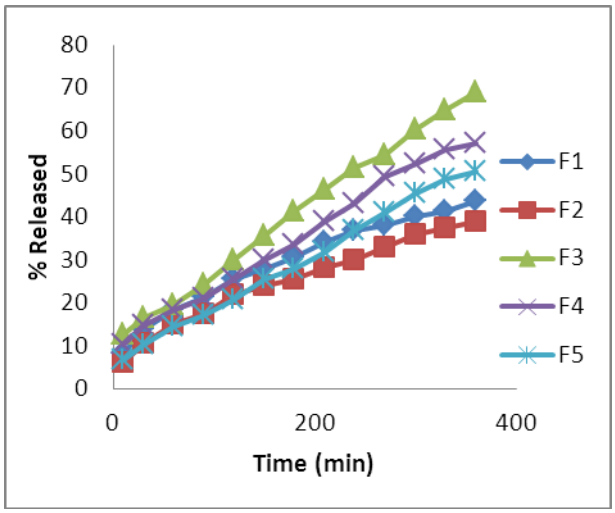
The maximum incorporation efficiencies of theophylline into microspheres were 51.41% for F10, they were found to be significantly different ($p<.05$) depending on the variation of drug concentration and stirring rate. The highest incorporation efficiency of F10 formula can be explained due to the amount of theophylline per unit polymer is greater (Table 2). [13]

Table 2: The values of the decision parameters and the resulting encapsulation efficiency and release percent

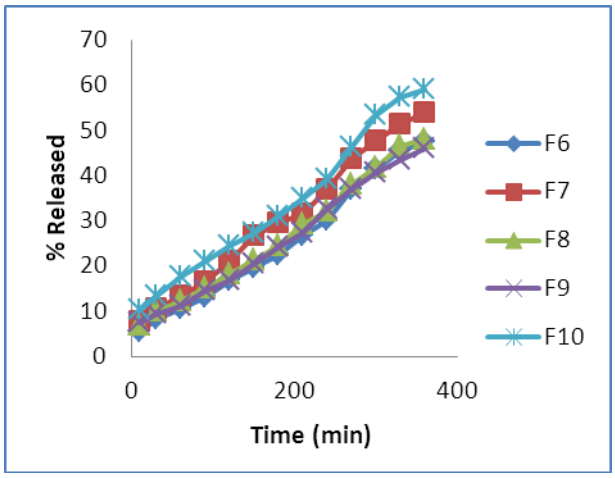
Form. No.	X1	X2	X3	Y1	Y2
F1	0.6	400	0.6	27.94	43.70
F2	1.8	400	0.6	47.34	39.00
F3	0.6	1200	0.6	31.99	69.00
F4	1.8	1200	0.6	45.33	57.00
F5	0.6	800	0.1	50.11	50.47
F6	1.8	800	0.1	51.00	47.30
F7	0.6	800	1	26.48	54.00
F8	1.8	800	1	46.80	48.00
F9	1.2	400	0.1	45.41	46.00
F10	1.2	1200	0.1	51.41	59.00
F11	1.2	400	1	43.27	41.00
F12	1.2	1200	1	29.87	62.00
F13	1.2	800	0.6	39.30	56.00
F14	1.2	800	. 0.6	39.00	57.70
F15	1.2	800	0.6	41.00	59.00

X1: drug concentration
X2: stirring rate
X3: span concentration,
Y1: encapsulation efficiency and
Y2: released percent

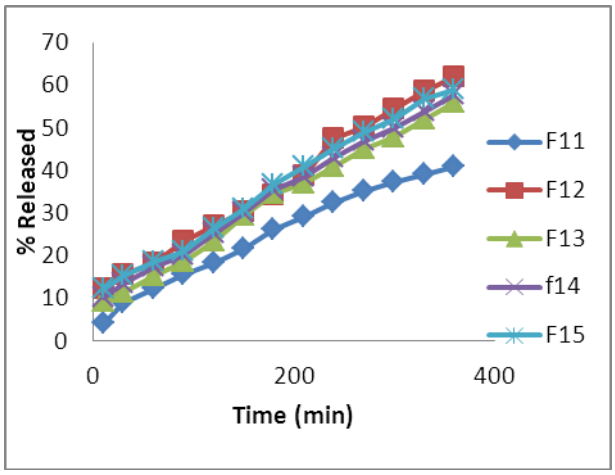
The cumulative percent release of theophylline from different formulations is shown in Figures 2a–c, Theophylline release from all the formulations was slow and sustained over 6h. The drug release rate was increasing on increasing the stirring rate. By the end of 6h formulation F 2 and F 4 released 39 and 57% of loaded drug, respectively. Theophylline release was higher in the case of microspheres prepared at a higher stirring rate but at low stirring rate the release rate was slow, at a lower stirring rate had a larger surface area exposed to dissolution medium, giving rise to faster drug release. The difference in drug release was statistically significant at different stirring rate[14]



