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Original Research Article

Applicability of Taguchi Design in Development of Microparticles for Pulmonary Delivery

Riddhi A. Shah[1], Vaishali T. Thakkar[1]*, Mukesh C. Gohel[1], Purvi Shah [2], Lalji H. Baldaniya[1]
[1]Department of Pharmaceutics, Anand Pharmacy College, Anand-388001 Gujarat, India
[2]Department of Quality assurance, Anand Pharmacy College, Anand-388001 Gujarat, India

***Corresponding Author:**
Dr. Vaishali T. Thakkar
Email: vtthakkar@rediffmail.com

Abstract: Biodegradable gemifloxacin microparticles were prepared to eradicate the resistant type pneumonia. For preparation of microparticles different techniques (solvent evaporation, solvent-evaporation crosslinking, double emulsion and spray drying) were used and compared. The process of spray drying using poly(d,l-lactic co-glycolic)acid was choosen considering the particle size and percentage yield. The critical processes as well as product related parameters were identified by Quality by Design approach. Optimization of formulation was performed by Taguchi design using particle size, percentage yield and percentage encapsulation efficiency as dependent variable while drug to polymer ratio, aspirator rate, inlet temperature and flow rate as independent variable. Optimized formulation was evaluated for particle size, percentage yield, percentage entrapment efficiency, in-vitro diffusion study and aerodynamic behavior. Taguchi design suggest that the batch containing drug to polymer (2:1), aspirator rate(50), inlet temperature (65°C) and flow rate (10ml/min) gives highest encapsulation efficiency (84%) and lowest particle size (2.75µm). The DSC and FTIR study revealed that there was no significant interaction between drug and polymer. The result of diffusion study revealed that the optimized batch was released at a controlled rate. Overall, the proposed formulation can be explored for inhalation route by the patient to achieve better targeting in deeper parts of lungs.
Keywords: Dry powder inhaler, Microparticles, Gemifloxacin, Lung target, Taguchi design.

INTRODUCTION

Pneumonia is a disease in which the alveoli get inflamed (irritated and swollen) and fill with fluid which makes difficulty in breathing. Pneumonia can be generally caused by bacteria, viruses, fungi or irritant things which get inhaled in the lungs. There are four types of pneumonia i.e. community acquired pneumonia, hospital acquired pneumonia, aspiration pneumonia and opportunistic pneumonia [1]. Multiple drug-resistant isolates of Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, and Enterobacteria species cause 16% of all health care-associated infections, putting the medical community on the defensive [2]. Due to the rapid spread of these isolates and the difficulty of treating infections caused by antibiotic-resistant bacteria, there is an urgent need for novel bactericides and the newer effective drug delivery system [3].

Gemifloxacin is a novel synthetic broad-spectrum fluoroquinolone that exhibits bactericidal activity by inhibiting DNA-gyrase [4,5]. It found to have good activity on the resistant acquired species of bacteria like Streptococcus species and Staphylococcus species. Hence, here a step was taken to prepare gemifloxacin microparticles for pulmonary delivery as empirical therapy in pneumonia [4,5].

Pulmonary delivery of drug has become an attractive target and of tremendous scientific and biomedical interest in the health care research area as the lung is capable of absorbing pharmaceuticals either for local deposition or for systemic delivery. Lungs provide high surface area to the particles which get inhaled in the body. Thus, pulmonary delivery is able to provide good target to the site. Dose of drug administration is tremendously decreases and chances of acquiring resistance also decreases due to its targeted delivery also it will not allow bacteria to acquire resistance [6].

Microparticles are defined as the solid colloidal particles which include both microspheres and microcapsules. They are prepared by using preformed polymers. The biodegradable polymers are having their important role as they improve the target-ability, control drug release, protect inactivation of compounds,

increases intracellular penetration and also enhance pharmacological activities. So, polymers like chitosan, poly-lactic acid (PLA) and poly(d,l,lactic co-glycolic) (PLGA) acid have been tried [7–10].

Now, in this era of competition one has to work smartly hence use of sophisticated techniques is very much important so in the preparation of microparticles concept of quality by design is applied. As this leads to build quality in product which is quite safe and effective in less time utilizing less resources with less wastage. For this Taguchi is the best suited design as one can do screening and optimization of parameters simultaneously and the best thing about this it will act directly to achieve the target. In this design, orthogonal arrays arrange the affecting parameters and their levels in the way, most likely to affect the process. Unlike factorial design, where all the possible combinations are tested, Taguchi employs a minimal number of trials by testing pairs of combinations. As 3^4 Full Factorial design gives 81 run while Taguchi design gives only 9 run so, by using Taguchi design approach one can effectively works with less resources and that too in less time. The optimal parameters obtained from these trials are insensitive to environmental changes and other noise factors. Other key process parameters such as the drying air flow, the product variables (raw material characteristics) were kept constant. Here, signal to noise ratio is of prime importance as its value is high it means variability is less. So, we can say that Taguchi design concentrates on product robustness against uncontrollable factors (i.e. noise).

MATERIAL AND METHOD

Materials

Gemifloxacin was procured from Swapanroop pharmaceuticals and chemicals ltd. Mumbai, India. Chitosan, Polylactic acid (PLA) and Poly (d,l-lactic co-glycolic) acid (PLGA) (50:50, 75:25) was procured from Evonik industries, Mumbai, India. Potassium hydrogen orthophosphate, disodium hydrogen phosphate and sodium chloride were procured from Astron Chemicals Pvt. Ltd, Ahmedabad, India. All the solvents were procured from S.D. Fine Chem ltd., Mumbai, India.

Experimental design

During preliminary study of the gemifloxacin microparticles number of initial trials were conducted to screen polymer (PLGA (50:50)), method (Spray drying) and solvent system (DCM : Methanol) hence by using PLGA (50:50) polymer with DCM : Methanol solvent system for spray drying method it was found that good characteristic microparticles were prepared in terms of yield, particle size, reproducibility and flowability. Here, Taguchi design was choosen to find out the most optimum condition. Taguchi design L9 orthogonal array experimental design was used for screening and optimization of process variables affecting the characteristics of PLGA loaded microparticles. A standard design using four independent variables named drug to polymer ratio, aspiratory rate, inlet temperature and flow rate (X_1, X_2, X_3 and X_4) and particle size, % yield and % entrapment efficiency (Y_1, Y_2 andY_3) were selected as dependent variables as shown in Table 1-2 by selecting L9 orthogonal array which suggests total nine run of experiment.

Response Surface Analysis

The results are expressed as two factor interaction polynomial equation of the following term:

$$Y_1= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 \quad \text{(Eq.6)}$$

$$Y_2= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 \quad \text{(Eq.7)}$$

$$Y_3= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{24}X_2X_4 \quad \text{(Eq.8)}$$

Where Y_1, Y_2 & Y_3 are the predicted response, b_0 is the airthmetic mean response of 9 runs **(Table 3).** The main effect of X_1, X_2, X_3 and X_4 represents the average result of changing one factor at a time from its low value to its high value. The interaction effect shows how the factors will affect when they are change simultaneously. The co-efficient corresponding to linear effect (b_1, b_2, b_3 and b_4) and interaction (b_{12}, b_{13}, b_{23} and b_{24})were determined from the results of the experiment (STAT-EASE, Design-Expert, 10.0, Trial version). To assess the reliability of the model, a comparision between the experimental and predicted values of the responses is also presented in terms of % Error in **Table 4**.

