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Formulation, Optimization and Evaluation of Sustained Release Microspheres using Taguchi Design

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Abstract The aim of present study is to prepare microspheres of eudragit RL 100 loaded with Nefopam Hydrochloride by single emulsion solvent evaporation technique. Taguchi L9 orthogonal array design has been used to optimize the composition and operating conditions for preparation of formulations. Nine batches (F1-F9) were prepared by taking three independent variables (X_1- drug: polymer ratio, X_2- stirring speed and X_3- stirring time) at three levels (+1, 0, -1). Response variables studied for batches (F1-F9) were mean particle size (μm) (Y_1), drug entrapment efficiency (% w/w) (Y_2) and drug loading (% w/w) (Y_3). Drug- polymer compatibility study was carried out by DSC and FTIR spectroscopy and indicates no physicochemical interaction. Microspheres were analyzed for morphological characteristics, mean particle size, drug entrapment efficiency, drug loading and *in-vitro* drug release. Percentage cumulative drug release for optimized batch F5 was found to be 85.421 ± 0.054 and followed higuchi model for release of drug.

Keywords: Taguchi, Response Variables, Independent Variables, Drug Entrapment Efficiency, Drug Loading, Higuchi Model.

1. INTRODUCTION

An ideal drug delivery system should be able to deliver effective amount of the drug for a sufficient period of time. Polymeric microsphere is an ideal formulation for sustained drug delivery (Neelesh *et. al*., 2004; Saharan *et. al*., 2009; Kumar and Majeti, 2000). Such systems offer numerous advantages over traditional methods of drug delivery including tailoring of drug release rates, protection of fragile drugs, increased bioavailability of drugs, decreased frequency of administration and increased patient comfort and compliance (Kumar *et. al*., 2011; Prasanth *et. al*., 2011; Gholap *et. al*., 2010).

Statistical experimental designs provide a coherent plan for carrying experimentation so that many factors can be studied simultaneously.

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Factorial design, response surface methodology and taguchi design are frequently applied experimental designs (Lewis *et. al*., 2005; Myers *et. al*., 2003). Taguchi design can optimize many factors simultaneously and extract more quantitative information by performing only a few experimental trials (Varshosaz *et. al*., 2009; Taguchi, 1987). Optimization of process implies the use of an experimental design in order to identify factors affecting the formulation, determine levels of factor resulting in an optimum response and decrease variability in process without presiding over or excluding causes of variation. Optimization of process parameters by taguchi design is an effort to minimize the variation in quality as well as to attain the quality near to the intended value simultaneously (Jose *et. al*., 2012).

Nefopam Hydrochloride is a centrally acting non-opioid analgesic and anti-inflammatory drug of the Benzoxazocine class having 30-40% bioavailability, high local and systemic gastro-intestinal disturbances and high first pass metabolism. It has an absorption region from upper gastro intestinal tract and an elimination half-life of 3.5 h (Aymard *et. al*., 2003; Alfonsi *et. al*., 2004; Del *et. al*., 2009; Starek *et. al*., 2011). It has log P value 3.519 which contribute to lipid solubility and hydrophilicity. It is used for the relief of acute and chronic pain.

The aim of present research work is to prepare microspheres of eudragit RL 100 loaded with Nefopam Hydrochloride using taguchi L9 orthogonal array design by single emulsion solvent evaporation technique to obtain sustained release of drug. Nine batches (F1-F9) were prepared by taking three independent variables (X_1- drug: polymer ratio, X_2- stirring speed and X_3- stirring time) at three levels (+1, 0, -1).

2. MATERIALS AND METHODOLOGY

2.1 Materials

Nefopam Hydrochloride was purchased from Prince Scientific and Surgical, Hyderabad. Eudragit RL100 was obtained as a gift sample from Evonik Industries AG, Mumbai, India. Light liquid paraffin, n-hexane and acetone were obtained from Merck Specialties Private Limited, Mumbai. Magnesium Stearate and methanol were obtained from Loba Chemicals Private Limited, Mumbai, India.

2.2 Experimental Design and Analysis

Taguchi orthogonal array L9 design was used to optimize the composition and operating conditions for preparation of microspheres. Factors selected for

study were drug: polymer ratio, stirring speed and stirring time taken at low, medium and high levels are given in Table 1.

