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Original Article

# FORMULATION AND EVALUATION OF NAPROXEN-EUDRAGIT® RS 100 NANOSUSPENSION USING $3^2$ FACTORIAL DESIGN

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**ABSTRACT**

**Objective**: The objective of the present investigation was to develop drug loaded Eudragit® RS100 nanosuspension as a sustained release carrier.

**Methods**: All the nanosuspensions of Naproxen loaded Eudragit® RS100 were prepared using the quasi emulsion solvent diffusion technique at different drug: polymer ratios. The formulation was optimized using design of experiments by employing a 2-factor, 3-level factorial design. The drug: polymer ratio ($X_1$) and speed of homogenization($X_2$), were the independent variables; particle size ($Y_1$), zeta potential ($Y_2$) and entrapment efficiency ($Y_3$) as dependent variables. The nanosuspensions were studied for particle size analysis, X-ray diffraction analysis and surface morphology by scanning electron microscopy. The *in vitro* release study of Naproxen from nanosuspension was carried out using dialysis bag with molecular weight cut-off value of 12,000 to 14,000 Daltons.

**Results**: Average particle size of nanosuspension was between 159 to 435nm and zeta potential ranges from 20.7 to 53.5 mV. The statistical analysis of data revealed that drug: polymer ratio($X_1$) has a significant positive influence on particle size (p=0.0077) whereas a negative influence on zeta potential (p=0.0045) and Entrapment efficiency (p=0.0003). The developed model was validated using two check point formulations and found no significant difference between the predicted and observed values. An optimized formulation was also identified during the study.

**Conclusion**: This investigation demonstrated the potential of the experimental design in understanding the effect of formulation variables on the development of Nanosuspensions. The results assures, nanosuspension are promising sustained release system to the naproxen and many other drugs.

**Keywords:** Nanosuspension, Naproxen, Eudragit.

## INTRODUCTION

In recent years, research in the field of nanotechnology and its applications in drug delivery has gained a momentum. They were identified to be a promising drug delivery systems for a wide range of drugs starting from low molecular weight to macromolecules, peptides, proteins or genetic materials targeted to particular cells or tissues[1].Nanoparticles are also preferred for their improved bioavailability and stability of drug molecules against enzymatic degradation. Eudragit RS100 is a co-polymer of poly (ethyl acrylate, methyl–methacrylate and chlorotrimethyl–ammonioethyl methacrylate). The ammonium groups are present as salts, and they are responsible for permeability, which is independent of pH in the physiological region. Eudragit® RS100 was proved to be a promising polymer for controlled and prolonged localized delivery of desired medicine to some physiologic fluids. Nanoparticles as sustain release drug delivery system using Eudragit RS 100 was reported for drugs like Acyclovir [2], Aceclofenac [3,4,5].

Naproxen (NPX) is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness caused by conditions such as osteoarthritis, rheumatoid arthritis, psoriatic arthritis, gout, injury (like fractures), tendinitis and bursitis. Naproxen's usefulness is limited by a short duration of action (8 h) when administrated orally. Repeated administrations are required for maintenance of the pharmacological action. Patients with chronic inflammatory diseases require long term therapy with such NSAIDs. But chronic usage of NASIDS may lead to gastrointestinal disorder, gastritis, ulcer and bleeding. A sustained release Nanoparticles formulation based on Eudragit RS 100 could retard the release of drug extending the pharmacological action of the NPX and reducing the frequency of administration which in turn reduces the drug related adverse effects. [6, 7].

These days Statistical models are extensively used by scientist of formulation and development to strengthen the art of drug formulations. A $3^2$ factorial design is a well established model to study the effect of selected formulation variables on the characters of the drug products. In the present investigation the drug/polymer ratio ($X_1$), speed of homogenization($X_2$), were the independent variables particle size ($Y_1$), zeta potential ($Y_2$) and entrapment efficacy ($Y_3$) were the dependent variables.

