SIGNIFICANCE OF BOX-BEHNKEN DESIGN FOR OPTIMIZATION OF PMMA LOADED CIPROFLOXACIN NANOCARRIERS BY SILVERSON HOMOGENISER

Jaya Raja Kumar

Unit of Pharmaceutical Technology, Faculty of Pharmacy, Asian Institute of Medicine, Science and Technology (AIMST) University, Bedong 08100, Kedah, Malaysia

ABSTRACT

In this work, we have utilized a 17 factorial design approach for optimizing ciprofloxacin-loaded PMMA nanoparticles with a specific interest in high shear mixers. Ciprofloxacin-loadedPMMA nanoparticles were prepared by using the single emulsion solvent evaporation technique. In vitro characterizations of the ciprofloxacin-loaded nanoparticles revealed that the mean particle sizes of the nanoparticles ranged from 211 nm to 275 nm, the percentage of yield values were in the range of 56.5 to 66.6%and the optimized formulation showed maximum drug release 90.21 % at the end of 24th hours. The properties of the optimized Ciprofloxacin-loaded PMMA nanoparticles predicted by the 17 factorial design approach correlated very well with the experimentally determined particle size of 245 nmand yield 62.46%. The mathematical model generated for % Yield was found to be significant model. The Model F-value of 575.31 implies the model is significant.

Keywords: Ciprofloxacin Nanocarriers, Box-Behnken Design, RP-HPLC, Silverson Model L4RT,PMMA

INTRODUCTION

Ciprofloxacin is a broad spectrum second generation fluoro-quinolone antibiotic which is effective against gram positive and gram negative bacteria. It kills bacteria by interfering with topoisomerase which stops synthesis of DNA and of protein [1]. It is practically insoluble in water and sensitive to sunlight losing its antibacterial activity.The formulation development and efficient delivery of poorly water-soluble drugs has always been a challenge. It has been estimated that more than 40% of the discovered drugs are poorly water-soluble [2]. Bioavailability and performance of such drugs are usually low as their absorption is dissolution rate limited. Moreover, such drugs often have an incomplete or erratic absorption profile thus highly variable bioavailability. Subsequently, prediction, estimation and control of pharmacological response are often difficult. Significant food effect is also very common on bioavailability of poorly soluble drug. Thus it is evident that solubility of the drug is a critical factor in achieving an optimum formulation of any drug so as to get better therapeutic profiles [3, 4].
Synthetic polymers are widely used materials for the design of functional nanoparticles because they

provide unique structural diversity and functionality [5]. In this study, we have used a silverson emulsifier, available for alternative production process, for producing drug-loaded polymer nanoparticles.

EXPERIMENTAL

Materials:
The ciprofloxacin was obtained as gift sample from Dynapharm (M) SdnBhd, Malaysia.Polyvinyl alcohol (PVA) and Poly (methyl methacrylate) was purchased fromSigma Aldrich (USA). Dichloromethane were obtained from E-Merck (Germany). All chemicals and solvents used in this study were of HPLC grade.

PREPARATION OF CIPROFLOXACIN LOADED PMMA NANOPARTICLES

Preparation of ciprofloxacin loaded PMMA nanoparticles by single emulsion solvent evaporationtechnique [5] using asilversonemulsifier model L4RT [6-8]. Briefly,different amounts of PMMA were dissolved in dichloromethane(DCM). Accurately weighed quantity of drug was dissolvedin PMMA solution and added to aqueous phase (1% w/v PVA) slowly,mix for 3 hours at 8000 rpm using a Silverson L4RT emulsifiersat 25ºC. Ciprofloxacin loaded PMMA nanoparticles were separated from bulk aqueous phase by centrifugationat 15000 rpm for 30 min (Beckman Coulter‐Avanti J‐26XP,USA) followedby subsequent washing with cold distilled water and

Address for correspondence:
Jaya Raja Kumar,
Faculty of pharmacy,
AIMST University,
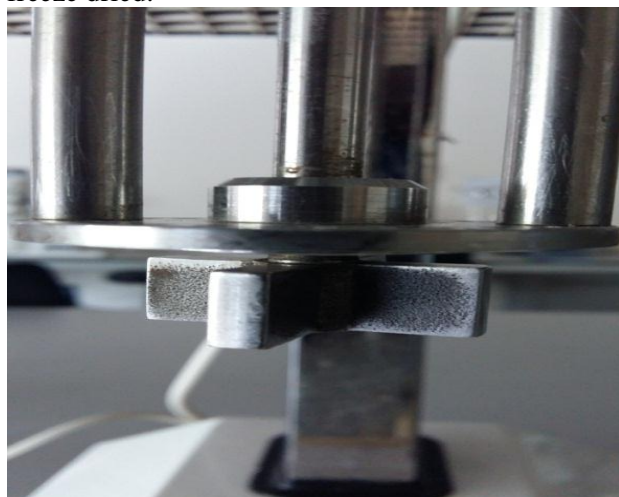Semeling, Bedong,Malaysia.
Email: jayaraj2775@gmail.com

freeze dried.