Response variables studied for prepared batches were mean particle size (μm) (Y_1), drug entrapment efficiency (% w/w) (Y_2) and drug loading (% w/w) (Y_3) are given in Table 2. Taguchi design layout with coded values of independent variables is given in Table 3. The experimental trials were performed in random order.

Table 1: Levels of Independent Variables

S. No.	Independent Variables	Codes -1(Low)	0 (Medium)	+1 (High)
1.	Drug: ERL (w/w), X_1	1:2	500	2
2.	Stirring Speed, (rpm), X_2	1:3	1000	3
3.	Stirring Time (hrs), X_3	1:4	1500	4

Table 2: Response variables

S. No.	Codes	Response variables
1.	Y_1	Mean Particle Size (μm)
2.	Y_2	Drug Entrapment Efficiency (% w/w)
3.	Y_3	Drug Loading (% w/w)

Nine batches of microspheres (F1-F9) were prepared as per experimental design layout of taguchi L9 orthogonal array design. Calculations and statistical analysis of the results were carried out by multiple linear regression analysis (MLRA) to determine which factors has statistically significant effect on the response parameters.

2.3 Methods

2.3.1 *Preparation of Microspheres*

Single emulsion solvent evaporation technique was selected for preparation of microsphere because it is one of the most common methods for creating

Table 3: Experimental design layout as per taguchi L9 orthogonal array design

Batch	X_1 (Drug: ERL)	X_2 (Stirring Speed, rpm)	X_3 (Stirring Time, hrs)
F1	-1	-1	-1
F2	-1	0	0
F3	-1	+1	+1
F4	0	-1	0
F5	0	0	+1
F6	0	+1	-1
F7	+1	-1	+1
F8	+1	0	-1
F9	+1	+1	0

microspheres from preformed polymers and due to its relative ease. Moreover, selected drug and polymer are soluble in a common solvent and drug polymer

Nefopam Hydrochloride and eudragit RL 100 (1:2, 1:3 and 1:4), were dissolved in mixture of methanol and acetone (1:1). This mixture was stirred with magnetic stirrer to produce a transparent solution. Drug-polymer solution was slowly added with continuous stirring by a magnetic stirrer to light liquid paraffin taken as continuous phase containing span 60 (0.1% w/v) as an emulsifier. Prepared microspheres were filtered and washed with petroleum ether. Successive washings were continued till all the organic solvent was removed. Microspheres were lyophilized and stored till further use (Das and Das, 1998; Lee *et. al*., 2000; Phutane *et. al*., 2010).

2.3.2 *Mean Particle Size Analysis*

Particle size distribution of prepared microspheres was measured in a particle size analyzer by laser diffraction (Mastersizer, Malvern Instruments, United Kingdom). Approximately 10 mg of microspheres were suspended in deionised water and sonicated with an ultra-sound probe for 1 min. This homogeneous suspension was determined for equivalent volume diameter. Measurements for particle size were taken in triplicate (Srivastava *et. al*., 2005).

2.3.3 *Drug Entrapment Efficiency (%)*

Accurately weighed 100 mg of microsphere was extracted in 100 ml phosphate buffer, pH 7.4 for 24 h. Extracted solution was filtered and a sample of 5 ml

was withdrawn from this solution. Sample was diluted to 50 ml with distilled water and assayed spectrophotometrically (Systronics AU2701) at 266 nm to determine the content of Nefopam Hydrochloride in microsphere. All the determinations were performed in triplicate (Arindam and Biswanath, 2006). Drug entrapment efficiency (%) of the microspheres was calculated using the equation:

Drug Encapsulation Efficiency (%) = Drug Content × 100 Weight of drug

2.3.4 *Loading Efficiency (%)*

Accurately weighed 100 mg of microsphere was extracted in 100 ml phosphate buffer, pH 7.4 for 24 h. Extracted solution was filtered and a sample of 5 ml was withdrawn from this solution. Sample was diluted to 50 ml with distilled water and assayed spectrophotometrically (Systronics AU2701) at 266 nm to determine the content of Nefopam Hydrochloride in microsphere. All the determinations were performed in triplicate (Arindam and Biswanath, 2006). Drug Loading (%) of the microspheres was calculated using the equation:

Drug Loading (%) = Drug Content × 100 Weight of Drug + Polymer

2.3.5 Statistical analysis

Response variables were analyzed using Microsoft Excel spreadsheet, 2007. Multiple linear regression analysis was used to develop equations for mean particle size (Y_1), % drug entrapment efficiency (Y_2) and % drug loading (Y_3).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

Where, Y is estimated response of response variable, β_0 is arithmetic mean response of nine batches, β_1 is estimated coefficient for factor X_1, β_2 is estimated coefficient for factor X_2 and β_3 is estimated coefficient for factor X_3.

2.3.6 *Differential scanning Calorimetry*

Differential scanning calorimetry measurements were done on DSC Q10V 9.0 build 275 model Water Limited instrument by heating the samples from 25 °C to 250 °C at the rate of 20 °C /minute in a nitrogen atmosphere (flow rate, 10 mL/min).

2.3.7 *Fourier Transform Infra-red Spectroscopy*

FTIR spectra were taken on FTIR- Shimadzu instrument to investigate the possible chemical interactions between drug and polymer. 1 mg of sample was triturated with 300 mg of KBr in a mortar. A little amount of triturated sample was compressed at 10 kg/cm^2 into a pellet maker. Pellet was kept on sample holder and scanned from 4000 cm^{-1} to 400 cm^{-1}.

2.3.8. In-vitro dissolution studies

In-vitro dissolution studies were performed on microspheres at 37°C (± 0.5°C) at 100 rpm with USP Dissolution Apparatus II (LABINDIA DF 8000). An accurately weighed amount of microspheres was dispersed in dissolution media consisting of 900 ml Phosphate buffer, pH 7.4 and dissolution study was carried for 24 h. Aliquots of dissolution fluid was withdrawn at stated time intervals to assay the released drug spectrophotometrically at 266 nm. Each graphical data point was mean of dissolution data from three samples. Corrections were made for withdrawal of samples (Srivastava *et. al*., 2005; Arindam and Biswanath, 2006; Umer *et. al*., 2011; Barratt, 2000).

2.3.9 *Scanning Electron Microscopic Analysis*

Microspheres were plated with gold palladium for 150 seconds to achieve a 20 nm film under an atmosphere of air (Coater Polaron, 18mA current at 1.4 kV). Coated sample was examined using scanning electron microscope (Variable Pressure Scanning Electron Microscope, Hitachi S3400N). Spherical shape of microspheres was established by SEM.

3. RESULTS AND DISCUSSION

Taguchi L9 orthogonal array design was used to prepare microspheres of eudragit RL 100 loaded with Nefopam Hydrochloride by single emulsion solvent evaporation technique to obtain a sustained release of drug. Microspheres were prepared to study the effect of drug polymer ratio, stirring speed and stirring time on response variables of microspheres. Nine formulations (F1–F9) were developed and calculated values of response variables are shown in Table 4.

Model, developed from multiple linear regression analysis, to estimate effect Y_1, Y_2 and Y_3 can be depicted mathematically as;

$$Y_1 = 122.2 + 45.29\ X_1 - 10.92\ X_2 - X_3$$

$$Y_2 = 62.76 + 7.94\ X_1 + 1.18\ X_2 + 0.36\ X_3$$

$$Y_3 = 15.96 - 1.68\ X_1 + 0.35\ X_2 + 0.14\ X_3$$

From values of coefficients of linear equations, it was concluded that mean particle size (μm) was increased with the increase in drug-polymer ratio whereas