## MATERIALS AND METHODS

### Materials

Naproxen was purchased from Himedia laboratories Pvt. Ltd. (Mumbai).Eudragit RS 100 was a kind gift sample from Evonik Degussa. Tween 80 was obtained from Sisco research laboratories Mumbai. All other chemicals and materials were of analytical grade and were used as procured.

### Preparation of nanoparticles

Nanoparticles were prepared by an adaptation of the Quasi-emulsion solvent diffusion method (QESD). Drug and polymer were co-dissolved at room temperature in 4 ml of ethanol. Ethanol solution produced by co-dissolving the drug and the polymer was perfectly clear. The solution was slowly injected (0.5 ml / min) with a syringe connected to a thin Teflon tube, into a 10 ml of water containing tween 80 (0.02%, w/v) as a hydrophilic emulsifier in a cylindrical vessel maintained at low temperature by means of an ice-cooled water bath to avoid a rapid ethanol evaporation. During injection, the mixture was stirred at specified RPM (10,000, 15,000, and 20,000 rpm) by high shear homogenizer (an Ultra-Turrax T 25, Ika Labortechnik, Staufen, Germany). Stirring was kept at the same rate for 15 min. The solution immediately turned into a pseudo-emulsion of the polymer ethanol solution in the external aqueous phase. The counter diffusion of ethanol and water out of and into the emulsion micro droplets, respectively, and the gradual evaporation of the organic solvent determined the *in situ* precipitation of the polymer with the formation of matrix- type Nanoparticles.

Ethanol residues evaporated off during a further slow stirring at 200 rpm for 24 h of the nanosuspension at room temperature [8].

**$3^2$ Full Factorial Designs**

A $3^2$ full factorial design was applied to examine the combined effect of two formulation variables on particle size, stability and entrapment efficiency. 9 combinations of Naproxen loaded Eudragit RS 100 nanosuspension were prepared according to the design given in Table 1.

The drug/polymer ratio ($X_1$), speed of homogenization($X_2$), were used as independent variables and particle size ($Y_1$), zeta potential ($Y_2$) and entrapment efficacy ($Y_3$) as dependent variables. The experimental, design coded values is given in Table 1. The levels of parameters used are given in Table 2. The graphs and mathematical models were computed using Design Expert 8.0.4.1 (Stat- Ease, USA) software.

**Table 1: Formulation of batches in $3^2$ factorial designs (coded values)**

| Trail no. | Variable levels in coded forms | |
|---|---|---|
| | $X_1$ | $X_2$ |
| 1 | -1 | 1 |
| 2 | 0 | 1 |
| 3 | 1 | 1 |
| 4 | -1 | 0 |
| 5 | 0 | 0 |
| 6 | 1 | 0 |
| 7 | -1 | -1 |
| 8 | 0 | -1 |
| 9 | 1 | -1 |

**Table 2: Actual values of independent variables in $3^2$ factorial design**

| Coded level | -1 | 0 | 1 |
|---|---|---|---|
| $X_1$:Drug: Polymer ratio | 1:1 | 1:2 | 1:4 |
| $X_2$: RPM | 10000 | 15000 | 20000 |

**Evaluation parameters**

**Zeta potential**

The Zeta-potential of drug loaded nanoparticles was measured by Zeta sizer (Malvern Zetasizer version 6.20). To determine the zeta potential, nanoparticles samples were diluted with 0.1 mM solution of potassium chloride and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analyzed in triplicate.

**Particle size analysis and surface morphology**

The particle size distribution was analyzed by Malvern instrument (Zetasizer ver.6.20).