a



b



c

Fig. 2a–c: The USP dissolution profile for the 15 formulations.

The release mechanism of theoyphylline from these Eudragit microspheres was also evaluated on the basis of theoretical dissolution equations including zero-order, Higuchi equation and first order kinetic

models [9], since different release kinetics are assumed to reflect different release mechanisms. The results are shown in Table 3. It shows that the release pattern of theophylline from all formula Eudragit RS microspheres corresponded best to the Higuchi equation and diffusion model. i.e. Mechanism of drug release from Eudragit RS microspheres is often dominated by drug diffusion from microsphere matrix, which makes Eudragit RS microspheres suitable for long-term drug release system [15]. The correlation coefficient (r^2) was in the range of 0.99-0.98 for various formulations .

Box–Behnken design was applied in this study to optimize the theophylline EE with constraints on the percent released after 6hrs.The constraints applied were to control the percent release and to maximize the entrapped percent .The observed responses for the 15 formulations are given in Table 2.

Based on the experimental design, the factor combinations provided different entrapment efficiency. The entrapment percent was 26.48 for F7 (minimum) and 51.41% in F 10 (maximum). The percent of the drug released after 6 hrs ranged from 39 F 2 (minimum) to 69% in F 3 (maximum) respectively.

In order to obtain a formulation having higher entrapment and controlled release, RSM optimization was used to determine the levels of these factors. The mathematical relationship in the form of factors' coefficients and its corresponding *P*-values for the measured responses is listed in Table 4. Coefficients with *P*-value less than 0.05 had a significant effect on the prediction efficacy of the model for the measured response. The polynomial equations relating the responses *Y*1and Y2 and the independent variables were:

$$Y1= 39.76 + 6.74\ X1 - 0.67X2 - 6.43X3 - 1.51\ X1X2 + 4.85\ X1X3 - 4.84X2X3 - 0.25\ X_1^2 - 1.32\ X_2^2 + 4.08\ X_3^2$$

$$Y2= 59.24 - 3.23X1 + 9.66X2 + 0.27X3 - 1.82X1X2 - 0.70X2X3 + 2.00X1X3 - 3.72\ X_1^2 - 1.66\ X_2^2 - 3.89X_3^2$$

Table 3. The kinetic parameters for release of Theophylline in deionized water

F	Zero-order		Higuchi model		First order	
	K	R	K	R	K	R
F1	0.11	0.98	2.91	0.99	$6.90*10^{-3}$	0.96
F2	0.12	0.95	3.17	0.98	$7.74*10^{-4}$	0 .92
F3	0.15	0.98	3.81	0.99	$1.08*10^{-3}$	0.94
F4	0.15	0.99	4.71	0.99	$1.66*10^{-3}$	0.99
F5	0.13	0.92	3.38	0.99	$8.19*10^{-3}$	0.98
F6	0.17	0.98	4.33	0.99	$1.23*10^{-3}$	0.96
F7	0.15	0.99	4.71	0.99	$1.62*10^{-3}$	0.99
F8	0.20	0.92	5.23	0.98	$1.94*10^{-3}$	0.99
F9	0.15	0.99	4.71	0.99	$1.62*10^{-3}$	0.99
F10	0.20	0.98	5.18	0.99	$2.41*10^{-3}$	0.93
F11	0.20	0.98	5.20	0.99	$2.71*10^{-3}$	0.96
F12	0.13	0.95	3.20	0.99	$1.70*10^{-3}$	0.96
F13	0.15	0.98	3.65	0.99	$3.80*10^{-3}$	0.92
F14	0.18	0.98	4.32	0.99	$5.10*10^{-3}$	0.93
F15	0.25	0.98	4.99	0.99	$1.60*10^{-3}$	0.93

Table 4: Regression equations for the responses.