Table 4: Microspheres (F1-F9) and their response variables

Batch code	Factors Code X_1	X_2	X_3	Y_1 Mean Particle Size (µm)	Y_2 Drug Entrapment Efficiency (% w/w)	Y_3 Drug Loading (% w/w)
F1	-1	-1	-1	85.43	46.12	15.37
F2	-1	0	0	80.33	53.11	17.70
F3	-1	+1	+1	75.65	48.22	16.07
F4	0	-1	0	140.42	68.11	17.03
F5	0	0	+1	100.38	78.02	19.50
F6	0	+1	-1	104.42	76.11	19.02
F7	+1	-1	+1	180.23	65.43	13.08
F8	+1	0	-1	172.41	67.23	13.44
F9	+1	+1	0	160.52	62.45	12.50

decreased with the increase in stirring speed and stirring time. Drug entrapment efficiency (% w/w) was increased with the increase in drug-polymer ratio, stirring speed and stirring time. Drug loading (% w/w) was decreased with the increase in drug-polymer ratio whereas increased with the increase in stirring speed and stirring time. Formulation (F5) was found to be the optimized batch with mean particle size (100.38 ± 1.12 µm), drug entrapment efficiency (78.02 ± 0.99 % w/w) and drug loading (19.50± 0.55 % w/w).

3.1 Differential scanning Calorimetry

DSC studies were performed to investigate physicochemical interaction between drug and polymer. DSC thermograms of pure Nefopam hydrochloride, eudragit RL 100 and mixture of drug with eudragit RL 100 were evaluated.

Peak corresponding to t_m of Nefopam Hydrochloride was present in the mixture of drug and polymer indicating absence of physicochemical interaction between Nefopam Hydrochloride and eudragit RL 100. DSC thermograms of Nefopam Hydrochloride, Eudragit RL 100 and mixture of Nefopam Hydrochloride and eudragit RL 100 are given in Figure 1 (a), 1 (b) and 1 (c) respectively.

3.2 Fourier Transform Infra-red Spectroscopy

FTIR studies were performed to investigate physicochemical interaction between drug and polymer. FTIR spectra of pure Nefopam Hydrochloride and mixture of drug with eudragit RL 100 were evaluated. Characteristic peaks of

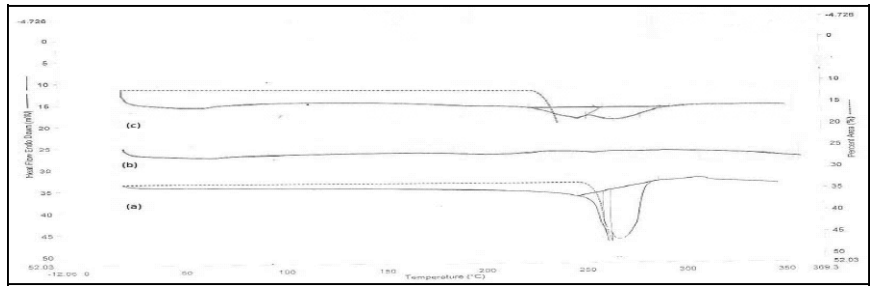

Figure 1: DSC thermogram of (a) Pure Nefopam Hydrochloride (b) Eudragit RL 100 (c) Mixture of Nefopam Hydrochloride and eudragit RL 100

drug were observed at 3429 cm^{-1} (C-O-C of cyclic ring), 2910 cm^{-1} (C-H(s) of alkane), 1446 cm^{-1} (C=C of aromatics), 1346 cm^{-1} (C-N of amines) and 759 cm^{-1} (aromatic ring). It was found that there was neither shifting nor overlapping of characteristic peaks of drug in FTIR spectra of mixture of drug with eudragit RL 100 which indicates absence of physicochemical interaction between drug and polymer. FTIR spectra of Nefopam Hydrochloride and mixture of drug with eudragit RL 100 are given in Figure 2 and Figure 3 respectively.