The formula for %Error is as follows:

$$\textbf{\% Error}= \frac{\textit{Predicted value} - \textit{Actual value}}{\textit{Predicted value}}$$

Preparation of feed for spray drying

Gemifloxacin loaded PLGA 50:50 microparticles were prepared by spray drying technique. The polymer was first dissolved in DCM (15ml) and on other side gemifloxacin was dissolved in methanol (5 ml) then mix both of them and homogenize at 5000 rpm. A clear transparent yellowish solution was obtained.

Spray drying

The feed was spray dried using a LU-222 Labultima advanced, India. Spray dry the feed solution at inlet temperature 55-65°C, feed rate 5-10ml/min and aspirator rate 40-50. The prepared microparticles were collected in the collection chamber.

EVALUATION OF MICROPARTICLES

In-vitro Diffusion study

The in vitro drug release test was performed by using Franz diffusion cell using Dialysis membrane 110. The receptor compartment was filled with 22.7 ml Phosphate buffer pH 7.4 and maintained at 37^0C ± 0.5^0C. Samples were periodically withdrawn from the receptor compartment & replaced with the same amount of fresh buffer solution, and were assayed U. V. spectrophotometrically at 267nm [12].

Particle size analysis

Particle size was determined by particle size analyzer Malvern ZS 2000 Gorveywood road, Engima business park, U.K. Microparticles were dispersed in distilled water and analyzed the particle size under particle size analyzer [17]. Mean particle size and polydispersion index value was calculated.

$$\text{Polydispersibility index}=\frac{(D0.9-D0.1)}{D0.5} \quad \text{..... (Eq.2)}$$

Aerodynamic study

The aerodynamic properties of the powder were investigated using cascade impactor 20 mg of each sample loaded into a hard gelatine capsule manually. The experiment was carried out at an air flow rate of 60 l/min. A capsule filled with particles will loaded and an actuation time of 4 s was allowed for each capsule to completely disperse all the particles. Particles remaining in the capsule, inhaler, throat, pre-separator, individual impaction plates, and stages were extracted using phosphate buffer. Amount of drug deposited on capsule, inhaler, throat, pre-separator, individual impaction plates, and stages was assayed using UV-spectrophotometrically. Mass Median Aerodynamic Diameter (MMAD), Geometric Standard Deviation (GSD) and % Emitted dose was calculated[15,16].

Production yield

The production yield was calculated as the weight percentage of the final product after drying, with respect to the initial total amount of ingredients used for the preparations [18]. Production yield was calculated by using equation 1.

$$\textbf{\% Production yield}=\frac{W1}{W2}\times 100 \quad \text{..... (Eq.3)}$$

W1= weight of dried microparticle
W2= dry weight of starting materials

Encapsulation efficiency

Accurately weighed 5 mg of drug-loaded microparticles was dissolved in 10 ml of methanol. The resulting mixture was shaking for 24 h in an orbital shaker incubator (Remi, RIS-24BL, and India). After suitable dilution with methanol analyzed by the U.V spectrophotometrically at 272nm [18-20]. Encapsulation efficiency (%) was calculated by using equation 3.

$$\textbf{\% Encapsulation Efficiency}=\frac{\text{drug content in microparticles}}{\text{Initial drug feed}}\times 100 \quad \text{..... (Eq.4)}$$

$$\textbf{\% Loading Efficiency}=\frac{\text{drug content in microparticles}}{\text{total weight of microparticles gain}}\times 100 \quad \text{..... (Eq.5)}$$

SOLID STATE CHARACTERISTICS

Scanning Electron Microscopy (SEM)

Surface morphology of spray dried powder was obtained using Scanning electron microscope (Philips, Philips XL 30 ESEM). Sample was fixed on an aluminum stub with conductive double-sided adhesive tape and coated with gold in an argon atmosphere (50 Pa) at 50 mA for 50 sec to obtain the surface morphology [12, 21].

Fourier Transform Infrared Spectroscopy (FTIR)

The drug-polymer containing microparticles and the pure drug (Gemifloxacin) were subjected to the Fourier-transform infrared spectroscopy in order to detect the existence of interaction between drugs and polymers. The procedure consisted of dispersing a sample (Drug alone, Polymers alone and Physical mixture and Formulation) in KBr to prepare 10% of mixture and ground generally in mortar-pestle with KBr before being compressed into pellets. This pellet was placed in light path and spectrum was recorded at a resolution of 2 cm-1 over a frequency range of 4000 to 400 cm-1. The background spectrum of KBr was used as blank for determination [22].

Differential Scanning Calorimetry (DSC)

DSC was performed using Perkin Elmer instruments, (Perkin Elmer DSC-7, Norway, USA.) to study the thermal behavior of Gemifloxacin mesylate and polymers Poly (d,l-lactide-co-glycolic acid) and mixture of drug and polymers. The instrument comprised of calorimeter (DSC-60), flow controller (FCL-60), Thermal analyzer (TA-60) and operating software (TA-60). The samples (2-4 mg) was heated in hermetically sealed flat-bottomed aluminum pans under nitrogen flow (20ml/min) at a scanning rate of 10^0c/min from 25^0C to 200^0C. Empty aluminum pans were used as the reference standard [23].

Stability study

Short term stability study was carried out at accelerated stability condition. Powder was stored at ambient conditions in capped amber vials (40 ˚C/75% RH).Encapsulation efficiency as well as % CDR were examined [14].

RESULT AND DISCUSSION

Statistical design and analysis

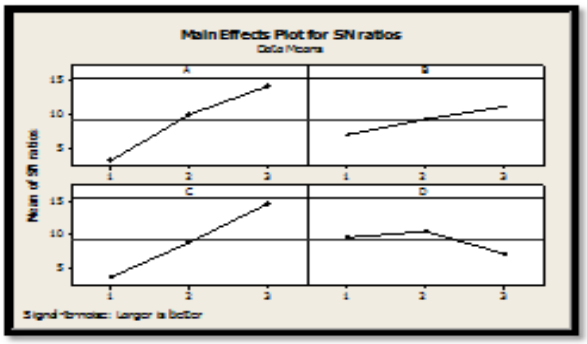
Taguchi design is a six sigma approach which directly works for achieving target with very less number of trials at three levels in L9 orthogonal array i.e. high (+1), medium (0) and low(-1) level shown in table 1 and dependent variables with range shown in table 2. The factor range was selected based on the prior knowledge about the system under study. In Taguchi design the selection was done on the basis of signal to noise ratio plot generated using Minitab software which gives us indication about the most prominently affected factor based on the delta value and rank given to the independent variables.

From the Figure 2(d), shows that there was almost negligible effect of Flow rate on the formed microparticles using different experimental condition. But in oppose to this the Drug: Polymer concentration affects it significantly; lower the concentration of solids lower the particle size of the microparticulate as high concentration leads to evaporation of solvent easier and thus they form more rigid and dense particulate which is not desired. The same thing was concluded from the Delta value and Rank given in the following figure 1(a). As the inlet temperature increases the particles formed in irregular shape and size as the droplets when introduced it directly comes in to contact of high temperature where they dried rapidly and this may cause formation of agglomerates of smaller unit particles. So, the temperature employed in spray drying operation has to be compatible with the material to be dried and the solvent used. High pumping rates during the spray drying process results in large nebulized solution to be dried. Also it may be possible that liquid droplets will not transform rapidly in to solid microparticles, results in formation of larger/irregular particles that are not completely dried after leaving the desiccating chamber. Also incomplete atomization may lead to wider droplet size distribution.