**Entrapment efficiency**

Aliquots of 2ml of the freshly prepared nanosuspension were centrifuged at 11,000 rpm for 15 min, and the amount of unincorporated drug was measured by UV analysis of the supernatant. Some samples were submitted to a second centrifugation cycle. The pellets obtained after centrifugation was then re-suspended and further dialysis process was used to measure any un-entrapped Naproxen might be precipitated in the system.

$$Entrapment\ efficiency\% = \frac{Amount\ of\ NPX\ actually\ present\ in\ nanoparticles}{\text{Amount of NPX actually used}} \text{ X } 100$$

***In vitro* drug release**

*In vitro* release studies were carried out for all the formulations by using the HIMEDIA DM70 dialysis membrane (pore diameter of 2.4 nm and cut off of 12-14 kD) in phosphate buffer (300ml, pH 6.8). Earlier, one side of the membrane was tied with a thread and nanosuspension (2ml) was then placed inside the membrane bag, the other side was tied properly, and placed in 300ml pH 6.8 phosphate buffer in a beaker with stirring for 12h. 5ml of the aliquot was withdrawn at predetermined intervals up to 12 h. The required dilutions were made with pH 6.8 phosphate buffer and the solution was analyzed for the drug content spectrophotometrically at 330nm. Equal volume of the dilution medium was replaced after each withdrawal to maintain sink condition.

The release studies were carried out in triplicate for all the formulations and the average, and standard deviation values were calculated. Cumulative percentage drug releases for all the formulations were calculated and they were plotted against function of time to study the pattern of drug release.

Drug release kinetics

To analyze the mechanism of drug release from the matrix tablets, the release data were fitted to the following equations:

Zero-order equation [9]: $R = k_0 t$

Where, R is the amount of drug released at time t, and $k_0$ is the release rate.

First-order equation [10]: $\text{Log UR} = \text{Log UR}_0 - k_1 t / 2.303$

Where, UR is the amount of drug un-dissolved at t time, UR0 is drug concentration at t = 0 and $k_1$ is the release rate constant.

Higuchi's equation [11]: $R = k_2 t^{1/2}$

Where, R is the percent of drug release at time t, and $k_2$ is the diffusion rate constant.

Hixson-Crowell equation: $(Ro^{1/3} - Rt^{1/3}) = k_4 t$

Where, Qt is the initial amount of drug, Qo is cumulative amount of drug release at time t, $k_4$ is Hixson-Crowell release constant and t is time in hours.

Korsmeyer-Peppas equation [12]: $\text{Log } (R_t / R_f) = \text{Log } k_3 + n \text{ Log } t$

Where, $R_t$ is the amount of drug release at time t; $R_f$ is the amount of drug release after infinite time; $k_3$ is a release rate constant incorporating structural and geometric characteristics of the tablet; and n is the diffusion exponent indicative of the mechanism of drug release. To clarify the diffusion exponent (n) for different batches of matrix tablet, the log value of percentage drug released was plotted against log time for each batch. A value of $n \leq 0.45$ indicates Fickian (case I) release; > 0.45 but < 0.89 for non-Fickian (anomalous) release; and > 0.89 indicates super case II type of release. Case II generally refers to the erosion of the polymeric chain, and anomalous transport (Non-Fickian) refers to a combination of both diffusion and erosion-controlled drug release [13].

**Fourier transform infrared spectroscopy (FT-IR)**

FT-IR spectra for pure drug and drug-excipient mixtures were done by means of FT-IR spectrophotometer (Shimadzu 4300, Japan) using the KBr disc method.

**RESULTS AND DISCUSSION**

The responses observed for all the formulations such as particle size ($Y_1$), zeta potential ($Y_2$) and entrapment efficiency ($Y_3$). Nine formulations of naproxen loaded Eudragit nanosuspension were prepared according to a $3^2$ full factorial design. The formulations were analyzed for response like as particle size ($Y_1$), zeta potential ($Y_2$) and entrapment efficiency ($Y_3$).

