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QUALITY-BY-DESIGN APPROACH FOR DEVELOPMENT AND OPTIMIZATION OF NEFOPAM HYDROCHLORIDE LOADED POLY-(ε-CAPROLACTONE) AND POLY-3-HYDROXYBUTYRATE MICROSPHERES

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Keywords:

Response surface methodology, Second-order polynomial equations, Desirability function, Quality - by - design, Central composite design, Optimization

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ABSTRACT: The objective of present study was to investigate optimized nefopam hydrochloride loaded microspheres (NPH-MS) by investigating the relationship between independent and dependent variables using response surface methodology. Central composite design with thirty three batches was constructed using drug: polymer (X_1), PHB: PCL (X_2), stirring speed (X_3), stirring time (X_4) and polyvinyl alcohol (PVA) (X_5) level as independent factors. NPH-MS were manufactured using polyhydroxybutyrate (PHB) and poly ε-caprolactone (PCL) by double emulsion solvent evaporation technique. The response variables were % entrapment efficiency (Y_1), mean diameter (Y_2), % drug loading (Y_3), and % yield (Y_4). Second-order polynomial equations for (Y_1-Y_4) were developed by Design-expert® 9.0.5.1 software. Positive and negative signs of regression coefficient indicated synergistic and antagonistic effect on response variables, respectively. Optimized NPH-MS estimated by design-expert software have highest desirability function, D = 0.911 and X_1, X_2, X_3, X_4 and X_5 were 1: 2.86, 1:1.19, 1501 rpm, 2.98 h and 0.54 % w/v, respectively. The model predicted values of Y_1, Y_2, Y_3 and Y_4 for optimized NPH-MS were 83.80%, 100.78µm, 21.50% and 77.99% respectively. Check point batch analysis validated the authenticity of predictive power of designed model as % bias between experimental and model predicted values was < 5%. It was concluded that quality-by-design approach has great utility in formulation optimization.

INTRODUCTION: Novel drug delivery system involves formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. Recurrent dosing, systemic toxicity and reduced bioavailability are major obstacles in delivery of an active pharmaceutical entity.

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Polymeric microspheres have been outstanding approach in domain of sustained drug delivery with superior safety and biocompatibility [1, 2]. Nefopam hydrochloride is a non-opioid, non-steroidal, centrally acting analgesic drug having chemical name 5-methyl-1-phenyl-1, 3, 4, 6-tetrahydro-2, 5-benzoxazocine hydrochloride [3, 4].

It is cyclized analog of orphenadrine and diphenhydramine representing a new class of centrally acting skeletal muscle relaxants. It has oral bioavailability approximately 30-40% and an elimination half life of about 3-5. The adverse effects of drug such as nausea, vomiting, dizziness and patient non-compliance has limited its application [5, 6].

In this research, nefopam hydrochloride loaded microspheres (NPH-MS) were synthesized employing mixture of poly-3-hydroxybutyrate (PHB) and poly-ε-caprolactone (PCL) by double emulsion solvent evaporation method [7]. Central composite quality-by-design (QbD) approach has been applied for optimizing critical formulation / process variables with least number of experiments. Selection of appropriate regression model was performed through statistical analysis *i.e.* model *F*-value, lack of fit *F*-value and correlation coefficient (r^2). The purpose of present research was to study effect of independent variables on response prameters and numerical optimization to investigate optimized NPH-MS [8-11]. Skeletal muscle relaxant activity of optimized NPH-MS was investigated using rota-rod apparatus.

MATERIALS AND METHODS:

Materials: Nefopam hydrochloride ($C_{17}H_{20}ClNO$, 5- methyl- 1- phenyl -1, 3, 4, 6- tetrahydro- 2, 5- benzoxazocine hydrochloride, Mw 289.8 g mol^{-1}, CAS NO-23327-57-3, 99.57% purity) was purchased from Hangz Hou- Daying- Chem. Company Ltd., China. Poly ε-caprolactone (CAS NO-24980-41-4, $(C_6H_{10}O_2)_n$, average Mw ~ 14,000, average M_n ~ 10,000 by GPC) and polyhydroxybutyrate (CAS NO-29435-48-1, poly-(R)-3-hydroxybutyric acid) were purchased from Sigma-Aldrich Chemie, Gmbh, Steinhelm, USA. All other chemicals used were of analytical grade.

Quality-by-Design: CCD was utilized for optimizing critical formulation/process variables in production of NPH-MS [12, 13]. CCD design consists of 2^n two-level full-factorial or 2^{n-1} fractional factorial design points which forms backbone of experiment, 2n axial ($\pm\alpha$) points to check curvature and n_o center points to estimate experimental reproducibility. In this research, CCD with fractional factorial design was utilized for investigation of effect of five independent variables (n = 5) on response parameters of NPH-MS (**Table 1**). A 2^{5-1} (sixteen experimental runs) fractional factorial design formed backbone of experiments with variables taken at low and high levels, which were depicted by coded values -1 and +1, respectively. Seven replicate runs (n_o) were performed at centre point, coded value 0 and ten additional runs ($2n$) were carried out at axial (star) points, coded α (in this study, α = 2 using following formula):

$$\alpha = (\text{Total number of experimental runs in FFD})^{1/4} \quad \text{.....Eq. (1)}$$