From the Figure 2(e), shows that there was negligible effect of Drug: Polymer ratio on the % yield of microparticles using different experimental condition. But in oppose to this the Inlet temperature affects it significantly, also the flow rate have significant effect. Both the inlet temperature and flow rate works simultaneously as soon as the material was sprayed and comes in contact to the inlet temperature solvent evaporates from the droplets and forms dried microparticles. Higher the flow speed less time was taken by the solvent in the feed pipe hence there was less loss as compare to lower feed rate. The same thing was concluded from the Delta value and Rank given in the following figure 1(b). As the inlet temperature increases the sprayed droplets in the spraying chamber dries rapidly and with this if the aspirator rate is high then it will lead to instant collection of the dried particles from spray chamber to the collector and this two will lead to maximize the yield of the final formulation. Also when the flow rate is high then the solution which is to be sprayed get less time to flow from the feed inlet to the sprayed chamber this will reduce the %loss which can occur while transferring of the solution because here organic solvents are used which are having high volatility at room temperature that can cause decrease in %yield.

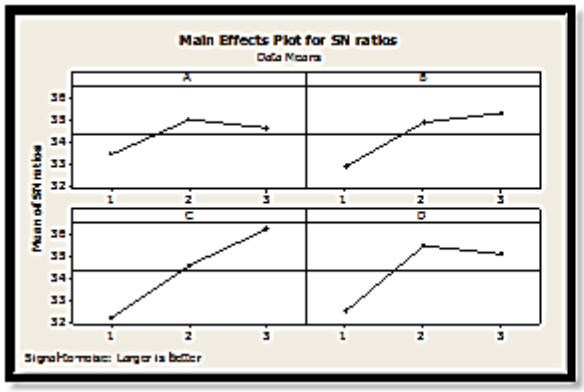
From the Figure 2(f), shows that there was negligible effect of Inlet temperature on the entrapment efficiency of microparticles using different experimental condition. But in oppose to this the Drug: Polymer concentration and aspirator rate have shown significant impact. The same thing was concluded from the Delta value and Rank given in the following table 1(c). We think that there should be maximum entrapment with the higher concentration of polymer used but here the polymer may undergo intermolecular hydrogen bonding with the drug molecule and when high amount of polymer is present with drug molecules then every polymer molecule compete for drug molecules and that leads to formation of improper bonding results in irregular microparticle formation and that leads to less entrapment efficiency. As the flow rate increases the entrapment efficiency may get decreased but when with the flow rate if aspirator rate increases the entrapment efficiency will be increased. Similarly when inlet temperature increases with the aspirator rate the entrapment efficiency will be increased.

Here, low level of Drug to Polymer ratio is selected because of their high encapsulation power as compared to other ratio and also there was negligible effect of the concentration on % yield of the microparticles. So, it is acceptable to go with low level of parameter when it had no significant impact on the output.



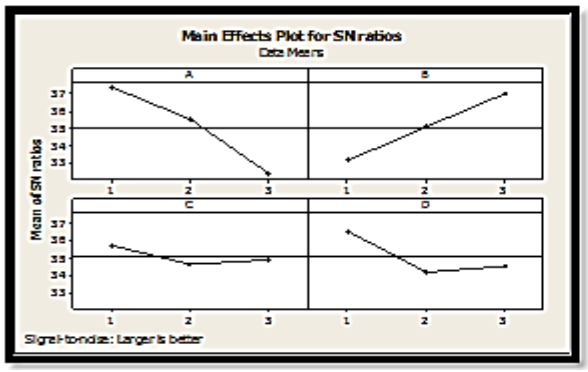
Level	X1	X2	X3	X4
1	3.184	7.034	3.851	9.693
2	10.114	9.338	8.983	10.504
3	14.205	11.131	14.669	7.306
Delta	11.021	4.097	10.819	3.199
Rank	1	3	2	4

Fig–1(a): Taguchi analysis for main effects Particle size versus Drug: Polymer, Aspirator rate, Inlet temperature and Flow rate



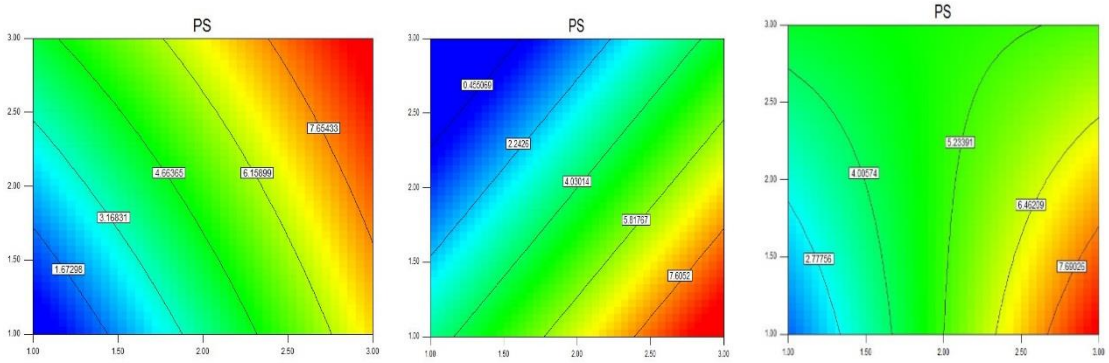
Level	X1	X2	X3	X4
1	33.43	32.89	32.22	32.51
2	35.02	34.90	34.61	35.49
3	34.66	35.32	36.29	35.12
Delta	1.59	2.43	4.07	2.98
Rank	4	3	1	2

Fig–1(b): Taguchi analysis for main effects Percentage yield versus Drug: Polymer, Aspirator rate, Inlet temperature and Flow rate

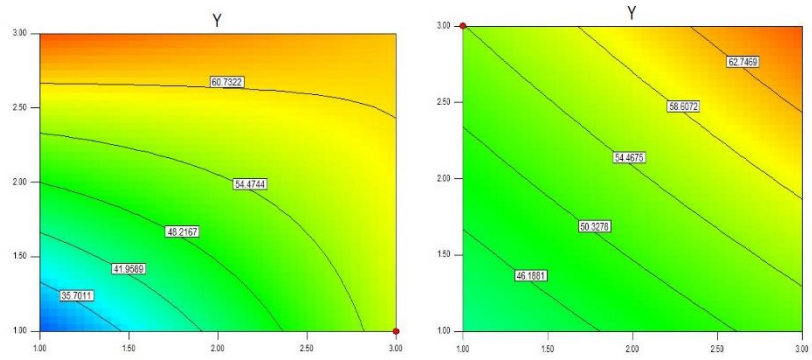


Level	X1	X2	X3	X4
1	37.34	33.15	35.72	36.52
2	35.50	35.10	34.62	34.17
3	32.37	36.98	34.88	34.53
Delta	4.97	3.83	1.10	2.35
Rank	1	2	4	3