Figure-1: Silverson-L4RT emulsifiers
Particle size analysis:
Particle size of nanoparticles was determined using Anton Paar particle size analyzer (Litesizer™ 500).
In vitro drug release:
In vitro release studies were performed using Franz diffusion cell. Dialysis membrane having pore size 2.4 nm, molecular weight cut off 12,000–14,000 was used. Membrane was soaked in double-distilled water for 12 h before mounting in a Franz diffusion cell. 1ml of ciprofloxacin loaded PMMA nanoparticles suspension, containing an amount equivalent to 2.5mg of CP was transferred into the donor compartment and the receptor compartment was filled with 22 ml of dialysis medium consisting of phosphate buffer pH 7.4. An aliquot of 2 ml of sample was withdrawn from receiver compartment through side tube at time intervals of 0.15,0.3, 1, 2, 3, 4,5,6,8,10,12,16 and 24 h. Fresh medium was replaced each time to maintain constant volume. Samples were analyzed by RP HPLC method.
The solution was determined by RP HPLC method. RP HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50μL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, ACN: 10mmA.A (pH 4.5) (60:40, v/v), and detection was made at 280nm. The mobile phase was prepared daily, filtered through a 0.45μm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5μ) was used for the separation.

OPTIMIZATION OF PROCESS VARIABLES
In this work, we report the successful outcome on the formulation of ciprofloxacin nanoparticles. Through preliminaryexperiments the Drug (A), Polymer (B) and Stirring Speed (C) were identified asthe most significant variables influence the particle size and percentage of yield.Design of experiments has been used as a strong approach to reduce the variation in a processand, ultimately, to produce high % yield withuniform particle size distribution. Hence, design ofexperiments (DOEs) concept was used in the present work, which gives optimized parameters using lesser number of experimental runs.[9-11]. Among various design approaches, the Box-Behnken design was used to optimize and evaluate main effects, interactioneffects and quadratic effects of the process variables on the particle size and% yield.
Seventeen runs were required for the response surface methodology based on the (BBD). Based on the experimental design, the factor combinations produced different responses as given in Table 1. These results clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 17 runs. Mathematical relationship generated using multiple linear regression analysis for the studied variables are expressed as shown in Table 2.
These equations represent the quantitative effect of Drug (A), Polymer (B) and Stirring Speed (C) and their interaction on Particle size (R1) and % Yield (R2). The values of thecoefficient A, B and C are related to the effect of these variables on the responses R1 and R2.A positive sign represents a synergistic effect, while a negativesign indicates an antagonistic effect. A backward elimination procedure was adopted to fit the data to thequadratic model. Both the polynomial equations were found to be statistically significant (P <0.01), as determined using ANOVA as per the provision of Design Expert software (DX9).
Particle size analysis of ciprofloxacin nanoparticles was found to be in the range of 211–278nm as shown in Table 1. The Model F-value of 554.08 implies the model is significant. There is onlya 0.01% chance that an F-value this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms as shown in Table 3. The "Lack of Fit F-value" of 5.50 implies there is a 6.66% chance that a "Lack of Fit F- value" this large could occur due to noise. The "Pred R-Squared" of

0.9815 is in reasonable agreement with the "Adj R-Squared" of 0.9968; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 71.922 indicates an adequate signal. This model can be used to navigate the design space.

The normality of the data could be proved through the normal % probability plot of the externally studentized residuals (R1&R2). If the points on the plot lie on a straight line, the residuals are normally distributed as confirmed in Figure 2&9.

The assumption of constant variance was tested by plotting externally studentized residual versus predicted values (R1&R2) as illustrated in below figures. The studentized residuals are located by dividing the residuals by their standard deviations. According to evident from this Figure 3 and 10, the points are scattered randomly between the outlier detection limits +4.25 to -4.25.Residuals vs. Run were scattered randomly as shown in Figure 4 &11.Valueof λ = 1.00 indicates that no transformation neededand produces results identical to original data shown in Figure 5& 12.