Item	A	X1	X2	X3	X1 X2	X1 X3	X2 X3	$X1^2$	$X2^2$	$X3^2$
Y1 P- value	39.76 0.0001	6.74 0.0007	-0.67 0.4889	-6.43 0.0008	-1.51 0.2863	4.85 0.0123	-4.84 0.0124	-0.25 0.8545	-1.32 0.3501	4.08 0.0271
Y2 P- value	59.24 0.0001	-3.23 0.0112	9.66 0.0001	0.27 0.7494	-1.82 0.1789	-0.70 0.5711	2.00 0.1475	-3.72 0.0280	-1.66 0.2286	-3.89 0.0238

The above equations represent the quantitative effect of process variables ($X1$, $X2$, and $X3$) and their interactions on the both response ($Y1$ and $Y2$). The values of the coefficients $X1$–$X3$ are related to the effect of these variables on the response ($Y1$ and $Y2$) Table 4.

Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships, respectively. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). In ANOVA, the "Prob > F" parameter is the observed significance probability (P-value) of obtaining a greater F-value by chance alone if the specified model fits no better than the overall response mean. Observed significance probabilities of 0.05 or less are often considered evidence of a regression effect. A Prob > F of 0.0032 and F of 0.0022 indicated a significant effect of the independent factors on the response ($Y1$) and ($Y2$) respectively. Concerning the P-value of the coefficients, $X1$, $X3$, $X1X2$, $X2X3$, $X1X3$ and X_3^2 were found to have significant effects on the performance of the model for the prediction of the entrapment efficiency and $X1$, $X2$, X_1^2 and X_3^2 of the percent release. A coefficient with positive sign represents a synergistic effect of the factor on the response, while a negative sign indicates an antagonistic effect.

The relationship between the dependent and independent variables was further elucidated using contour and pareto plots. The effect of $X1$ and $X3$ and their interaction at a middle level of $X2$ on $Y1$ and the effect of $X1$ and $X2$ and their interaction at a middle level of $X3$ on $Y2$ is given in Figures 3 and 4. At low levels of X1(drug conc.) $Y1$ increased from 41.09 to56.08% when the amount of theophylline ($X1$) increases from 0.6 to 1.8 mg. Conversely, at high levels of $X3$, $Y1$ decreases from 47 to 26 %. At low levels of X1,$Y2$ increased from 28 to54% , at high levels of $X3$, $Y2$ decreases from 54 to 30 %. The standardized Pareto charts depict the main effect of the independent variables on the encapsulation efficiency and percentage of drug released after 6hrs. The length of each bar in graph indicates the effect of these factors on the responses. The highest effect was observed