**Table 3**: **Formulations with their responses and constrains**

| Formulation Code | Variables | | Responses | | |
|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $Y_1$ | $Y_2$ | $Y_3$ |
| NNE1 | 50 | 20000 | 159.7 | 53.5 | 93.17 |
| NNE2 | 67 | 20000 | 208.5 | 42.5 | 78.82 |
| NNE3 | 80 | 20000 | 329.3 | 40.5 | 71.19 |
| NNE4 | 50 | 15000 | 259.5 | 51.2 | 91.6 |
| NNE5 | 67 | 15000 | 379.9 | 37.8 | 84.4 |
| NNE6 | 80 | 15000 | 435.1 | 29.4 | 76.59 |
| NNE7 | 50 | 10000 | 180.5 | 46.3 | 89.92 |
| NNE8 | 67 | 10000 | 196.7 | 45.9 | 86.68 |
| NNE9 | 80 | 10000 | 309.6 | 20.7 | 77.14 |
| $X_1$=Drug: Polymer ratio | | | | | |
| $X_2$=RPM | | | Constrains (Goal, Limits) | | |
| $Y_1$=Particle size (nm) | | | Minimum, 150-250nm | | |
| $Y_2$=Zeta potential (mV) | | | Maximum,25-50mv | | |
| $Y_3$=Entrapment efficiency (%) | | | Maximum, 85-90% | | |

**Particle size distribution**

The particle size distribution was analysis using Malvern zetasizer. The average particle size of all the formulations were of nano size, ranging from 159 to 435nm.Quardratic model was suggested for particle size distribution as given in Table 4. Anova analysis of the data is given in Table 5.

Values of "Prob>F" less than 0.0500 indicate the model terms are significant. Model equation with coefficient of factors was developed for particle size and given in Table 6. Drug: polymer ratio has a negative coefficient, which suggests that, the drug:polymer ($X_1$) ratio has negative influence on particle size, RPM($X_2$) of the homogeniser has no significant effect on particle size of the nanosuspension. These result coincides with the earlier studies reported [14,15]. The relationship between the dependable variables and two independent variables was elucidated by constructing a 3-D response surface. The response surface depicts the effect of amount of drug and polymer ratio at different levels on studied response particle size. The highest particle size recorded at the higher polymer concentration. The least particle size was obtained at the lower polymer concentration. Related interaction plots, contour plots & surface plot are shown in Figure 1(a-c).

**Table 4: Summary of results of regression analysis of particle size, zeta potential and entrapment efficiency**

| | Standard Deviation | R-Squared | Adjusted R-Squared | Predicted R-Squared | PRESS | Suggested model |
|---|---|---|---|---|---|---|
| **Particle size($Y_1$)** | | | | | | |
| Linear | 78.7380 | 0.4957 | 0.3276 | 0.0710 | 68527.85 | |
| 2FI | 85.7531 | 0.5015 | 0.2025 | -0.1605 | 85610.33 | |
| Quadratic | 30.2521 | 0.9627 | 0.9007 | 0.6785 | 23716.1 | Suggested |
| Cubic | 48.5397 | 0.9680 | 0.7444 | -5.1419 | 453044.3 | |
| **Zeta potential ($Y_2$)** | | | | | | |
| Linear | 5.5365 | 0.7889 | 0.7186 | 0.4964 | 438.8759 | Suggested |
| 2FI | 5.5373 | 0.8241 | 0.7185 | 0.2658 | 639.9073 | |
| Quadratic | 6.4513 | 0.8567 | 0.6180 | -0.6449 | 1433.73 | |
| Cubic | 4.1765 | 0.9799 | 0.8398 | -2.8481 | 3354.11 | |
| **Entrapment efficiency($Y_3$)** | | | | | | |
| Linear | 2.6955 | 0.9077 | 0.8769 | 0.74006 | 122.807 | Suggested |
| 2FI | 1.9916 | 0.9580 | 0.9328 | 0.8064 | 91.4578 | |
| Quadratic | 2.1188 | 0.9714 | 0.9239 | 0.6543 | 163.287 | |
| Cubic | 0.1675 | 0.9999 | 0.9995 | 0.9885 | 5.39509 | |

**Zeta potential**

Zeta Potential analysis is a technique for determining the surface charge of Nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the Nanoparticles surface. This double layer of ions travels with the Nanoparticles as it diffuses throughout the solution. The electric potential at the boundary of the double layer is known as the Zeta potential of the particles. Zeta potential is taken as a measure for stability of nanosuspension. The zeta potential of all the nine formulations was found to be ranging from 20.7 to 53.5mV.