The individual main effects of A, B and C on particle size are as shown in Figure 6. It is found that all the variables are having interactive effects for the response R1. The 2D contour plots of the response and the 3D response surfaces R1 are shown in Figure 7& 8 to illustrate the interactive effects of independent variables on response R1.

The mathematical model generated for %Yield (R2) was found to be significant model. The Model F-value of 575.31 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The "Lack of Fit F-value" of 1.01 implies the Lack of Fit is not significant relative to the pureerror. There is a 47.55% chance that a "Lack of Fit F-value" this large could occur dueto noise.

The perturbation plot (Figure 13) showing the main effects of A, B and C on the percentage yield (R2) of ciprofloxacin nanoparticles. The relationshipbetween the dependent and independent variables was further elucidated using 2D contour plots and 3D response surfaces as shown in Figure14& Figure 15.

The results of the FTIR spectroscopy confirmed the compatibility of drug and polymer. Ciprofloxacin compound formed the polymer active with no disturbance in the functional group, Therefore a polymerized active constituent has no change of effect after polymerization as shown in Figure 16.

Differential scanning calorimetry measures the heat loss or gain resulting from physical or chemical changes within a sample as a function of the temperature.The DSC spectra of ciprofloxacin showed endothermic peaks at 260.61ºC. The polymer PMMA peaks at 119.29 ºC. The physical mixture of ciprofloxacinand PMMA showed endothermic peaks at 259.36 ºC as showed in Figure 17.

The new batch of ciprofloxacin nanoparticles were prepared and measured according to these optimized levels. The observed values of responses were compared to the predicted values [Table 5] to validate the method. The observed value of NP1was in a very close agreement to the predicted ones. By this the validity of the optimization procedure was proven.

The percentage of drug release from nanoparticles over the period of 24 hours for optimized formulation was found to be 90% at the end of hours as shown in Figure 18 and 19.

These results reveal that the size of ciprofloxacin nanoparticles has direct relation with the concentration level of the polymer as shown in Figure 19.

The morphology and size distribution of the PMMA nanoparticleswas investigated by SEM &Anton Paar particle size analyzerare shown in Figure 19 &20. Typical images of nanoparticlesfrom PMMA prepared by single emulsion solvent evaporation technique. In optimized images, spherical-shaped particlesin the nanoscale range can be observed.

Table-1: Factorial design of Ciprofloxacin nanoparticle

Select	Std	Run	Factor 1 A:Drug mg	Factor 2 B:Polymer mg	Factor 3 C:Stirring sp... rpm	Response 1 Size nm	Response 2 yield %
	2	1	80	100	6500	215	57.4
	8	2	80	150	8000	240	65.4
	3	3	50	200	6500	274	66.6
	13	4	65	150	6500	245	62.4
	17	5	65	150	6500	244	62.6
	9	6	65	100	5000	220	56.5
	15	7	65	150	6500	245	62.7
	12	8	65	200	8000	271	68.4
	10	9	65	200	5000	278	65.1
	1	10	50	100	6500	212	58.8
	14	11	65	150	6500	245	62.3
	11	12	65	100	8000	211	60.1
	16	13	65	150	6500	246	62.3
	7	14	50	150	8000	242	65.3
	6	15	80	150	5000	247	61.7
	5	16	50	150	5000	246	62.9
	4	17	80	200	6500	275	66.5

Table-2: Regression equation for the response

Response Regression equation

Size= +245.00+0.37* A+30.00* B-3.38* C-0.50* AB-0.75* AC+0.50* BC-1.12* A2+0.13* B2-0.13* C2

Yield= +62.46-0.32* A+4.23* B+1.63* C+0.32* C+0.32* AB+0.33* AC-0.075* BC+0.58* A2-* B2-0.72* B2+0.78* C2

Table-3: ANOVA results of the quadratic model for the response particle size (R1)

Source variations	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F	R^2
Model	7301.99	9	811.33	554.08	< 0.0001	0.9986
A-Drug	1.13	1	1.13	0.77	0.4098	
B-Polymer	7200.00	1	7200.00	4917.07	< 0.0001	
C-Stirring speed	91.13	1	91.13	62.23	< 0.0001	
AB	1.00	1	1.00	0.68	0.4358	
AC	2.25	1	2.25	1.54	0.2551	
BC	1.00	1	1.00	0.68	0.4358	
A^2	5.33	1	5.33	3.64	0.0981	
B^2	0.066	1	0.066	0.045	0.8382	
C^2	0.066	1	0.066	0.045	0.8382	
Residual	10.25	7	1.46			

Lack of Fit	8.25	3	2.75	5.50	0.0666

Table-4: ANOVA results of the quadratic model for the response % of yield (R2)