In present investigation, thirty three batches of NPH-MS were manufactured according to CCD experimental design matrix (**Table 2**) as generated by Design-Expert software (Trial Version 9.0.5.1, Stat-Ease Inc., MN). Second order polynomial model (Eq. 2) was generated by multiple regression analysis using Design-Expert software (Montgomery, 2008).

$$Y = \beta_0 + \sum_{i=1}^{5}\beta_i X_i + \sum_{i=1}^{5}\beta_{ii} X_i^2 + \sum_{i=1}^{4}\sum_{j=i+1}^{5}\beta_{ij} X_i X_j + \epsilon \quad \text{.....Eq. (2)}$$

Where, Y denotes observed response variable, β_0 is constant coefficient and ϵ is residual correlated to experiments. β_i, β_{ii} and β_{ij} represents coefficients of linear, quadratic parameter and interaction parameters, respectively. X_i represents average outcome of changing one variable at a time from low to high, polynomial terms X_i^2 was employed to assess non-linearity effect of variable X_i, interaction terms X_iX_j illustrated how the response transforms when two variables X_i and X_j were altered concurrently.

TABLE 1: INDEPENDENT AND DEPENDENT VARIABLES FOR NPH-MS

	Coded levels of variables				
Independent variables	**–2 (–α)**	**–1**	**0**	**+1**	**+2 (+α)**
X_1= Drug: polymer (w/w)	1:1	1:2	1:3	1:4	1:5
X_2= PHB:PCL (w/w)	1:0	1:0.5	1:1	1:1.5	1:2
X_3= Stirring speed (rpm)	500	1000	1500	2000	2500
X_4= Stirring time (h)	1	2	3	4	5
X_5= PVA concentration (%, w/v)	0.2	0.4	0.6	0.8	1.0
Dependent variables			Constraints		
Y_1= Entrapment efficiency (% EE, w/w)			Maximize		
Y_2= Mean diameter (μm)			Minimize		
Y_3= Drug loading (% DL, w/w)			Maximize		
Y_4= Yield (%, w/w)			Maximize		

TABLE 2: CENTRAL COMPOSITE DESIGN MATRIX FOR NPH-MS

Standard order	Run order	Coded values of independent variables				
		X1	X2	X3	X4	X5
NPH-MS-1	11	–1	–1	–1	–1	+1
NPH-MS-2	17	–1	–1	–1	+1	–1
NPH-MS-3	22	–1	–1	+1	–1	–1
NPH-MS-4	19	–1	–1	+1	+1	+1
NPH-MS-5	2	–1	+1	–1	–1	–1
NPH-MS-6	14	–1	+1	–1	+1	+1
NPH-MS-7	3	–1	+1	+1	–1	+1
NPH-MS-8	16	–1	+1	+1	+1	–1
NPH-MS-9	12	+1	–1	–1	–1	–1
NPH-MS-10	5	+1	–1	–1	+1	+1
NPH-MS-11	13	+1	–1	+1	–1	+1
NPH-MS-12	9	+1	–1	+1	+1	–1
NPH-MS-13	20	+1	+1	–1	–1	+1
NPH-MS-14	1	+1	+1	–1	+1	–1
NPH-MS-15	10	+1	+1	+1	–1	–1
NPH-MS-16	6	+1	+1	+1	+1	+1
NPH-MS-17	8	0	0	0	0	0
NPH-MS-18	7	0	0	0	0	0
NPH-MS-19	21	0	0	0	0	0
NPH-MS-20	18	0	0	0	0	0
NPH-MS-21	15	0	0	0	0	0
NPH-MS-22	4	0	0	0	0	0
NPH-MS-23	28	0	0	0	0	0
NPH-MS-24	29	–2	0	0	0	0
NPH-MS-25	31	+2	0	0	0	0
NPH-MS-26	23	0	–2	0	0	0
NPH-MS-27	25	0	+2	0	0	0
NPH-MS-28	30	0	0	–2	0	0
NPH-MS-29	26	0	0	+2	0	0
NPH-MS-30	27	0	0	0	–2	0
NPH-MS-31	32	0	0	0	+2	0
NPH-MS-32	33	0	0	0	0	–2
NPH-MS-33	24	0	0	0	0	+2

Production Methodology: Double emulsion solvent evaporation method was employed for manufacturing NPH-MS [7]. NPH dispersed in distilled water: acetone (1:1) was extruded gradually through syringe #20 to solution of PHB, PCL and sorbitan monooleate (0.5% w/v) in dichloromethan. Subsequently, sonicated for 2 min *via* an ultrasonic probe sonicator (PCI analytical, India) to produce primary emulsion. The gradual addition of primary emulsion to 0.5% w/v PVA solution containing Tween 20 (1% w/v) produced double emulsion. Hardening of microspheres was accomplished through continuous stirring at 37 ± 0.5 °C on magnetic stirrer (REMI, Mumbai, India). Hardened NPH-MS were collected by membrane filtration through 0.45µm Millipore filter (Merck Life Science Pvt. Ltd, Mumbai, India), and ultra-centrifuged using cooling centrifuge (Remi, RIS-24 BL, Mumbai, India) at 10,000 rpm for 30 min at 4 °C. Lyophilization was achieved using D-mannitol as lyoprotectant at - 65 °C and 0.5 kPa for 24 h in lyophilizer (Allied Frost, India) (**Fig. 1**).