The linear model was suggested for Zeta potential. Suspensions with zeta potential more than +25mV and less than -25mV were found to be stable. NNE9 is not a stable suspension and remaining 8 formulations are stable. All formulations were found to stable based upon zeta potential. The positive zeta potential was obtained due to positive charge of polymer. The Statistical analysis of data revealed that drug:polymer ratio ($X_1$) has negative effect on zeta potential (Figure 2 a,b) where as RPM($X_2$) has no significant effect on zeta potential.

**Entrapment efficiency**

The drug entrapment efficiency for all the formulations ranged from 76.5% to 93.1%. The linear model was suggested for entrapment efficiency. Figure 3 shows the contour graph, 3D graph and interaction graph of relation to the response size. Drug: polymer ratio has a negative coefficient, which suggests that, the drug:polymer ratio ($X_1$) has negative effect on entrapment efficiency and RPM($X_2$) has no significant on entrapment efficiency.

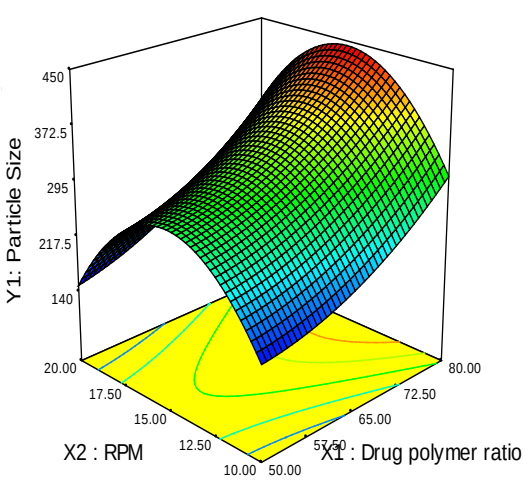
**Table 5: Anova table**

| Source | Sum of Squares | df | Mean Square | F Value | Prob. > F p value |
|---|---|---|---|---|---|
| $Y_1$=particle size ($R^2$ = 0.9628) | | | | | |
| Model(Quadratic) | 71024.67 | 5 | 14204.93 | 15.52131 | 0.0236 |
| $X_1$ | 37493.42 | 1 | 37493.42 | 40.96793 | 0.0077 |
| X2 | 10.42 | 1 | 10.42 | 0.011 | 0.9215 |
| $X_1$ $X_2$ | 430.05 | 1 | 430.05 | 0.011 | 0.9215 |
| $X_1^2$ | 1535.47 | 1 | 1535.47 | 0.47 | 0.5423 |
| $X_2^2$ | 32487.01 | 1 | 32487.01 | 35.49758 | 0.0095 |
| $Y_2$=zeta potential ($R^2$ = 0.7890) | | | | | |
| Model(Linear) | 687.7025 | 2 | 343.8513 | 11.21757 | 0.0094 |
| $X_1$-Drug polymer ratio | 594.8759 | 1 | 594.8759 | 19.40683 | 0.0045 |
| $X_2$-RPM | 92.33 | 1 | 92.33 | 3.03 | 0.01325 |
| $Y_3$=Entrapment efficiency ($R^2$ = 0.9077) | | | | | |
| Model(Linear) | 428.8564 | 2 | 214.4282 | 29.51019 | 0.0008 |
| $X_2$-RPM | 13.59 | 1 | 13.59 | 2.56 | 0.1609 |
| $X_1$-Drug polymer ratio | 410.2708 | 1 | 410.2708 | 56.46259 | 0.0003 |

df - degrees of freedom, $p < 0.05$ are statistically significant

**Table 6: Reduced equation for responses**

| Reduced model equation | $R^2$ Value |
|---|---|
| $Y_1$ = -492.27213-13.14470$X_1$+144.24501 $X_2$+0.13784$X_1$ $X_2$+0.12575$X_1$2-5.09800$X_2$2 | $R^2$ = 0.9628 |
| $Y_2$ =+72.52852-0.66186 $X_1$+0.78667 $X_2$ | $R^2$ = 0.7890 |
| $Y_3$ =+124.65251-0.54965$X_1$-0.35200$X_2$ | $R^2$ = 0.9077 |