Source variations	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F	R^2
Model	171.61	9	19.07	575.31	< 0.0001	0.9986
A-Drug	0.84	1	0.84	25.50	0.0015	
B-Polymer	142.81	1	142.81	4308.77	< 0.0001	
C-Stirring speed	21.13	1	21.13	637.39	< 0.0001	
AB	0.42	1	0.42	12.75	0.0091	
AC	0.42	1	0.42	12.75	0.0091	
BC	0.023	1	0.023	0.68	0.4371	
A^2	1.43	1	1.43	43.11	0.0003	
B^2	2.17	1	2.17	65.40	< 0.0001	
C^2	2.58	1	2.58	77.79	< 0.0001	
Residual	0.23	7	0.033			
Lack of Fit	0.10	3	0.033	1.01	0.4755	

Table-5: Optimized values obtained by the constraints applies on R1 and R2

Independent variables	Values	Predicted values		Batch	Observed values	
		P. Size	P. Yield		P. Size	P. Yield
Drug	65	245	62.46	NP1	245	62.4
Polymer	150					
Stirring speed	6500					
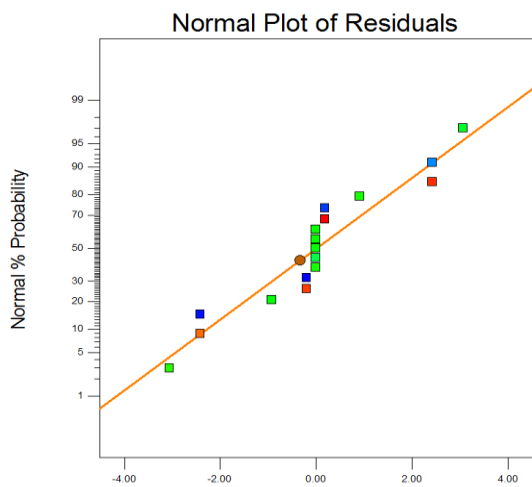