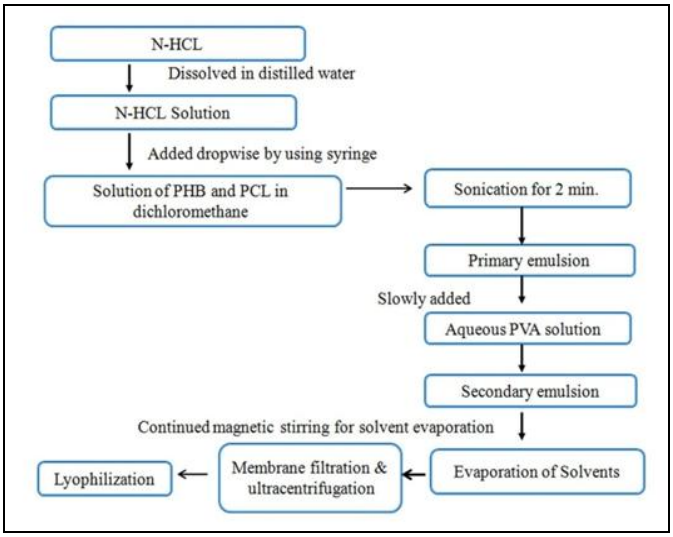

FIG. 1: PRODUCTION METHODOLOGY FOR NPH-MS

Evaluation of Response Variables of Microspheres (Y_1-Y_4): NPH-MS (50mg) was dispersed in phosphate buffer, pH 7.4 for 24 h followed by centrifugation (Remi, RIS-24 BL, Mumbai, India) at 5,000 rpm for 10 min. Supernatant was analyzed spectrophotometrically at 266nm using double beam UV-visible spectrophotometer (Systronics AU-2701, Ahmedabad, India) [14, 15]. Entrapment efficiency (% EE, w/w) and drug loading (% DL, w/w) of NPH-MS were calculated using following equations:

$$\% \text{ EE, w/w} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of drug}} \times 100 \quad \text{..... Eq. (3)}$$

$$\% \text{ DL, w/w} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of drug + weight of polymer}} \times 100 \quad \text{.....Eq. (4)}$$

Mean diameters of NPH-MS were determined by optical microscopy employing light microscope (Adeltavision Microscopes, India) at × 400 magnifications. Images were captured with APCAM USB2 digital cameras system (APCAM, India) and processed with Adelta Optec's APView imaging software. Measurement was executed in triplicate (n = 3) to obtain mean diameter [5]. The completely dried NPH-MS were accurately weighed and % yield of microspheres was calculated using following equation:

$$\% \text{ Yield, w/w} = \frac{\text{Weight of dried microspheres}}{\text{Initial weight of drug and polymer}} \times 100 \quad \text{.....Eq. (5)}$$

Response Surface Analysis of Variables (Y_1-Y_4) by Design-Expert Software: Three-dimensional response surface plots and corresponding two-dimensional contour plots were generated using model graphs tool of Design-Expert software to analyze transformation in response due to changes in level of independent variables [11, 16, 17].

Search for Optimum Batch of NPH-MS by Numerical Optimization Method: Optimal values of formulation and process variables for fabrication of NPH-MS were acquired on previously mentioned constrained criterion through numerical optimization method using Design-Expert software [16]. It involved setting optimization criteria followed by running optimization to generate desirability bar graph. Report of optimized composition and processing conditions for optimal formulation of NPH-MS along with predicted values of response variables was generated by Design-expert® 9.0.5.1 software. To acquire graphical view of region corresponding to highest overall desirability function, three-dimensional response surface graph and corresponding contour plot were generated for desirability coefficient as a function of changes in formulation variables.

Check Point Batch Analysis: Optimal values of formulation and process variables for synthesis of optimized NPH-loaded biodegradable microspheres with highest desirability coefficient were acquired using Design-expert® 9.0.5.1 software on set constrained criterion. For validation of optimization strategy, check point batch of NPH-MS was synthesized using optimal values of variables and evaluated for Y_1-Y_4 as per aforementioned procedures. Eq. 6 was used for calculating percent bias between experimental and model predicted values of response variables [10].

$$\% \text{ Bias} = \frac{|\text{Experimental value} - \text{Predicted value}|}{\text{Predicted value}} \times 100 \quad \text{.....Eq. (6)}$$

Statistical Analysis: All the experiments were performed in triplicate, and results were expressed as mean ± SD (n = 3). Statistical analysis of other data was performed using using GraphPad Prism version 5.01 (GraphPad Software, San Diego California, USA). Data were evaluated by Bonferroni post-test, and value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION:

Response Surface Analysis of Variables (Y_1-Y_4) by Design-Expert Software:

Entrapment Efficiency (% EE) (Y_1): A polymeric drug delivery system must be capable to entrap utmost possible quantity of drug with minimum amount of polymer, therefore, optimum drug: polymer for highest % EE should be acquired by evaluating the effects of formulation and process variables on % EE (Y_1). The effect of design variables on % EE can be described by following second-order polynomial equation:

$$Y_1 = 84.37 + 6.26X_1 + 0.99X_2 + 0.54X_3 + 0.041X_4 - 1.12X_5 + 0.19X_1X_2 - 0.60X_1X_3 - 0.41X_1X_4 + 0.31X_1X_5 - 0.60X_2X_3 + 1.73X_2X_4 - 1.10X_2X_5 - 0.48X_3X_4 - 0.34X_3X_5 - 0.88X_4X_5 - 8.23X_1^2 - 0.67X_2^2 - 6.99X_3^2 - 6.86X_4^2 - 3.24X_5^2 \ (r^2 = 0.9902) \ldots \text{Eq.(7)}$$

Model *F*-value and lack of fit *F*-value for % EE was found 55.29 ($p < 0.0001$) and 43.65 ($p > 0.05$), respectively, which implies that model is significant and lack of fit is non-significant relative to pure error indicating accuracy of data. Correlation coefficient (r^2=0.9902) for polynomial quadratic equation revealed that model fitted the data significantly.