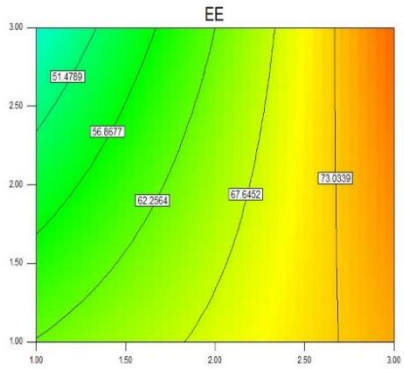
Fig–1(c): Taguchi analysis for main effects Percentage entrapment efficiency versus Drug: Polymer, Aspirator rate, Inlet temperature and Flow rate



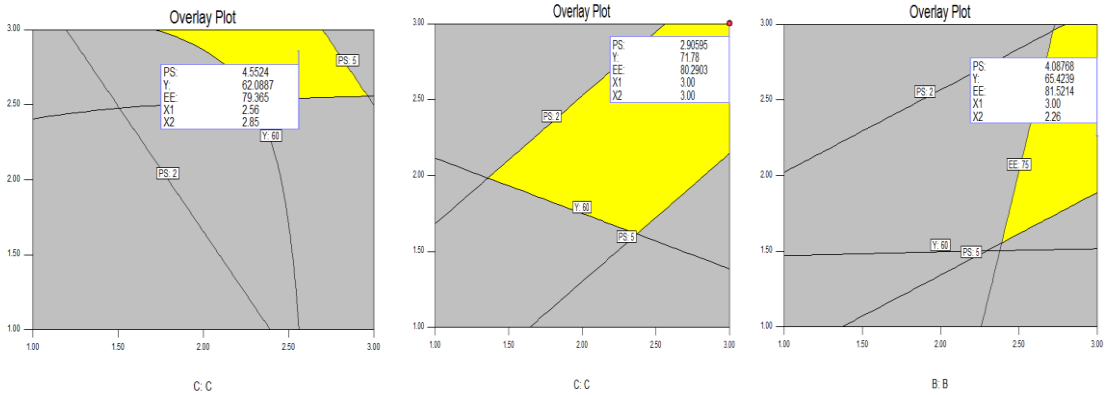
d) (i) Effect of Drug: Polymer concentration and Inlet Temperature on Particle size
(ii) Effect of Drug: Polymer concentration and Flow rate on Particle size
(iii) Effect of Drug: Polymer concentration and Aspirator capacity



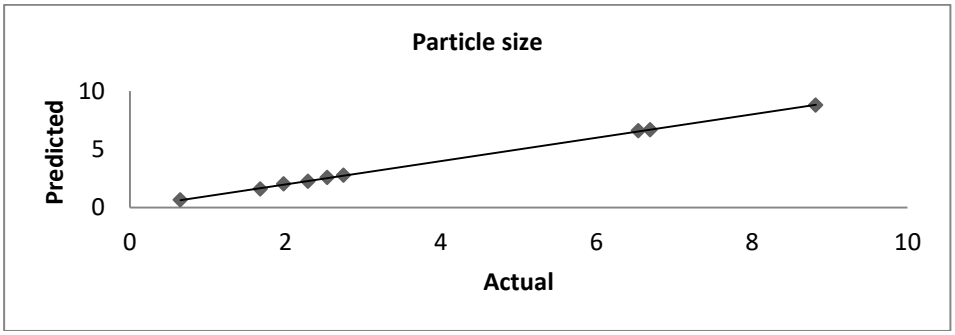
e) (i) Effect of Aspirator rate and Inlet temperature on % Yield
(ii) Effect of Aspirator rate and Flow rate on % Yield



f) (i) Effect of Inlet temperature and Aspirator rate on % Entrapment Efficiency
Fig–2: Interaction effect on dependent variables



g) (i) Temperature vs. Aspirator rate
(ii) Temperature vs. Flow rate
(iii) Aspirator rate vs. Flow rate
Fig-3: Overlay plot



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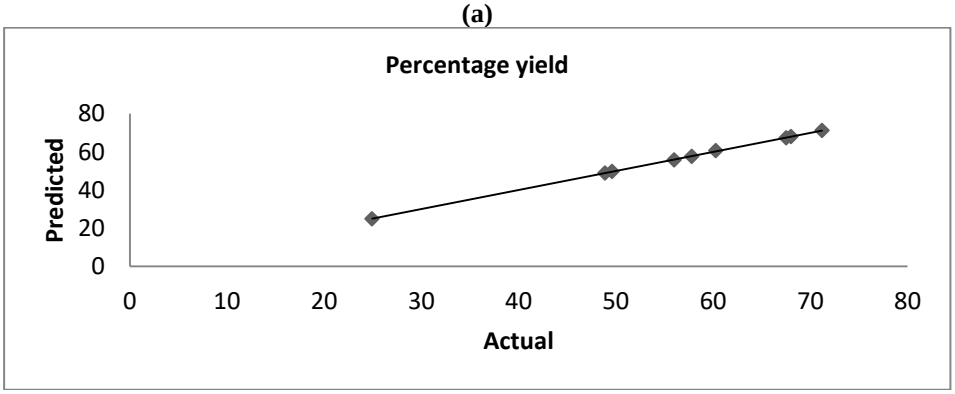

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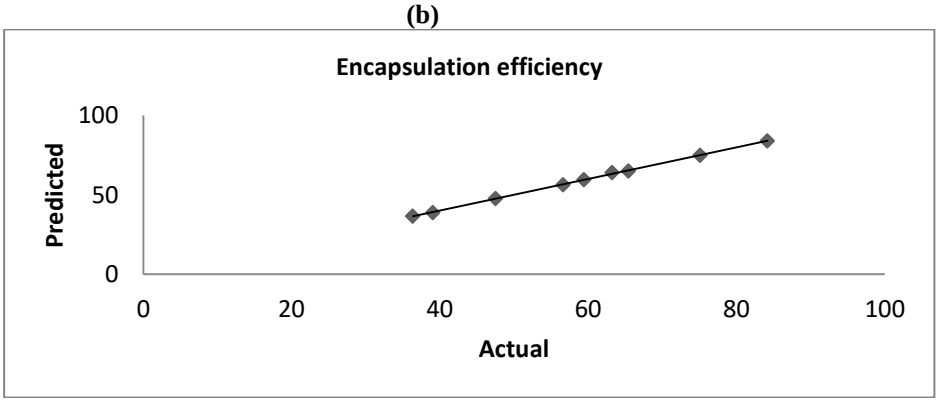
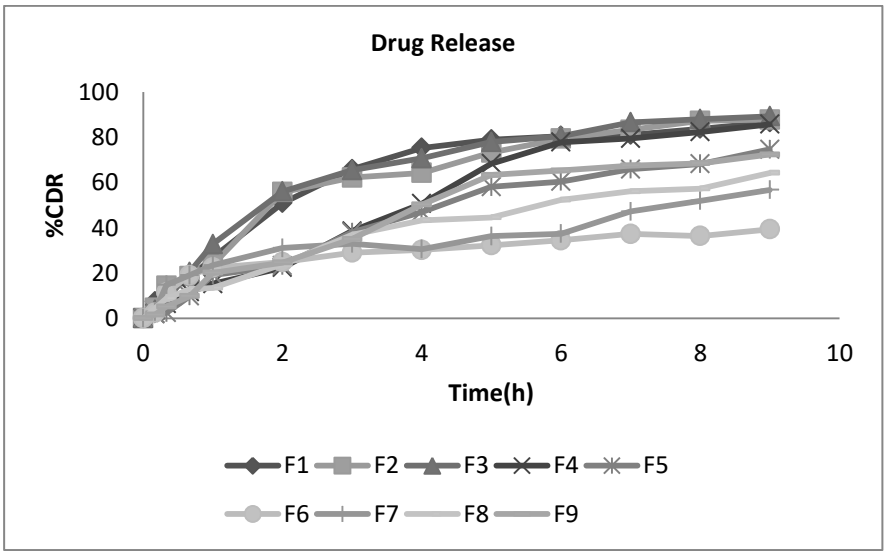
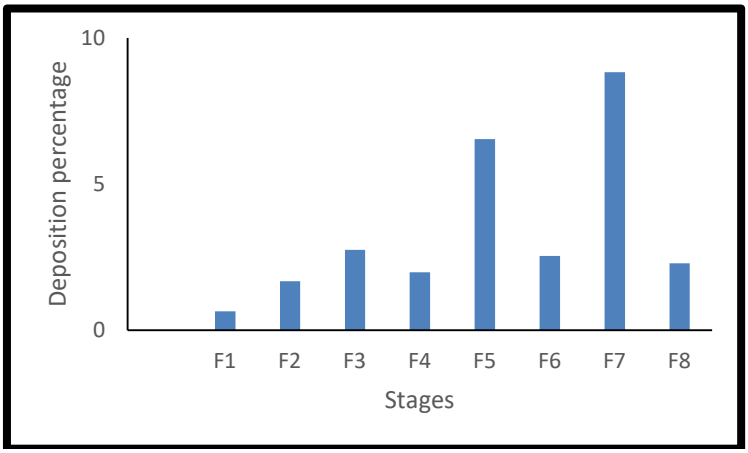