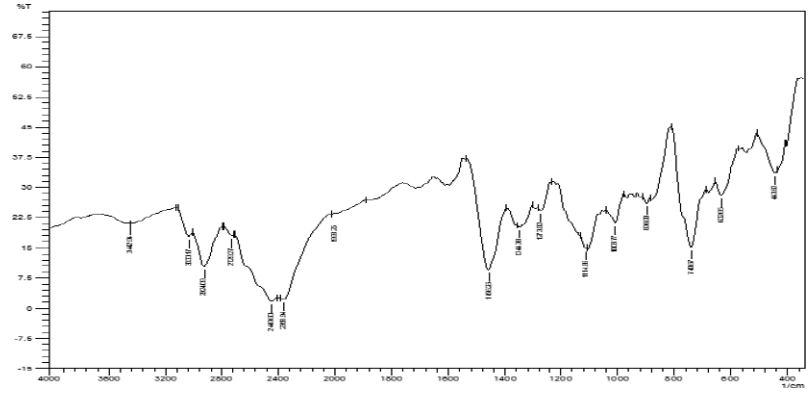

Figure 2: FTIR Spectra of Nefopam Hydrochloride

3.3 *In-vitro* dissolution studies

In-vitro drug release studies were performed for pure drug, batch F5 and F6 in phosphate buffer, pH 7.4. Batch F5 was found to be optimized batch which gives 85.421 ± 0.054 % cumulative release of the drug as compared to F6

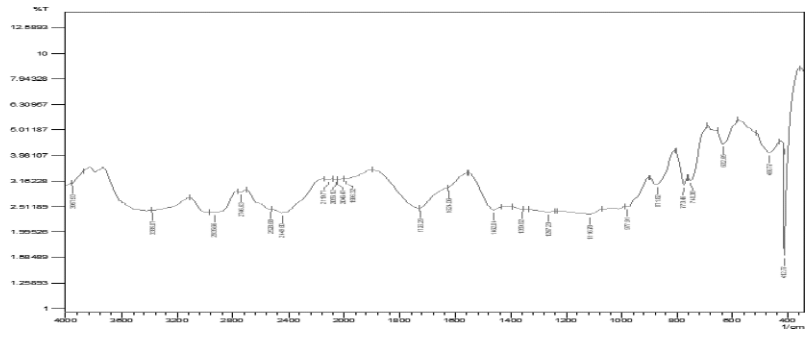

Figure 3: FTIR Spectra of mixture of Nefopam Hydrochloride and eudragit RL 100

which gives 80.342 ± 0.032 % release in 24 hours. *In-vitro* drug release pattern of pure drug, batch F5 and batch F6 is given in Figure 4.

In-vitro drug release data of optimized batch F5 was fitted to classic drug-release kinetics models such as zero-order model (% release *vs*. t), first order model (log% release *vs*. t), higuchi model (M_t/M_∞ *vs.* t) and korsmeyer peppas model ($M_t/M_\infty = kt^n$) (Costa *et. al*., 2001). Microsphere release mechanisms were assessed by comparing regression coefficients (r^2). Regression coefficient value for higuchi model was found to be 0.968. Drug release was shown to follow a higuchi model, with the release mechanism apparently controlled by a rapid diffusion-release process rather than polymer degradation. Higuchi plot of optimized batch F5 is given in Figure 5.