TABLE 3: REPORT OF ANALYSIS OF VARIANCE FOR % EE (Y_1), MEAN DIAMETER (Y_2), % DL (Y_3), AND % YIELD (Y_4) OF NPH-MS

Model term	Y_1		Y_2		Y_3		Y_4	
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Model	55.29	<0.0001*	47.91	< 0.0001*	67.27	< 0.0001*	26.02	< 0.0001*
b_1	182.39	<0.0001*	808.65	< 0.0001*	444.36	< 0.0001*	199.36	< 0.0001*
b_2	4.52	0.0570	0.057	0.8154	5.50	0.0388*	0.30	0.5932
b_3	1.36	0.2677	8.31	0.0149*	2.32	0.1556	5.83	0.0343*
b_4	7.916E-003	0.9307	14.56	0.0029*	0.045	0.8367	27.58	0.0003*
b_5	5.87	0.0339*	0.90	0.3641	8.32	0.0148*	35.21	< 0.0001*
b_{12}	0.11	0.7499	6.256E-004	0.9805	0.023	0.8810	2.52	0.1408
b_{13}	1.12	0.3119	0.18	0.6765	1.60	0.2323	4.65	0.0541
b_{14}	0.51	0.4886	3.76	0.0785	0.30	0.5961	2.69	0.1290
b_{15}	0.30	0.5967	1.33	0.2725	1.55	0.2390	0.028	0.8705
b_{23}	1.11	0.3138	0.025	0.8766	0.60	0.4543	0.23	0.6408
b_{24}	9.24	0.0113*	0.31	0.5878	13.81	0.0034*	2.21	0.1654
b_{25}	3.76	0.0787	0.37	0.5564	4.01	0.0706	1.58	0.2352
b_{34}	0.71	0.4189	0.85	0.3771	0.18	0.6816	3.32	0.0957
b_{35}	0.37	0.5579	0.11	0.7493	2.50	0.1424	5.45	0.0396*
b_{45}	2.42	0.1482	0.34	0.5728	2.23	0.1633	1.70	0.2194
b_1^2	396.64	<0.0001*	99.29	< 0.0001*	242.08	< 0.0001*	58.71	< 0.0001*
b_2^2	2.63	0.1334	9.88	0.0093*	3.36	0.0942	0.24	0.6324
b_3^2	285.89	<0.0001*	6.54	0.0266*	340.56	< 0.0001*	48.72	< 0.0001*
b_4^2	275.75	<0.0001*	1.63	0.2283	328.52	< 0.0001*	43.88	< 0.0001*
b_5^2	61.34	<0.0001*	13.86	0.0034*	73.67	< 0.0001*	110.98	< 0.0001*
Lack of Fit	43.65	0.1410	1.05	0.4880	2.23	0.1983	0.83	0.5933

*Statistically significant ($p < 0.05$).

Positive signs of regression coefficient X_1, X_2, X_3 and X_4 illustrated that % EE of NPH-MS increased with increasing values of these design variables. The factors affecting % EE with strong linear effect were X_1 (*F*-value, 182.39) and X_5 (*F*-value, 5.87) ($p < 0.05$). X_2 and X_4 had significant interaction effect on % EE (*F*-value, 9.24; $p < 0.05$). X_1, X_3, X_4 and X_5 had significant quadratic influence. The effect of design variables on % EE according to level of significance was $X_1 > X_5 > X_2 > X_3 > X_4$ (**Table 3**).

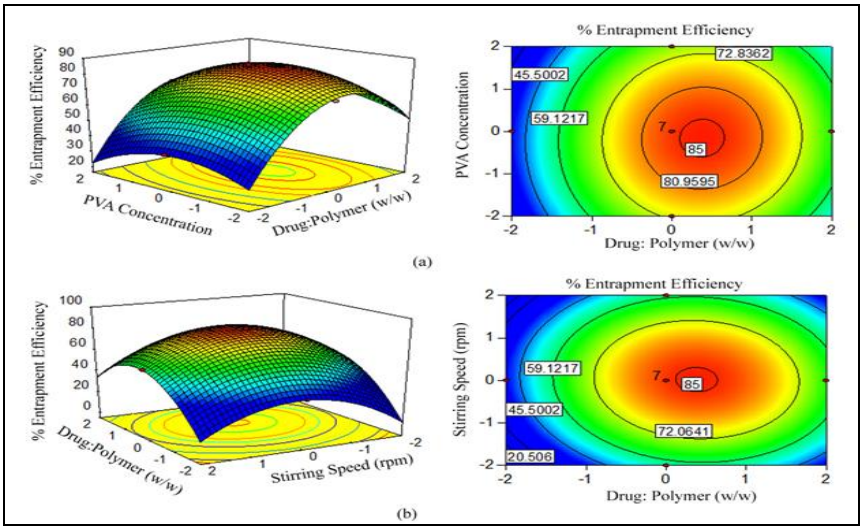

FIG. 2: RESPONSE SURFACE (3D) AND CORRESPONDING COUNTER (2D) PLOTS SHOWING THE EFFECT OF (A) DRUG: POLYMER AND PVA CONCENTRATION ON % EE, (B) DRUG: POLYMER AND STIRRING SPEED ON % EE