(c)

Fig–4(a-c): Actual response vs. Predicted response



Fig–5: Drug Release profile of Gemifloxacin microparticles



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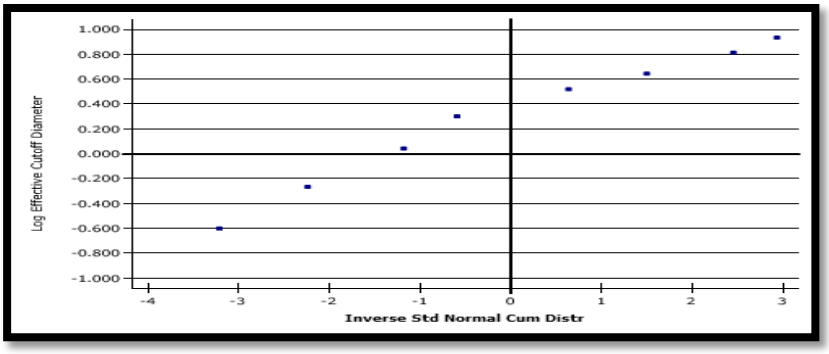
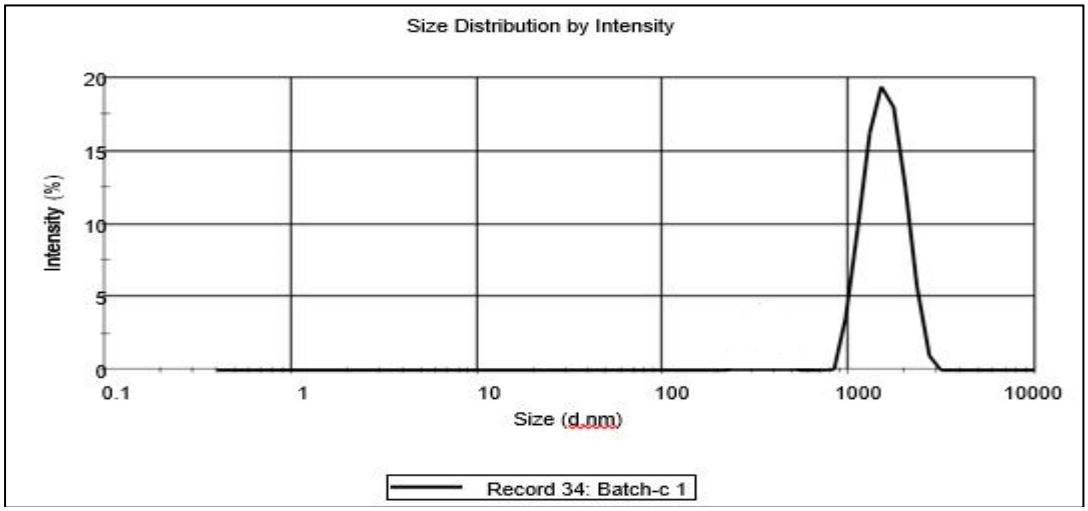
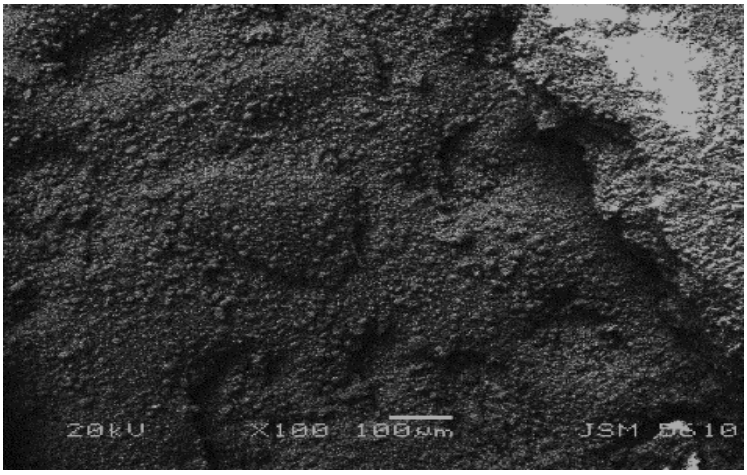


(b)

Fig–6: a) Bar graph representing amount of powder deposited at different stages b) log probability graph



Fig–7: Particle size distribution of optimized batch by intensity



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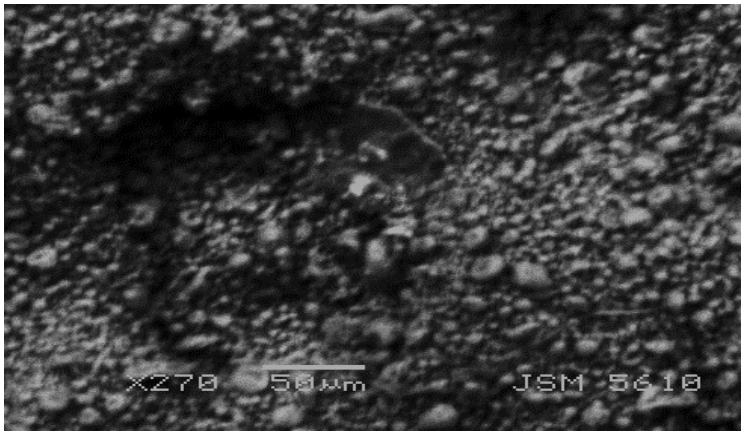