Figure-2: Normal % probability plot of the externally studentized residuals (R1)
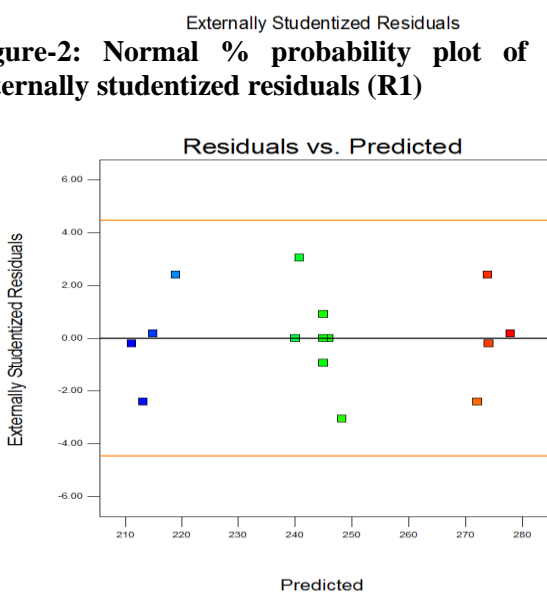
Figure-3: Residuals vs. Predicted (R1)
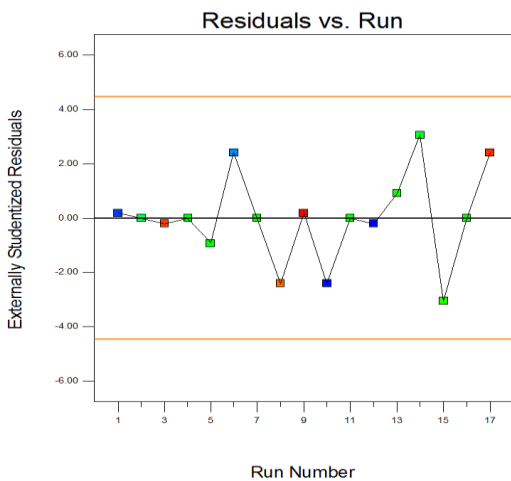
Figure -4: Residuals vs. Run (R1)
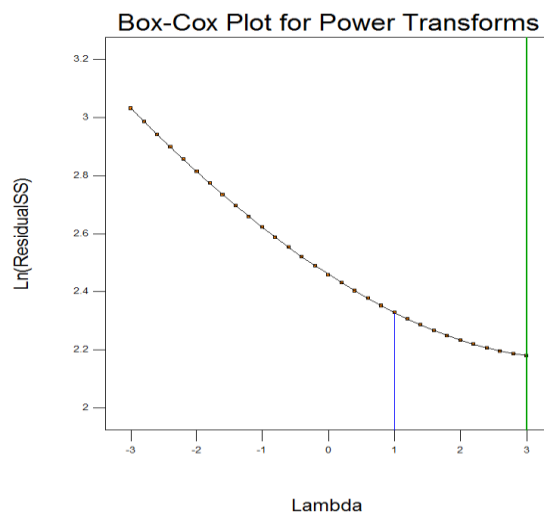
Figure -5: Box-Cox Plot (R1)
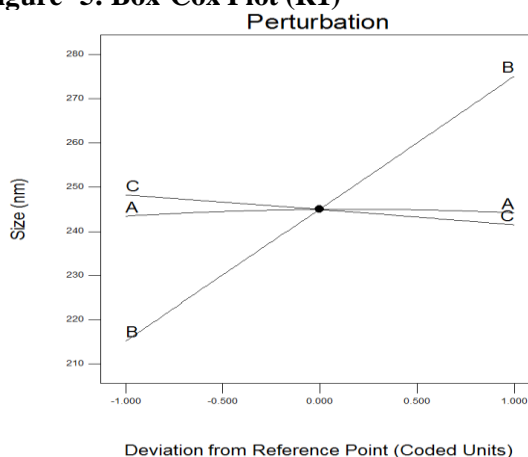
Figure-6 : Perturbation plot showing the main effect of drug (A), polymer (B) and Stirring speed (C) on particle size.
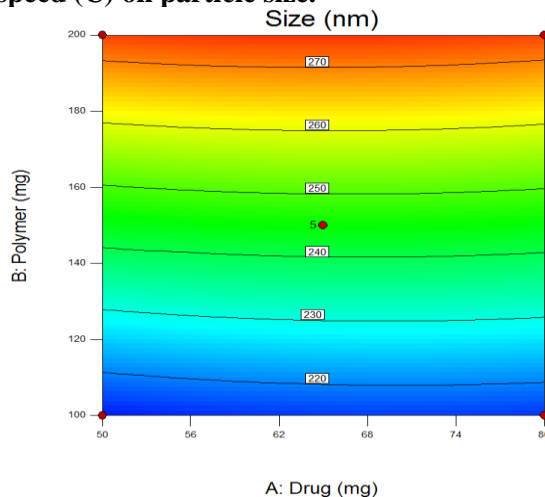
Figure-7: Response surface plot presenting the interaction between the drug and polymer affecting the particle size.
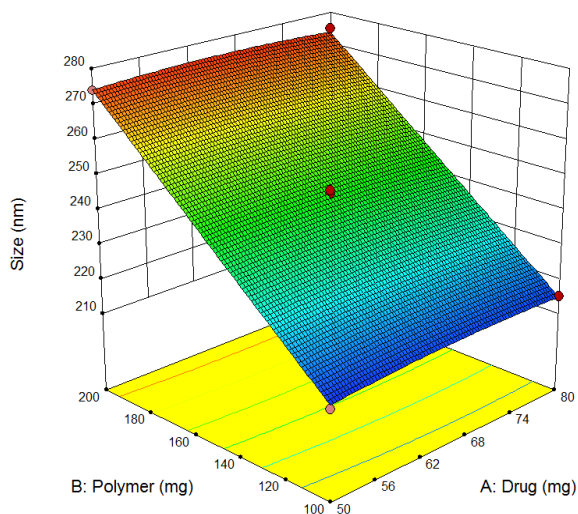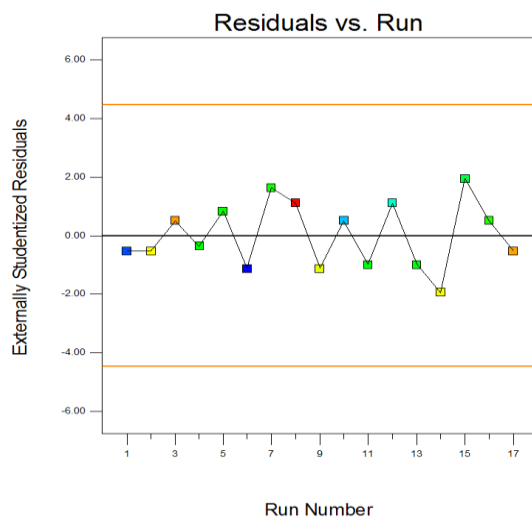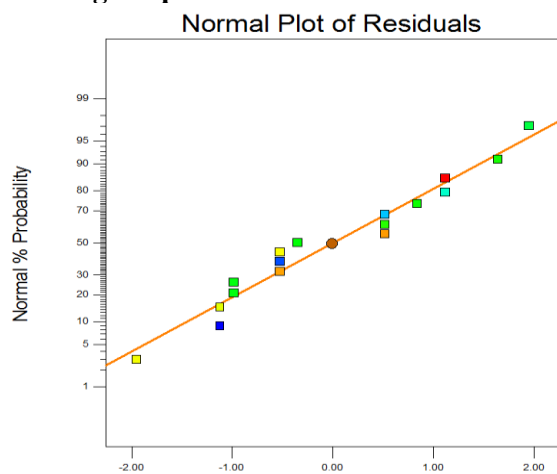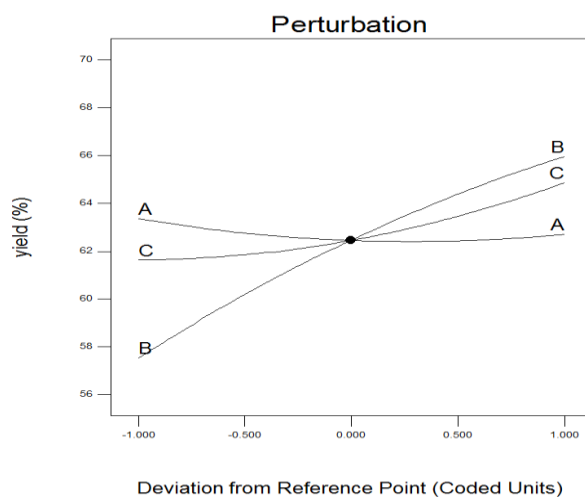
300