Fig. 2 suggested that increasing drug: polymer directly increased drug entrapment in NPH-MS which can be due to increased viscosity of droplets accommodating larger amount of drug. Secondly, viscous solution forms a barrier that might reduce the diffusion of NPH from into external aqueous phase. Furthermore, increasing amount of polymer would produce particles of larger volume to entrap greater amount of drug [11, 18-21]. Negative effect of PVA concentration on % EE can be illustrated by **Fig. 2a**. The increased concentration of PVA might result in reduction in free energy at interface leading to increased drug adsorption on particle surface, therefore reduced drug entrapment in microspheres [22].

Mean Diameter (Y_2): A second-order polynomial quadratic Eq. (8) developed by multiple regression analysis of mean diameter values can adequately describe the effect of selected variables on mean diameter.

$Y_2 = 108.73 + 68.46X_1 - 0.58X_2 - 6.94X_3 - 9.19X_4 + 2.28X_5 - 0.074X_1X_2 + 1.26X_1X_3 - 5.72X_1X_4 + 3.41X_1X_5 - 0.47X_2X_3 - 1.65X_2X_4 + 1.79X_2X_5 - 2.71X_3X_4 + 0.97X_3X_5 + 1.71X_4X_5 + 21.38X_1^2 + 6.74X22 + 5.49X32 + 2.74X42 + 7.99X52$ (r2 = 0.9886) .....Eq. (8).

F-test was performed to determine significance of regression model and *F*-value 47.91 (< 0.0001) indicated that model was statistically significant. The goodness of fit of model was revealed by non-significant lack of fit *F*-value 1.05 ($p > 0.05$) [23, 24]. From values of coefficient, it appeared that drug: polymer (X_1) and PVA concentration (X_5) positively affected the mean diameter. In contrast, PHB: PCL (X_2), stirring speed (X_3) and stirring time (X_4) had antagonistic effect on particle size of NPH-MS. None of the significant interaction effect of variables was observed for mean diameter. The effect of selected variables on mean diameter of NPH-MS can be arranged in sequence $X_1 > X_4 > X_3 > X_5 > X_2$ (**Table 3**).

Mean diameter of NPH-MS decreased dramatically with an increase in PVA concentration (**Fig. 3a**). The relationship between PVA concentration and mean diameter was quadratic as indicated by significant *p*-value of X_5^2 ($p < 0.05$). PVA molecules exist as monomers at lower concentration producing coalescence of globules to generate microspheres having greater mean diameter. At higher concentration (above CMC), PVA molecules self-associate to produce micelles which can align at interface to yield stable emulsion with fine droplets [25-27]. Mean diameter of microspheres decreased significantly with an increase in stirring speed (**Fig. 3a**). The shear force exerted at high rpm might result in formation of particles with smaller size [11].

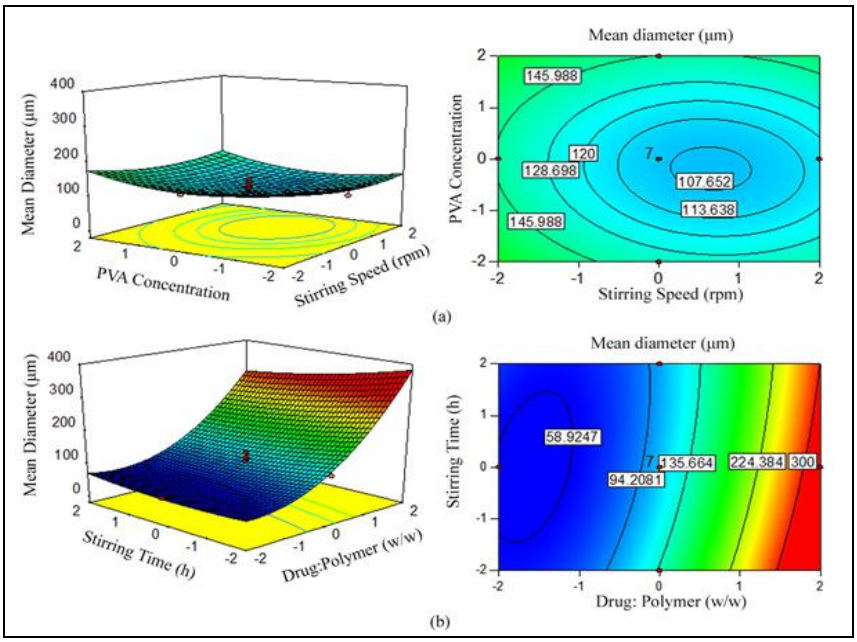

FIG. 3: RESPONSE SURFACE (3D) AND CORRESPONDING COUNTER (2D) PLOTS DISPLAYING THE EFFECT OF (A) STIRRING SPEED AND PVA CONCENTRATION ON MEAN DIAMETER, (B) DRUG:POLYMER AND STIRRING TIME ON MEAN DIAMETER

Fig. 3b demonstrated that an increase in drug: polymer linearly increased mean diameter of NPH-MS which could be owing to improved viscosity of organic phase which provide larger resistance to sonication, consequently promoting fabrication of larger droplets [25]. Furthermore, PVA was insufficient to stabilize the emulsion droplets at higher polymer concentration leading to coalescence of droplets [19-21].