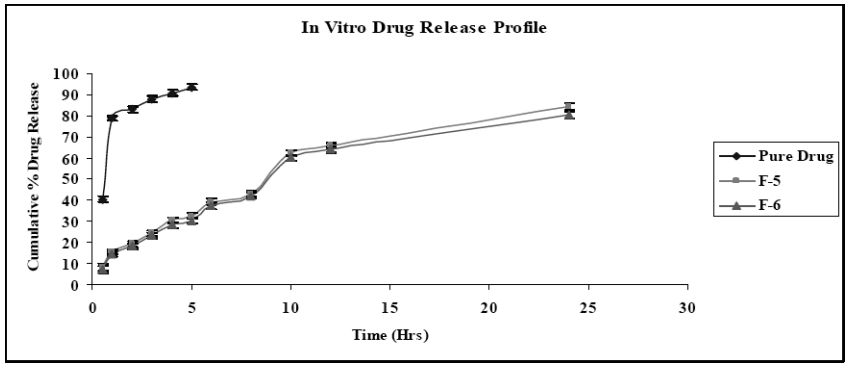

Figure 4: *In-vitro* drug release pattern of pure drug, batch F5 and batch F6

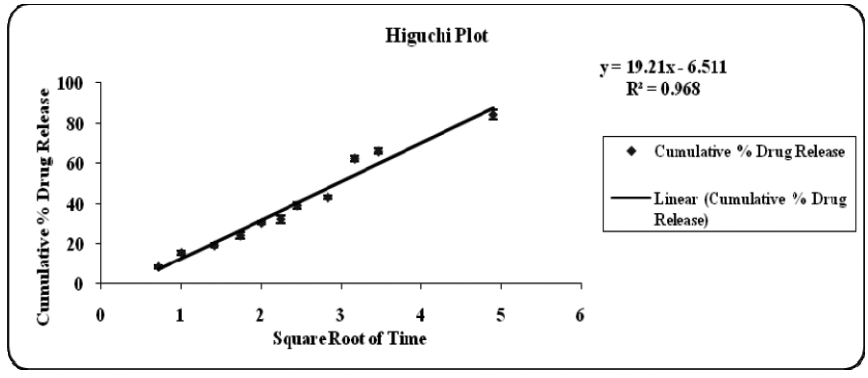

Figure 5: Higuchi plot of the optimized batch F5

3.4 Scanning electron microscopic (SEM)

Morphology of optimized batch F5 was examined by using scanning electron microscopy. SEM has been used to determine texture and examine morphology of surface. SEM study revealed that microspheres were spherical in shape shown in Figure 6.

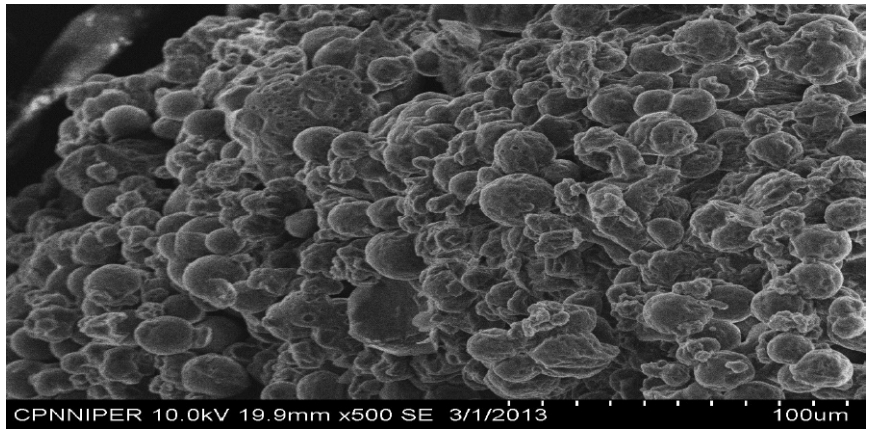

Figure 6: SEM Image of optimized batch F5 of microspheres loaded with Nefopam Hydrochloride

4. CONCLUSION

From the results of study, it was concluded that single emulsion solvent evaporation technique can be used to prepare sustained release microsphere of

Nefopam Hydrochloride using Eudragit RL 100 as matrix polymer. Microspheres were developed using taguchi L9 orthogonal array design. These investigations provided an understanding of the effects of drug-polymer ratio, stirring speed and stirring time on mean particle size, drug entrapment efficiency and drug loading. From the values of the coefficients of the linear equations developed by multiple linear regressions analysis, it was concluded that mean particle size (μm) increased with increase in drug-polymer ratio whereas decreased with increase in stirring speed and stirring time. Drug entrapment efficiency (% w/w) increased with increase in drug-polymer ratio, stirring speed and stirring time. Drug loading (% w/w) decreased with increase in drug-polymer ratio whereas increased with increase in stirring speed and stirring time. Based on the above findings, it was concluded that batch F5 with mean particle size (100.38 ± 1.12 μm), drug entrapment efficiency (78.02 ± 0.99 % w/w) and drug loading (19.50± 0.55 % w/w) was optimized formulation of microsphere. From *in-vitro* drug release studies, it was found that the optimized batch F5 gives sustained release of drug. *In-vitro* drug release data of batch F5 was fitted in various drug release kinetic models and it was found that it was best fitted in higuchi model with regression coefficient value 0.968. Spherical shape surface morphology of microspheres was confirmed by SEM. It was concluded that a sustained release microspheres of Nefopam Hydrochloride were successfully prepared using eudragit RL 100 by single emulsion solvent evaporation technique with the selection of appropriate experimental conditions. Prepared microsphere can be administered into a suitable capsule dosage form by filling powdered microsphere containing equivalent therapeutic dose of Nefopam Hydrochloride into hard gelatin capsule shell.

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