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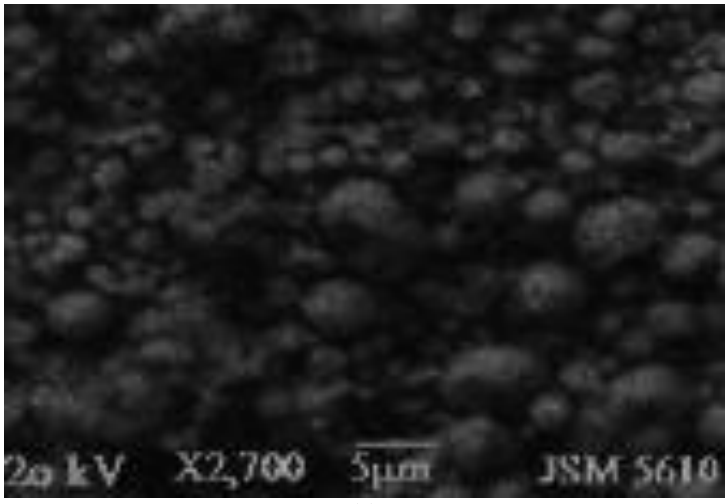
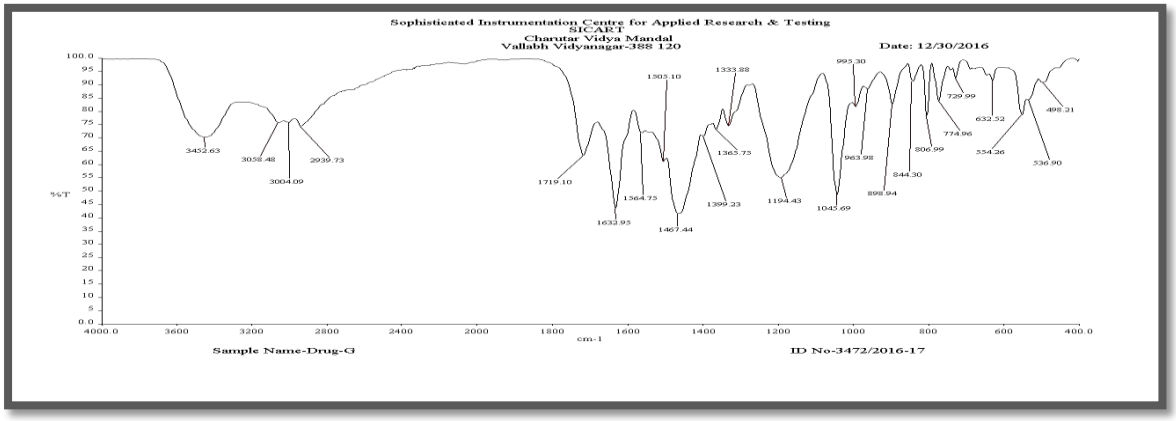


(c)

Fig–8(a-c): SEM micrographs of gemifloxacin microparticles of optimized batch



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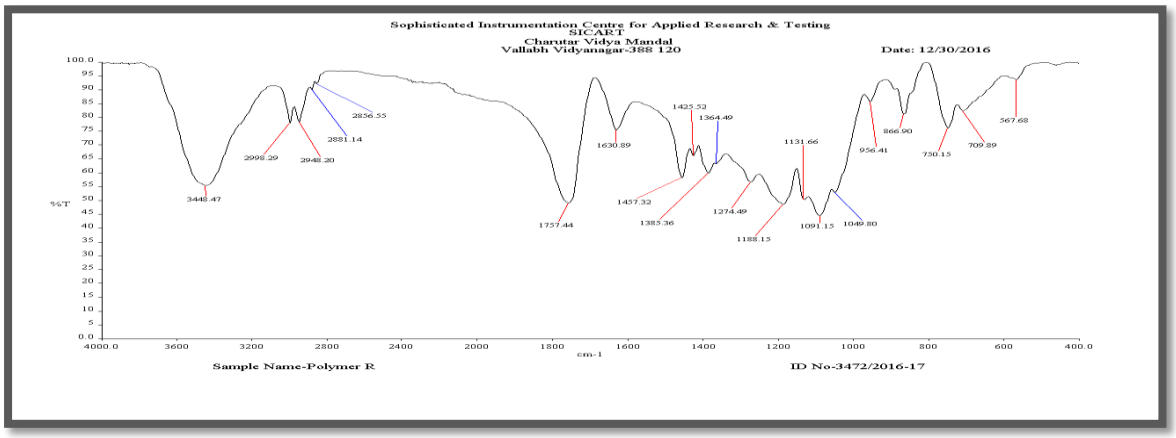

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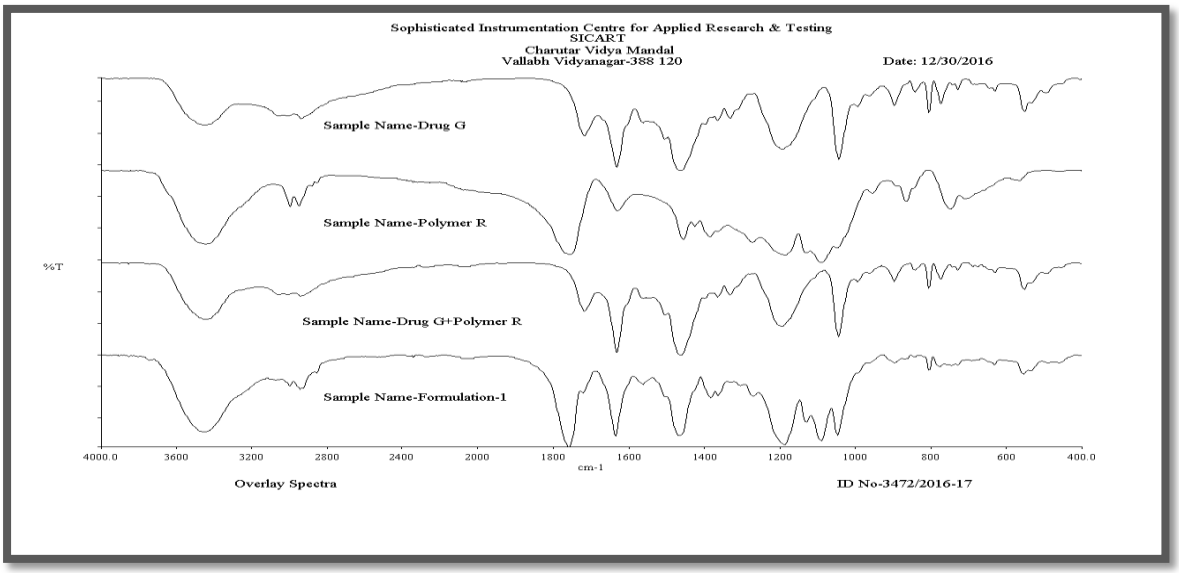
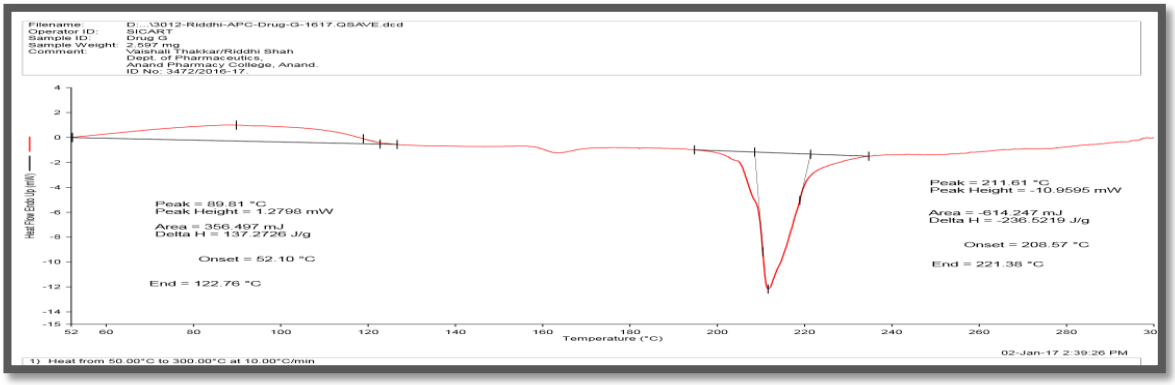