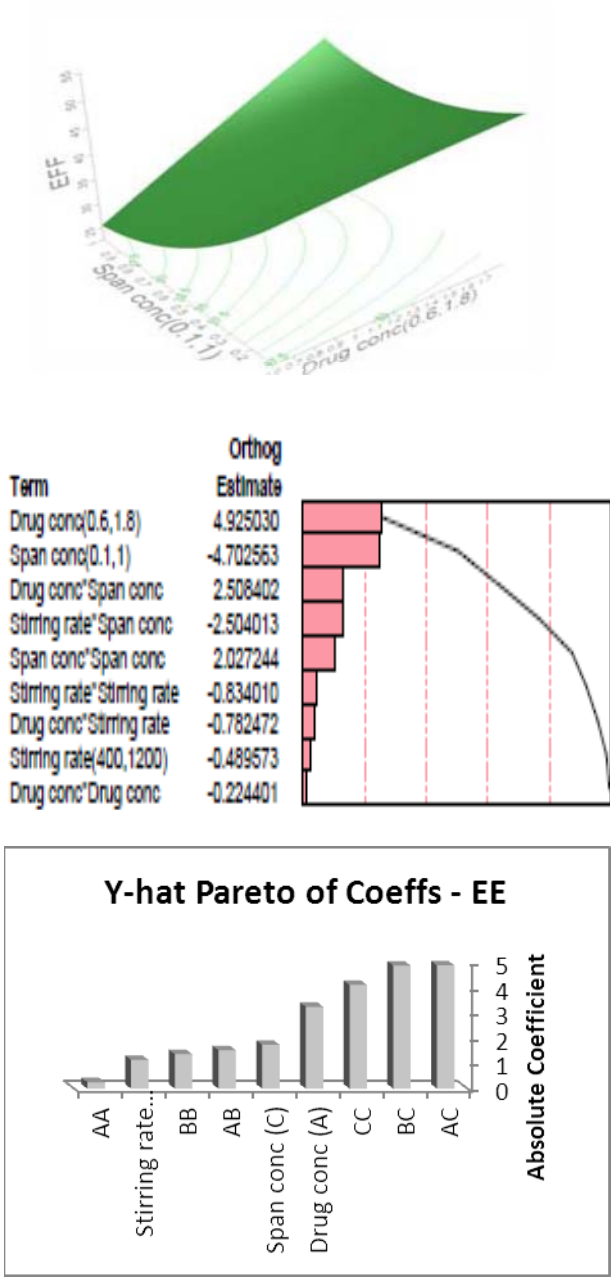


Fig. 3. Response surface (3D) , contour and Pareto plots showing the effect of the drug concentration ($X1$) and span concentration ($X3$) on the response $Y1$

for X_1 (drug conc.) and $X3$(span conc.) on encapsulation efficiency and, on the other hand, the highest effect was observed for X_1 (drug conc.) and X_2 (stirring rate) on percentage of theophylline released after 6 hrs, prediction of theoretical response values.

After establishing the relationship between the dependent and independent variables, the process was optimized. A computerized optimization procedure was used to obtain the levels of drug concentration, stirring rate and