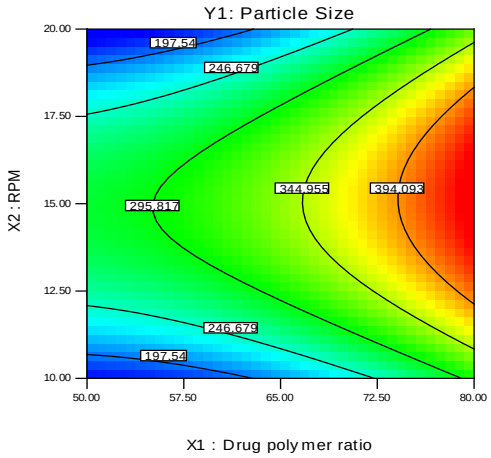
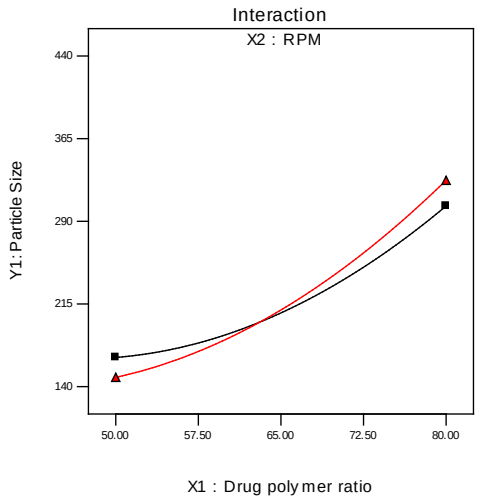




**Fig. 1: (a) Three-dimensional response surface plots for particle size. (b). Interaction Plot for particle size. (C). Contour surface plot of particle size**



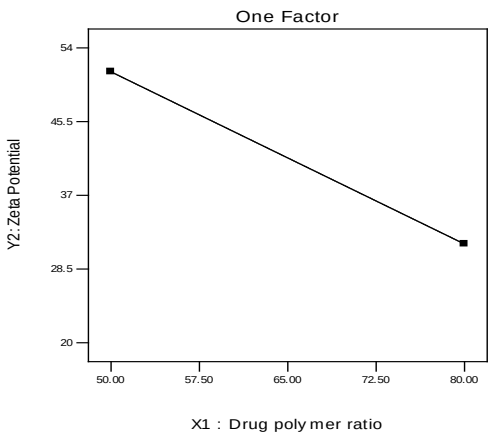




**Fig. 2a: Interaction plot for zeta potential.**

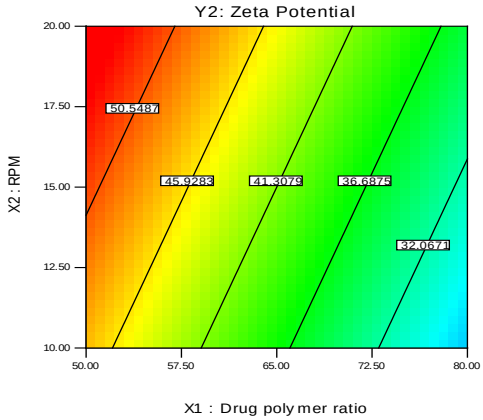
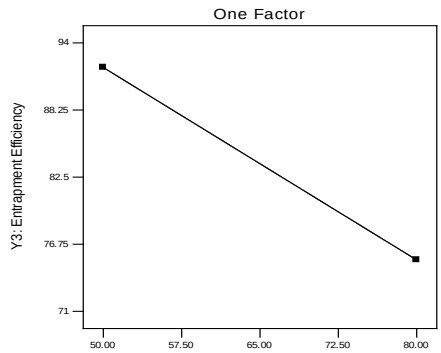