% Drug Loading (% DL) (Y_3): Model proposed quadratic polynomial equation for estimating the effect of selected variables on % DL (Y_3). Synergistic effects of X_2, X_3, X_4 and antagonistic effects X_1, X_5 was observed for % DL as illustrated by following equation:

$$Y_3 = 21.10 - 2.25X_1 + 0.25X_2 + 0.16X_3 + 0.022X_4 - 0.31X_5 - 0.020X_1X_2 - 0.17X_1X_3 - 0.071X_1X_4 + 0.16X_1X_5 - 0.10X_2X_3 + 0.49X_2X_4 - 0.26X_2X_5 - 0.055X_3X_4 - 0.21X_3X_5 - 0.19X_4X_5 - 1.48X_1^2 - 0.17X_2^2 - 1.75X_3^2 - 1.72X_4^2 - 0.82X_5^2 \ (r^2 = 0.9919)$$ ...Eq.(9)

Model *F*-value and lack of fit *F*-value for % EE was found 67.27 ($p < 0.0001$) and 2.23 ($p > 0.05$), respectively, which implies that model is significant and lack of fit is non-significant relative to pure error indicating accuracy of data. % DL was significantly influenced by X_1, X_2, X_5, X_2X_4, X_1^2, X_3^2, X_4^2, and X_5^2 ($p < 0.05$) (**Table 3**). **Fig. 4** depicted that increase in drug: polymer and stirring speed initially increased the % DL followed by decline implying maximum % DL can be achieved at center level [19-21, 28].

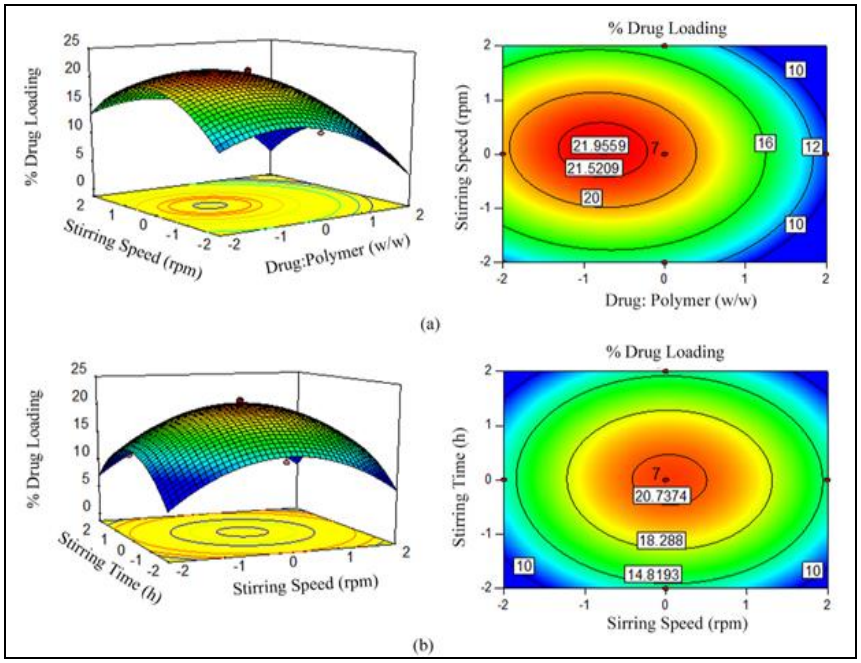

FIG. 4: RESPONSE SURFACE (3D) AND CORRESPONDING COUNTER (2D) PLOTS DISPLAYING THE EFFECT OF (A) STIRRING SPEED AND DRUG : POLYMER ON % DL, (B) STIIRING SPEED AND STIRRING TIME ON % DL

% Yield (Y_4): Results in **Table 3** signified that independent factors significantly affecting % yield were drug: polymer (X_1), PHB: PCL (X_2), stirring time (X_3) and PVA concentration (X_5) ($p < 0.05$). The effect can be elucidated through mathematical relationship depicted in following equation:

$$Y_4 = 78.47 + 5.57X_1 - 0.22X_2 - 0.95X_3 - 2.07X_4 - 2.34X_5 + 0.77X_1X_2 + 1.04X_1X_3 + 0.79X_1X_4 - 0.081X_1X_5 - 0.23X_2X_3 - 0.72X_2X_4 - 0.61X_2X_5 - 0.88X_3X_4 + 1.13X_3X_5 + 0.63X_4X_5 - 2.69X_1^2 - 0.17X_2^2 - 2.45X_3^2 - 2.33X_4^2 - 3.70X_5^2 \ (r^2 = 0.9793)$$..Eq. (10).