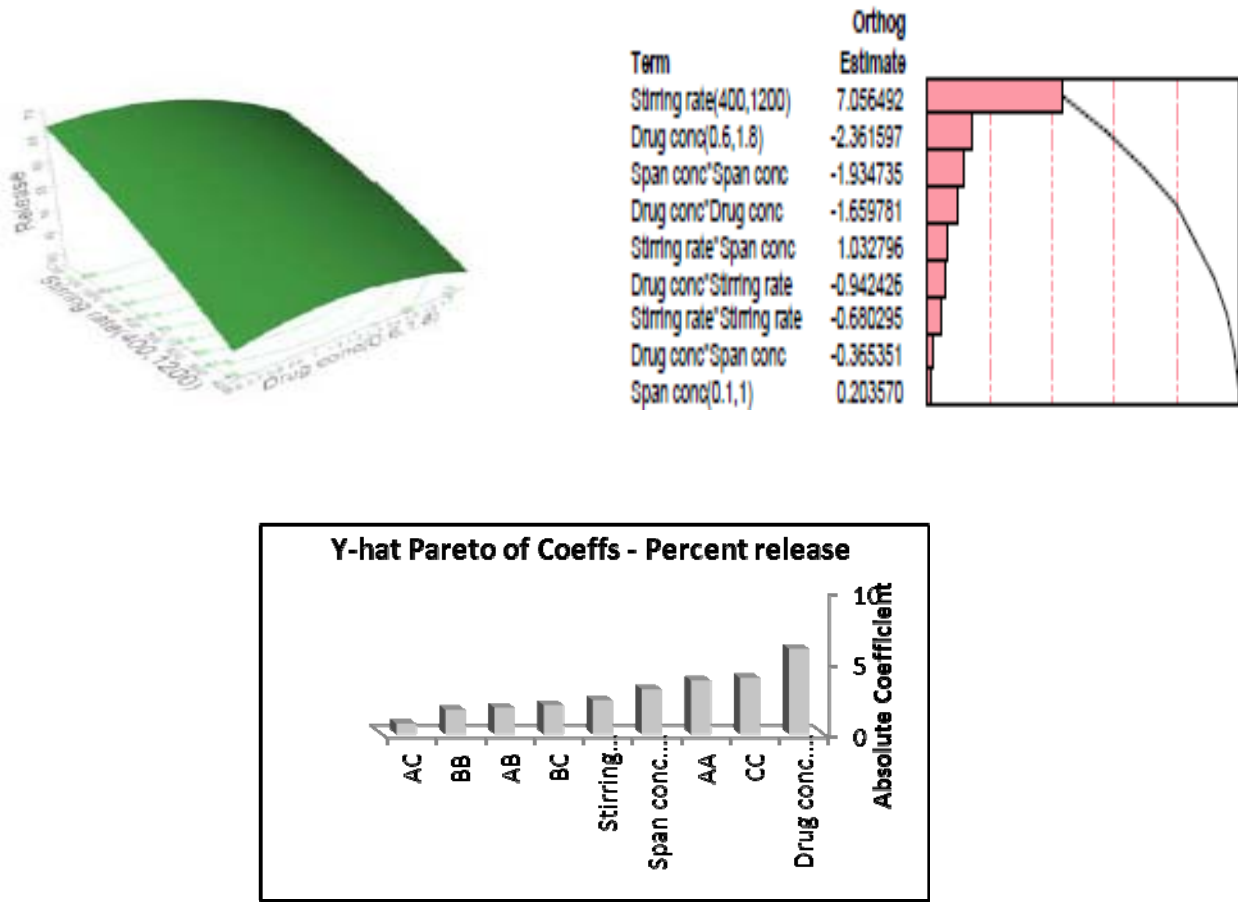


Fig. 4. Response surface (3D) , contour and Pareto plots showing the effect of the drug concentration (*X*1) and stirring rate (*X*2) on the response *Y*2

Table 5. Observed and predicted values and analysis of variance parameters for the responses *Y*1 and *Y*2.

Form No.	Observed y1	Predicted y1	Residual	Observed Y2	Predicted Y2	Residual
F1	27.94	30.56	-2.62	43.70	43.92	-0.22
F2	47.34	48.15	1.70	39.00	52.19	-1.72
F3	31.99	25.57	0.92	69.00	54.16	-0.16
F4	45.33	32.25	-0.26	57.00	66.90	2.10
F5	50.11	44.75	0.66	50.47	44.06	1.94
F6	51.00	41.57	1.69	47.30	40.61	0.38
F7	26.48	39.77	-0.46	54.00	57.57	-1.57
F8	46.80	39.77	-0.76	48.00	57.57	0.13
F9	45.41	39.77	1.23	46.00	57.57	1.43
F10	51.41	53.11	-1.70	59.00	59.38	-0.38
F11	43.27	30.53	-0.66	41.00	63.94	-1.94
F12	29.87	47.08	0.26	62.00	41.10	-2.10
F13	39.30	51.92	-0.92	56.00	47.14	0.16
F14	39.00	48.76	-1.96	57.70	46.28	1.72
F15	41.00	42.71	2.62	59.00	56.78	0.22

Source	d.f.	Sum of squares	Mean square	*F* ratio	Prob > *F*
			ANOVA for *Y*1		
Model	9	969.59	107.73	16.70	0.0032
Error	5	32.24	6.44		
Cumulative total	14	1001.84			
			ANOVA for *Y*2		
Model	9	966.92	107.43	19.69	0.0022
Error	5	27.27	5.45		
Cumulative total	14	994.20			

*Y*1: encapsulation efficiency *Y*2: cumulative percent release at 6hrs and d.f.: degree of freedom.

span 80 concentration at which a maximized entrapment percent and sustained controlled released could be obtained. The combination of factor levels leading to attainment of maximum response was 1.8 , 774, and 1% for drug concentration, stirring rate and span 80 concentration, respectively. The predicted optimum values found were 49.21 and 45.64 % for entrapment and released percent respectively.

The values of *X*1–*X*3 were substituted in the equation to obtain the theoretical values of *Y*1 and Y2. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen from Table 5. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). In ANOVA, the "Prob > *F*" parameter is the observed significance probability (*P*-value) of obtaining a greater *F*-value by chance alone if the specified model fits no better than the overall response mean. Observed significance probabilities of 0.05 or less are often considered evidence of a regression effect. A Prob > *F* of 0.0032 indicated a significant effect of the independent factors on the response (*Y*1) and a Prob > *F* of 0.0022 indicated a significant effect of the independent factors on the response (*Y*2).