**Fig. 2b: Contour surface plot of zeta potential.**



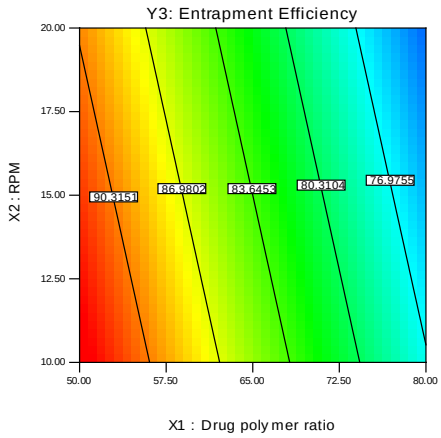




**Fig. 3(a): Interaction plot for entrapment efficiency. (b).Contour surface plot of entrapment efficiency**

### Check point analysis

Model equations were developed for responses and two check point formulations were made to confirm the reproducibility and reliability of the equations. Predicted as well as actual values showed no significant difference proving the reliability of the developed model. The comparisons of actual and predicted values are given in Table 7.

### Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra data of pure naproxen and Eudragit RS 100 were shown in Figure 5. The C-H stretching was appeared at 3199 $cm^{-1}$. The C=O stretching was found to be at 1727 $cm^{-1}$. The C=C stretching was appeared at 1685.89 $cm^{-1}$. The drug and polymer has similar stretching values at 3198 $cm^{-1}$, 1728.34 $cm^{-1}$ and 1689 $cm^{-1}$. So the naproxen and Eudragit RS 100 has no significant interactions.

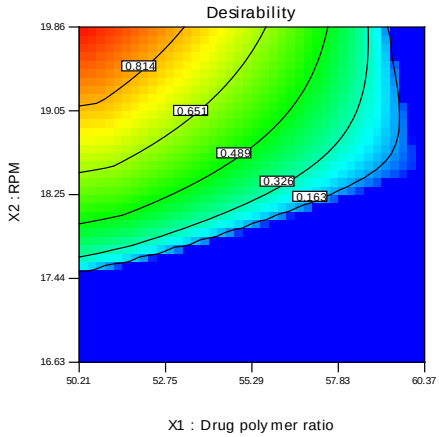



**Fig. 4: Desirability graph of predicted values**.

### *Invitro* drug release

*In vitro* drug release from the nanosuspension in a phosphate buffer pH 6.8 was performed using the diffusion bag technique. The in vitro release of the five batches of nanosuspension showed an interesting bi-phasic release with an initial burst effect. In the first hour, drug released was 12.9%, 29.5%, 17.1%, 13.2% and 15.3% for NN4, NNE5, NNE6, NNE7, NNE8, respectively (Figure 5). Afterwards the drug release followed a controlled release pattern. The burst release in the first hour can be attributed to the drug loaded on the surface of Nanoparticles. The amount of drug incorporation in the formulation and drug entrapment efficiency has a direct effect on the drug release profile from the formulations.

### Kinetics of Drug Release

Data obtained from dissolution studies were fitted to various kinetic models. The kinetic models used were zero order (percentage unreleased vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi's (cumulative percentage of drug released vs. square root of time), Hixon-Crowell cube root law and Korsmeyer (log cumulative percentage of drug released vs. log time) equation. The data of average values were described in the Table 8. Based on the highest regression values (r), the best-fit model for NNE5 and NNE7 followed higuchi and peppas model respectively, NNE1, NNE2, NNE3, NN4, NNE6, NNE8 and NNE9 followed Hixson-crowell model. Here it can be assumed that the release rate was limited by the drug particles dissolution rate and erosion of the polymer matrix [16].