F-test was performed to determine significance of regression model and *F*-value 26.02 (< 0.0001) indicated that model was statistically significant. The goodness of fit of model was revealed by non-significant lack of fit *F*-value 0.83 ($p > 0.05$) [23, 24]. All the factors produced antagonistic effects on % yield of NPH-MS except drug: polymer as implied by positive sign of coefficient X_1. **Fig. 5** represented that increase in drug: polymer produced positive effect while stirring speed and stirring time illucidated negative impact on % yield of NPH-MS [15, 19-21, 28].

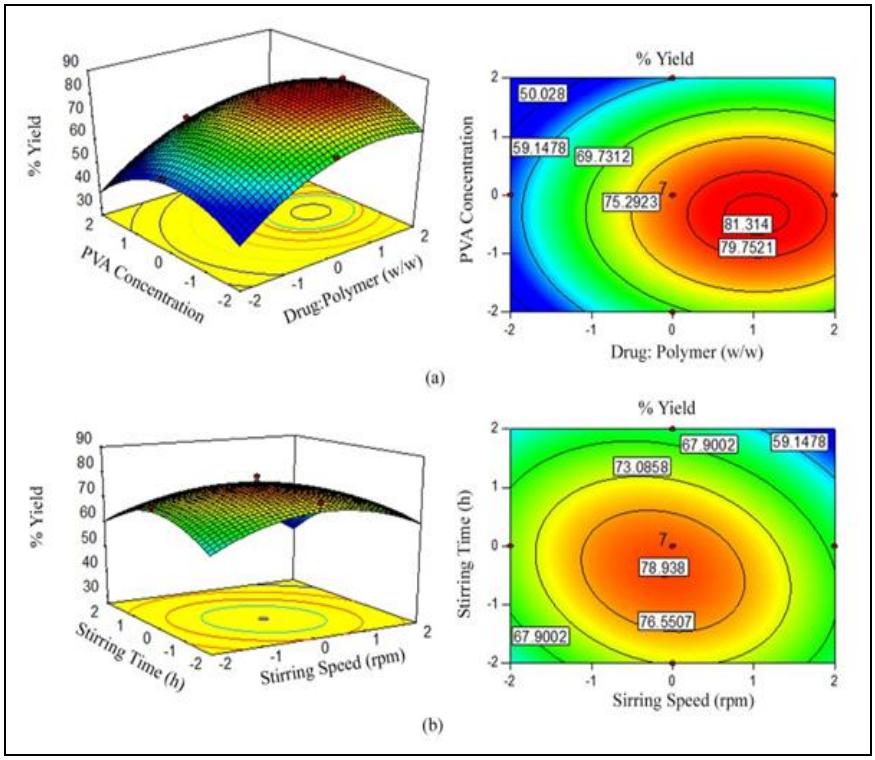

FIG. 5: RESPONSE SURFACE (3D) AND CORRESPONDING COUNTER (2D) PLOTS DISPLAYING THE EFFECT OF (A) DRUG: POLYMER AND PVA CONCENTRATION ON % YIELD, (B) STIRRING SPEED AND STIRRING TIME ON % YIELD

Optimized Batch of NPH-MS by Numerical Optimization Method: Optimal values of formulation and process variables producing highest overall desirability function, D = 0.911 was obtained by numerical optimization method using Design-Expert software (Table 4). Predicted values of response variables for optimized formulation have been represented in **Table 5**. **Fig. 6** represented desirability bar graph which showed that partial desirability function of response variables (d_1-d_4) as well as overall desirability function (D) for optimized NPH-MS predicted by Design Expert Software. **Fig. 7** depicted response surface plot and corresponding counter plot for overall desirability coefficient as a function of change in drug: polymer (X_1) and PHB: PCL (X_2) keeping stirring speed, stirring time and PVA concentration constant.