Conclusions

Eudragit RS microspheres containing theophylline can be prepared successfully by using an emulsion solvent evaporation technique. The surface structure of the microspheres was spherical and smooth . EE were up to 51.41 % and the release rate of Eudragit RS microspheres was slower obey Higuchi pattern.

The optimized formulation prepared using the predicted levels of factors provided the desired observed responses with *Y1* and *Y2* values of 49.21 and 45.64 for entrapment and released percent respectively. Consequently, through the rigorous analysis of the three independent variables and its effects on the investigated responses.

Acknowledgment:

The author greatly thank Dr Hany Ibrahim the demonstrator of organic chemistry in ERU for the help in performing this research

References:

[1] Fagerstroem, P. O.; Mellstrand, T.; Svedmyr, N. Absorption of theophylline from conventional and sustained-release tablets. Int J Clin Pharmacol Ther. Toxicol., 1981;19:131

[2] Mehta, K.A., Kislaloglu, M.S., Phuapradit, W., Malick, A.W. and Shah, N.H.,. Release performance of a poorly soluble drug from a novel Eudragit-based multi-unite erosion matrix. Int. J. Pharm. 2001;213:7–12

[3] Kibbe AH (ed.). 2000.Handbook of pharmaceutical excipients. Washington, D.C., USA, American Pharmaceutical Association; 401-406

[4] Apurba Sarker Apu1, Atiqul Haque Pathan1, Dilasha Shrestha2, Golam ,Kibria2 and Reza-ul Jalil Investigation of In vitro Release Kinetics of Carbamazepine from Eudragit® RS PO and RL PO Matrix Tablets Trop. J. Pharm.Res. 2009; 8 (2): 145-152

[5] Pao-Chu Wua, Yaw-Bin Huanga, Jui-Sheng Changa, Ming-Jun Tsaib and Yi-Hung Tsai, Design and evaluation of controlled release microspheres of potassium chloride prepared by Eudragit® .Eur. J. Pharm.Sci..2003; 19(2-3):115-122

[6] Pe´rez MH, Zinutti C, Lamprecht A, Ubrich N, Astier A, Hoffman M, Bodmeier R, Maincent P.. The preparation and evaluation of poly (e-caprolactone) micropartioles containing both a lipophilic and hydrophobic drug. J. Control. Release 2000;65:429–438.

[7] Xudong Wang, Yingjun Wang, Kun Wei, Naru Zhao, Shuhua Zhang, and Jingdi hen .Drug distribution within poly(-caprolactone) microspheres and in vitro release. J. Materials Processing Technology.2009;209;(1) 348-354.

[8] Box. G.E.P. , Behnken. D.W. Some new three level designs for the study of quantitative variables. Technometrics 1960; 2: 455–475.

[9] James, E., Singh, G., Larry, L., Vinod, P.,. Method to compare dissolution profiles and a rationale for wide dissolution specification for metoprolol tartrate tablets. J.Pharm. Sci. 1997;86: 690–700

[10] W u, P.C., Tsai, M.J., Huang, Y.B., Chang, J.S., Tsai, Y.H., In vitro and in vivo evaluation of potassium chloride sustained release formulation prepared with saturated polyglycolyed glycerides matrices. Int. J. Pharm. 2002;243 (1–2):119–124.

[11] Rainer Alex and Roland Bodmeier Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and formulation variables on drug entrapment J. Microencapsulation.1990;7: 3 , 347-355.

[12] Jyothi, N. V. Prasanna, M. Prabha, S. Seetha Ramaiah, P. Srawan, G. & Sakarkar, S. N. : Microencapsulation Techniques, Factors Influencing Encapsulation Efficiency: A Review. Int. J. Nanotechnology. 2009;3 :1.

[13] Müge Kılıçarslan and Tamer Baykara The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres .Int.J. .Pharm .2003;252:1-2. 99-109.

[14] Haznedar, S. and Dortunç, B. Preparation and in vitro evaluation of Eudragit microspheres containing acetazolamide Int.J. .Pharm.2004;269: 1, 131-140

[15] Bolourtchian,N. Karimi, K. and Aboofazeli, R. Preparation and characterization of ibuprofen microspheres. J. Microencapsulation 2005;22 (5) 529-538