Figure-8: Response surface plot presenting the interaction between the drug and polymer affecting the particle size.

Figure-9: Normal % probability plot of the externally studentized residuals (R2)

Figure-10: Residuals vs. Predicted (R2)

Figure -11: Residuals vs. Run (R2)
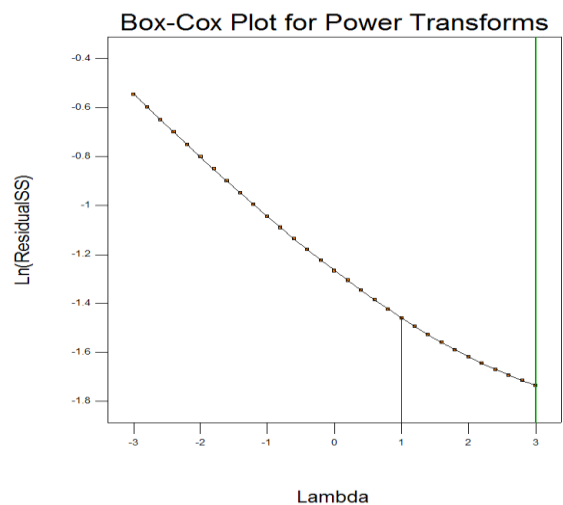
Figure -12: Box-Cox Plot (R2)

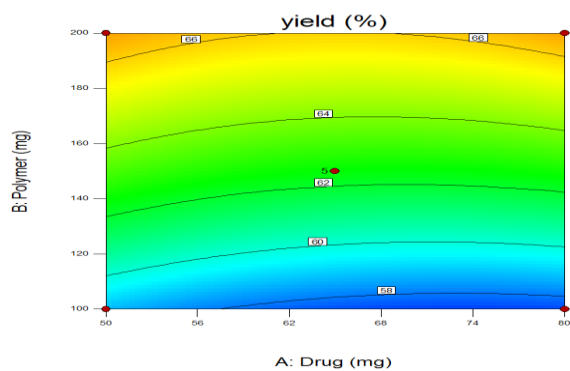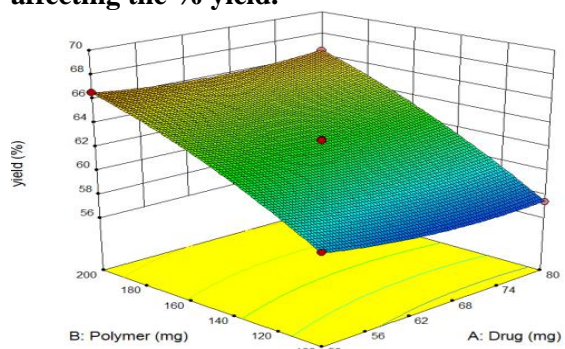**Figure-13: Perturbation plot showing the main effect of drug (A), polymer (B) and Stirring speed (C) on % yield.**

Figure-14: Response surface plot presenting the interaction between the drug and polymer affecting the % yield.