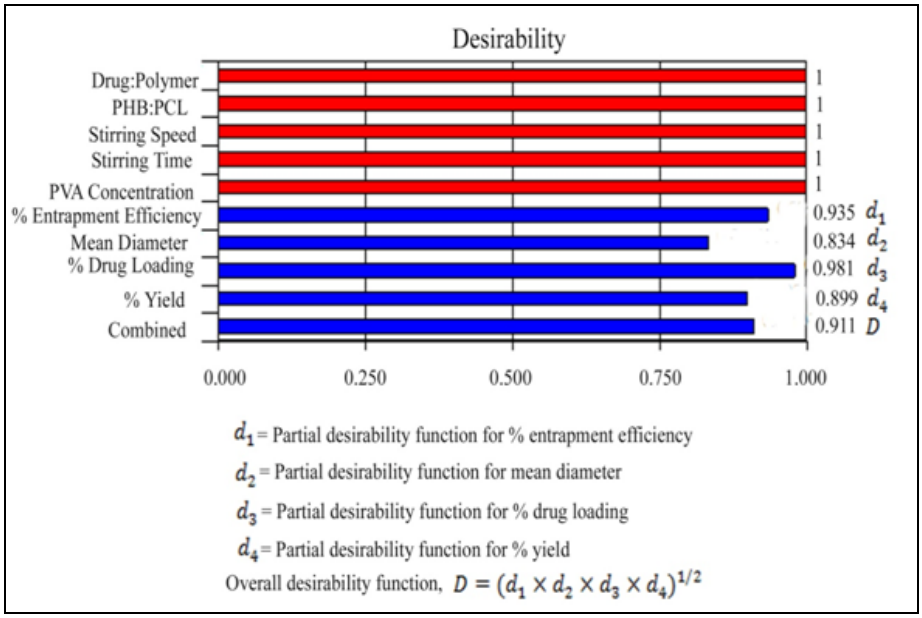

FIG. 6: DESIRABILITY FUNCTION BAR GRAPH FOR OPTIMIZED NPH-MS

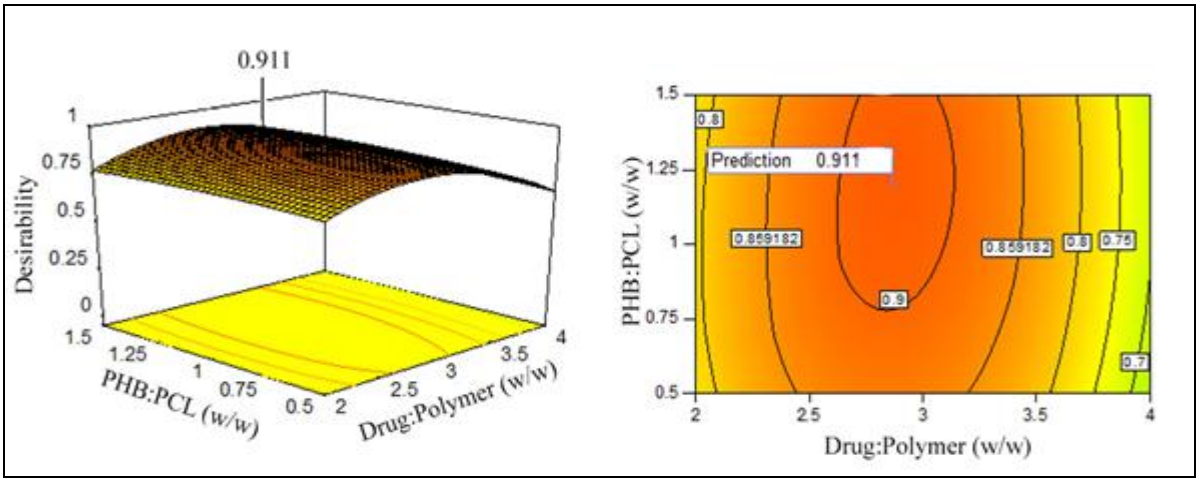

FIG. 7: RESPONSE SURFACE (3D) AND CORRESPONDING COUNTER (2D) PLOTS FOR DESIRABILITY FUNCTION OF OPTIMIZED MICROSPHERES

TABLE 4: REPORT OF FORMULATION AND PROCESS VARIABLES FOR OPTIMIZED BATCH OF NPH-MS PREDICTED BY DESIGN-EXPERT® 9.0.5.1 SOFTWARE

Independent variables	Criteria	Importance	Value	Desirability
X_1= Drug: polymer ratio (w/w)	In range	+++	1: 2.86	
X_2= PHB:PCL (w/w)	In range	+++	1.19	
X_3= Stirring speed (rpm)	In range	+++	1501.36	0.911
X_4= Stirring time (h)	In range	+++	2.98	
X_5= PVA concentration (%, w/v)	In range	+++	0.54	

Check Point Analysis and % Bias of Optimized Batch of NPH-MS: Comparison of experimental and model predicted values of response variables for optimized microspheres validated the authenticity of predictive power of designed model as indicated by % bias value < 5% (**Table 5**).

TABLE 5: COMPARISON OF EXPERIMENTAL AND MODEL PREDICTED VALUES OF RESPONSE VARIABLES FOR OPTIMIZED NPH-MS

Response variables	Predicted value	Experimental value	Bias (%)
Y_1= Entrapment efficiency (% EE, w/w)	83.80	81.29	2.99
Y_2= Mean diameter (μm)	100.78	105.63	2.82
Y_3= Drug loading (% DL, w/w)	21.50	21.05	2.09
Y_4= Yield (%, w/w)	77.99	76.84	1.47

CONCLUSION: Nefopam hydrochloride loaded poly-(ε-caprolactone) and poly-3-hydroxybutyrate microspheres were efficiently manufactured by double emulsion solvent evaporation technique. Central composite design response surface methodology could be successfully utilized for investigating the relationship between independent and dependent variables of formulation. Positive and negative signs of regression coefficient in second-order polynomial equations developed by Design-expert software indicated synergistic and antagonistic effect of selected factors on response variables, respectively. Optimized NPH-MS estimated by design-expert software have highest desirability function, D = 0.911. The present study conclusively manifested that the optimal formulations of NPH-MS contains drug: polymer (1: 2.86, w/w), PHB: PCL (1: 1.19, w/w), stirring speed (1501 rpm), stirring time (2.98 h) and PVA concentration (0.54%, w/v). The model predicted values of % entrapment efficiency (Y_1), mean diameter (Y_2), % drug loading (Y_3), and % yield (Y_4) for optimized NPH-MS were 83.80%, 100.78μm, 21.50% and 77.99%, respectively. Check point batch analysis illustrated that % bias between experimental and model predicted values was < 5% which authenticated the legitimacy of predictive power of designed model. Conclusively, it was manifested that quality-by-design approach could be effectively utilized for formulation optimization.

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