(c)

Fig−9(a-c): FTIR spectra of Gemifloxacin, PLGA 50:50 and Overlay spectra



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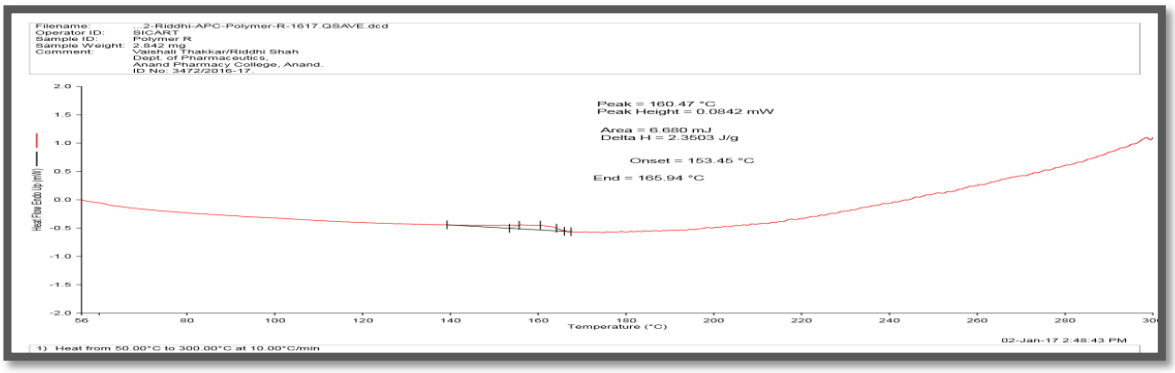

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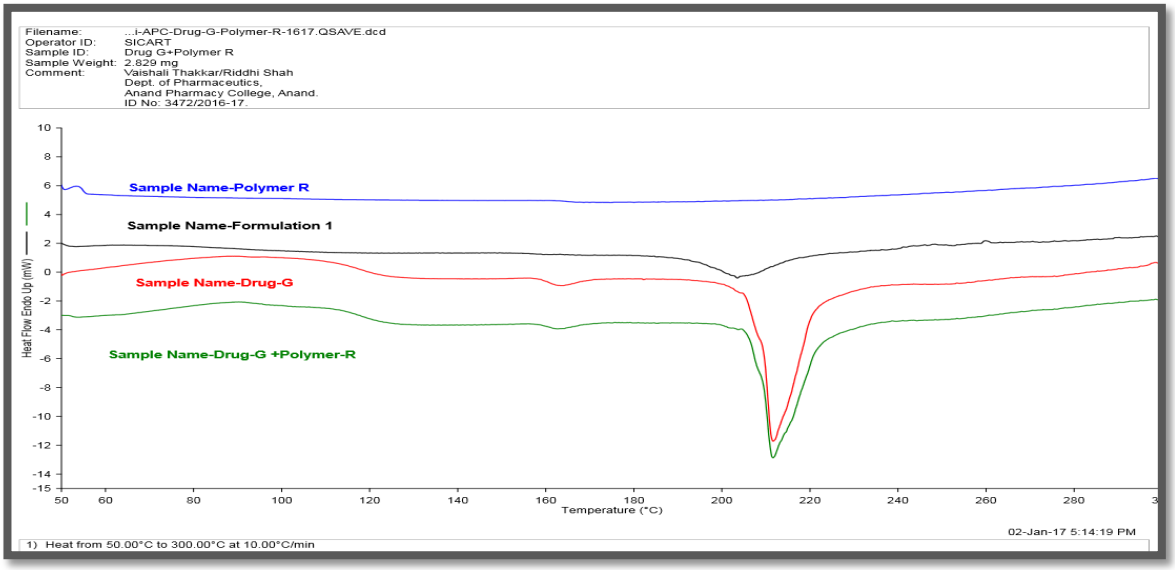

(c)

Fig–10(a-c): DSC thermogram of Gemifloxacin, PLGA 50:50 and Overlay spectra

Table–1: Independent variables for Taguchi Design

INDEPENDENT VARIABLES	LOW(-1)	MEDIUM(0)	HIGH(+1)
X_1- Drug:Polymer	1:0.5	1:1	1:1.5
X_2- Aspirator capacity	40	45	50
X_3-Temperature(˚C)	50	57.5	65
X_4-Flow rate(ml/min)	5	7.5	10

Table–2: Dependent variables for Taguchi Design

DEPENDENT VARIABLES	RANGE
Y1- Particle size	Less than 5 µm
Y2-% Yield	75-85%
Y3-% Entrapment efficiency	30-75%

Table–3: Experimental design performed by Taguchi Design and its Results

BATCHES	X1	X2	X3	X4	Particle size	% Yield	%E.E
F1	1 : 0.5	**40**	**50.00**	**5.0**	**0.65**	**24.93**	**75.1**
F2	1 : 0.5	**45**	**57.50**	**7.5**	**1.68**	**58.28**	**63.22**
F3	1 : 0.5	**50**	**65.00**	**10.0**	**2.75**	**71.2**	**84.18**
F4	1 : 1	**40**	**57.50**	**10.0**	**1.98**	**53.27**	**42.55**
F5	1 : 1	**45**	**65.00**	**5.0**	**6.54**	**60.26**	**69.02**
F6	1 : 1	**50**	**50.00**	**7.5**	**2.54**	**55.82**	**72.04**
F7	1 : 1.5	**40**	**65.00**	**7.5**	**8.82**	**64.71**	**26.35**
F8	1 : 1.5	**45**	**50.00**	**10.0**	**2.29**	**48.89**	**42.14**
F9	1 : 1.5	**50**	**57.50**	**5.0**	**6.69**	**50.02**	**58.04**

Table–4: Optimal regression equation for each response variable

Model	Co-efficient	Particle size	p-Value	% Yield	p-Value	% Entrapment efficiency	p-Value
	Intercept b0	3.95	0.0173	53.90	0.0202	58.11	0.0387
	A (b_1)	2.09	0.0069	-2.24	0.1886	-12.69	0.0299
	B (b_2)	-0.16	0.5613	5.69	0.0229	11.21	0.0268
	C (b_3)	1.58	0.0348	10.72	0.0069	-3.24	0.3328
	D (b_4)	-2.45	0.0178	6.73	0.0173	-3.43	0.3089
Linear	AB (b_{12})	-1.59	0.0729	-	-	-	-
	AC (b_{13})	-0.49	0.2360	-	-	-	-
	BC (b_{23})	-	-	-8.06	0.0275	4.96	0.3197
	BD (b_{24})	-	-	0.56	0.7200	-	-
	R^2	**0.9942**		**0.9932**		**0.9477**	
	Adjusted R^2	**0.9768**		**0.9729**		**0.8605**	
	PRESS	**104.90**		**405.27**		**3011.50**	