Figure-15: Response surface plot presenting the interaction between the drug and polymer affecting the % yield.
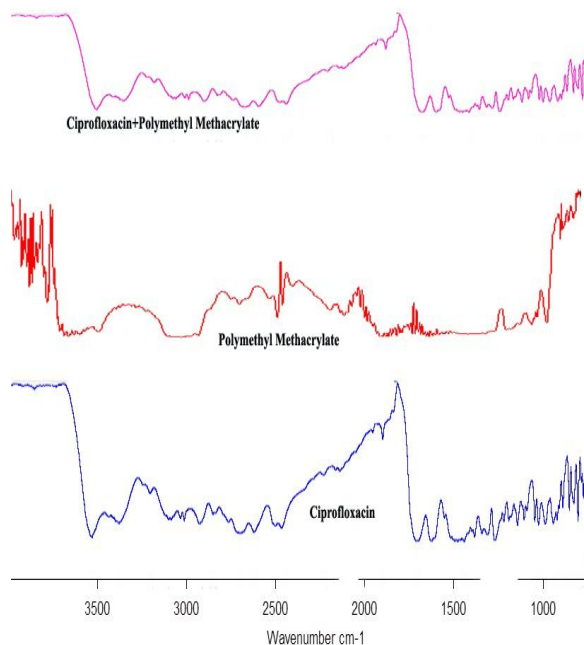

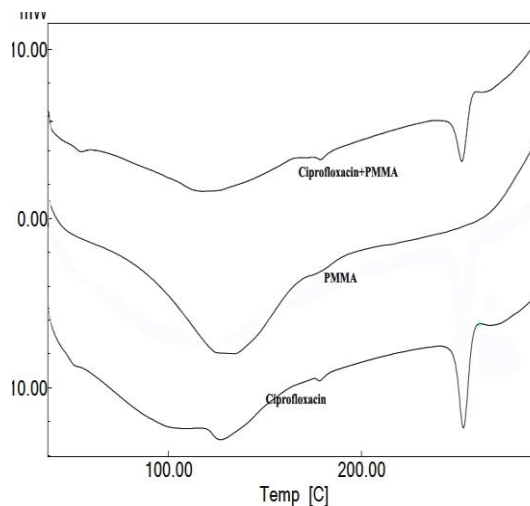**Figure-16: FTIR Spectra of ciprofloxacin, PMMA and ciprofloxacin+ PMMA**

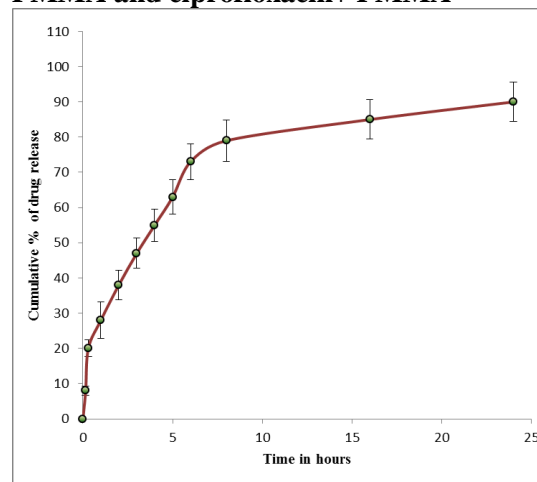**Figure-17: DSC Spectra of ciprofloxacin, PMMA and ciprofloxacin+ PMMA**
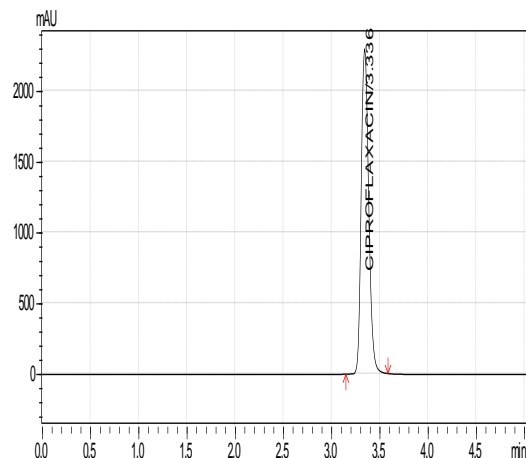