Table–5: Checkpoint batch predicted and observed values of response variables and percentage predicted error

Batch	X1	X2	X3	X4	Responses	Predicted	Actual	%Error
R1	1:0.5	52	56	8	Y1	4.55	4.65	-0.022
					Y2	62.08	60.14	0.031
					Y3	79.36	72.03	0.092
R2	1:0.5	55	65	10	Y1	2.91	2.75	0.054
					Y2	71.78	71.2	0.0080
					Y3	80.29	84.18	-0.048
R3	1:0.5	42	65	8	Y1	4.08	4.22	-0.034
					Y2	65.42	68.13	-0.041
					Y3	81.52	78.96	0.031

Table−6: Stability Study

Interval of testing	% encapsulation efficiency	% CDR
Initial	83.00±0.24	89.28±0.17
10 Day	84.02±0.15	89.07±0.13
20 Day	83.90±0.13	89.25±0.11
30 Day	83.55±0.12	89.00±0.14

Optimization of process parameters and validation of Taguchi design

After generating the polynomial equation relating to the independent and dependent variables, spray drying parameters were optimized for the responses. The optimum value for the variables were obtained by graphical and numerical analysis using Design-expert software which are based on criterion of desirability and overlay spectra which gives design space that is shown in figure 3(g). Percentage error was measured so as to find out the optimized spray drying condition. The observed values of the checkpoint batch were found quite closer to the predicted values as shown in table 5. Also from the optimal regression analysis in table 4 which also shows that the model is quite predictive in nature.

For validation of results the experimental value of responses were compared with the anticipated values and prediction values as shown in table 5. Linear correlation plots were drawn between the predicted and the experimental values were demonstrated high values of R^2 ranging between 0.9942, 0.9932 and 0.9477 indicating excellent goodness of fit $p<0.005$. Thus, the low magnitudes of error as well as the significant values of R^2 in the present study prove high ability of Taguchi design.

In-vitro drug release study

Franz diffusion cell was utilized for the drug release study of all Taguchi suggested batches of microparticles. The drug release profile of gemifloxacin microparticles showed initial burst release in 2h, followed by nearly sustained release profile of drug up to 9h. The initial burst release effect may ascribe to the release of drug on the surface of the microparticles followed by diffusion of drug. Drug release profile of gemifloxacin microparticles shown in figure 5. The drug release study indicates that the drug release was influenced by the concentration of PLGA. As concentration of PLGA increased it leads to decrease drug release rate from microparticles. Batch B_3 was found to have desired release profile as it was able to sustain the release of drug up to 9hrs.

Particle size analysis

The average particles size of the PLGA 50:50 loaded gemifloxacin microparticles were determined by particle size analyzer (Zetasizer Ver System; Serial Number: MAL1051945; Malvern instrument Ltd, Malvern UK) count rate (Kcps: 252.1) duration used (60sec) cell description (disposable sizing cuvette). The particle size of optimized batch was found to be 2.75µm that falls under the required range i.e. 0.5-5µm as shown in the figure 1-a and the poly-dispersibility index (PDI) was found to be 0.293 which indicates narrow size distribution on the basis of figure 7.

Aerodynamic study

Aerodynamic property of microparticle was investigated by Anderson cascade impactor. The optimized formulation was subjected to in vitro lung deposition study using Anderson cascade impactor. The deposition of total amount of remaining in capsule, device, throat, pre-separator and stages 0-7 microparticles was found to be 90%. Percentage deposition at stages 4-5 of the cascade impactor indicates Percentage respirable fraction was used to evaluate the possibility of delivering the particles to deep parts of the lung. Percentage respirable fraction was 62.16 % indicates the high aerosol performance of microparticles. The microparticles which was found at stages 6-7 was known as fine particle fraction i.e. 11.93 % which enhances aerosolization efficiency, which improve the flowability of the particles of the inhaler and promotes the disaggregation into fine particles. After plotting graph as shown in figure 6 (a, b). Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) was found to be 2.54µm and 1.50 respectively. Optimum range is defined as 0.5-5µm because particles <0.5µm are usually exhaled whereas particles >5.0µm are impacted in the oropharynx. Hence the powder is suitable for the delivery to the peripheral alveolar airway.

Production yield

The production yield of the PLGA 50:50 loaded gemifloxacin micro particles was ranged from 21.93 - 71.20 % as shown in table 3. Figure 1-b shows the effect of independent variables on the production yield which shows that increase in inlet temperature results in better production yield.

Encapsulation efficiency

The encapsulation efficiency of different experimental runs of microparticles was ranged from 46 - 83 % as shown in table 3. Figure 1-c shows the effect of independent variables on the encapsulation efficiency which shows that decrease in polymer concentration results in better encapsulation efficiency.

DRUG POLYMER COMPATIBILITY STUDY

Scanning Electron Microscope

The morphology of the optimized batch was examined by SEM. The SEM micrographs of the gemifloxacin loaded microparticles are shown in figure 8 (a, b, c). It was observed that the spray-dried microparticles were spherical in shape and that too with smooth surface.

Fourier Transform Infra-Red Spectroscopy

The possible interaction between the drug and polymer was studied by FTIR spectroscopy. The FTIR spectrum of all the tested samples shows the prominent peaks of Gemifloxacin and PLGA in figure 9 (a, b, c) respectively and they shows characteristic peaks at 1632.95 cm^{-1}(C=N), 1719.10 cm^{-1} (C=O aromatic), 1399.23cm^{-1}(C–F stretch), 1194.43 cm^{-1} (C-O stretch) in Gemifloxacin. In infrared spectra of physical mixture and formulation as shown in Figure.11, all similar peaks were found as that of gemifloxacin indicating the absence of any interaction.

Differential Scanning Calorimetry

The DSC spectra are represented in figure 10 (a, b, c). Exothermic peak of pure drug was found to be at 211.61°C and endothermic peak of PLGA 50:50 was found to be at 76.67°C. There was a negligible change in the melting exotherm and endotherm of the prepared physical mixture of Gemifloxacin and PLGA respectively. Melting temperature of physical mixture was found to be almost same but with a slight reduction which is not prominently significant. From the result of DSC it can be concluded that the drug is compatible with polymer. The DSC spectra of the prepared formulation were shown that there is decrease in intensity of the peak without shifting which denotes that the drug was entrapped.

Stability study

Stability study of the optimized batch was conducted at 40 ±2˚C and 75±5%RH for one month. Entrapment efficiency as well as %CDR was measured at frequent time interval as shown in the table 6 and there was no significant difference found.

CONCLUSION

This study demonstrates the ability of Taguchi design in optimization of gemifloxacin nanoparticles to overcome limitation of factorial design. The prepared nanoparticles of gemifloxacin was able to provide controlled release over a specific period of time with high encapsulation efficiency and good uniformity in particle size distribution.The prepared formulation may increase the efficiency of treatment with reduced dose.

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