Figure-18: Cumulative % of drug release
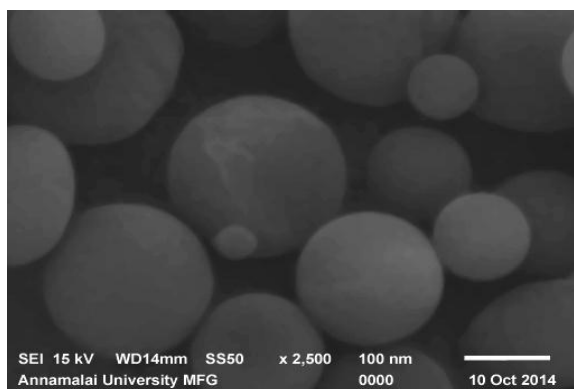

Figure-19: Typical chromatogram of ciprofloxacin

302

Figure-19: SEM photography of ciprofloxacin-loaded PMMA nanoparticles
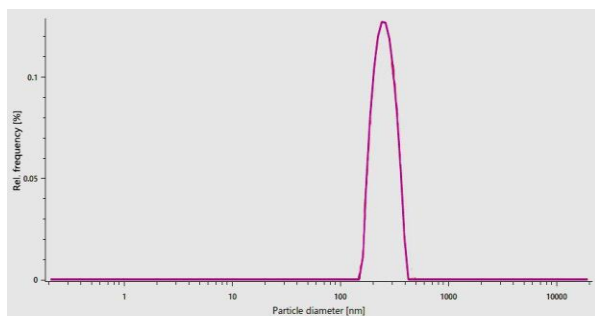

Figure-20: Particle size analysis of CP nanoparticles

CONCLUSION

Ciprofloxacin-loaded PMMA nanoparticles for colon delivery were successfully prepared with the emulsion solvent evaporation technique. The use of the 17 factorial design model enabled development of an optimized ciprofloxacin-loaded PMMA -based nanoformulation using minimum particle size and maximum percentage of yield. The observed responses of particle size (245 nm) and percentage of yield (62.4%) correlated well with the predicted values obtained from the 17 factorial design model. DSC studies confirmed the presence of ciprofloxacin in an amorphous or disordered-crystalline phase of molecular dispersion form or in a solid solution state in the polymer matrix. FT-IR studies confirmed that there are no substantial interactions between ciprofloxacin and PMMA. SEM images of the optimized ciprofloxacin -loaded PMMAnanocarriesrevealed that the NPs were spherical in shape, anddisplayed a good size distribution.

REFERENCES:

[1] YZ Pommier; E Leo; HZhang; CMarchand.*Chem.Biol*, 17 (5): 421-433(2010).

[2] VRVemula; VLagishetty; SLingala.Int. *J. PharmSciRev Res*, 5(1): 41-51(2010).

[3] T Loftsson; MEBrewster.*J. Pharm. Pharmacol*, 62(11): 1607-1621(2010).

[4] CA Lipinski.*J. Pharmacol.Toxicol Methods*, 44(1): 235-249 (2000).

[5] M Ashford, J T Fell, D Attwood, P Woodhead. *J. Int J Pharm*, 91: 241–245(1993).

[6] Yeap Su Yong, Jaya Raja Kumar, Hiew Mei Yi, Teo Johnson, Kasturi. A. The effect of independent variable on the isradipine nanoparticle, size and drug loading capacity evaluated by means of design-expert 9. Rapports De Pharmacie,1(2):90-98(2015).

[7] Hiew Mei Yi, Jaya Raja Kumar, Kasturi. A, Yeap Su Yong, Teo Johnson. Influence of dependent variable: particle size and yield on terbutaline nanoparticle by using surface quadratic model. Rapports De Pharmacie,1(2):109-117(2015).

[8] Lee Ali Leng, Jaya Raja Kumar, LiowHinTeng, Kanagambikai. R, MVRA MaivizhiSelvi. Formulation and optimization of ethylcellulose coated triamcinolone nanoparticles using 3-factor, 2-level box-behnken factorial design. Rapports De Pharmacie,1(2):99-108(2015).

[9] VJ Mokale, JB Naik, U Verma, JS Patil, SK Yadava. Preparation andcharacterization of biodegradable glimepiride loaded PLA nanoparticles byo/w solvent evaporation method using high pressure homogenizer: a factorialdesign approach.*SAJ Pharm. Pharmacol.*1 1–10(2014).

[10] MC Gohel, AF Amin. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design.*J Control Release,*51:115-22 (1998).

[11] S Nazzal, MA Khan. Response surface methodology for the optimization of ubiquinone self-nanoemulsified drug delivery system.*AAPS Pharm Sci Tech*,3:E3 (2002).