### Stability studies

The stability studies were carried out on optimized formulation. The samples were stored at 40°c±2°c and 75 ± 5% (accelerated testing) RH for three months to access their stability. After 1, 2 and 3 months samples were withdrawn and retested for particle size and zeta potential; the optimized formulation did not show any significant change in both parameters. It indicates that this formulation was able to retain its stability up to 3 months. Stability data is shown in Table 9.

**Table 7: Actual and predicated values of the responses**

| Standard order | Actual values | | | Predicted values | | | Residual | | |
|---|---|---|---|---|---|---|---|---|---|
| | $Y_1$ (nm) | $Y_2$ (mV) | $Y_3$ (%) | $Y_1$ (nm) | $Y_2$ (mV) | $Y_3$ (%) | $Y_1$ (nm) | $Y_2$ (mV) | $Y_3$ (%) |
| 1 | 159.7 | 53.5 | 93.17 | 149.58 | 55.12 | 90.11 | 10.12 | -1.62 | 3.058 |
| 2 | 180.5 | 46.3 | 89.92 | 166.43 | 47.3 | 93.65 | 14.069 | -1 | -3.73 |

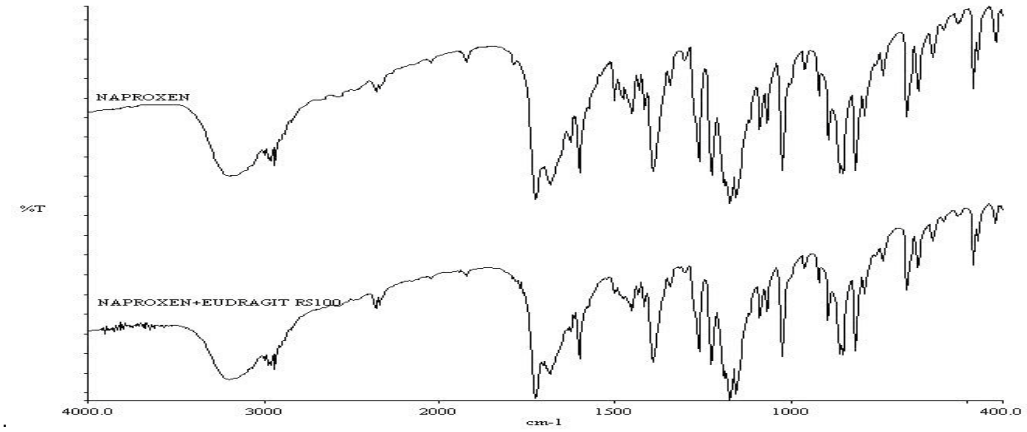



**Fig. 5: Fourier transform infrared spectra of naproxen and eudragit rs 100**

**Table 8**: ***In Vitro*** **release kinetics for naproxen loaded eudragit RS 100 nanosuspension formulations**

| Formulation Code | Zero Order | | First Order | | Higuchi | | Peppas | | | Hixson Crowel | | Best Fit Model |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $k_0$ | $R^2$ | $k_1$ | $R^2$ | $k_2$ | $R^2$ | n | $k_3$ | $R^2$ | $k_4$ | |
| NNE 1 | 0.951 | 9.251 | 0.981 | 0.215 | 0.974 | 26.761 | 0.977 | 0.885 | 12.538 | 0.995 | 0.051 | Hixson crowel |
| NNE 2 | 0.981 | 7.843 | 0.986 | 0.143 | 0.951 | 22.330 | 0.976 | 1.139 | 6.268 | 0.994 | 0.038 | Hixson crowel |
| NNE 3 | 0.990 | 7.361 | 0.984 | 0.127 | 0.937 | 20.843 | 0.958 | 1.495 | 2.914 | 0.992 | 0.034 | Hixson crowel |
| NNE 4 | 0.983 | 6.511 | 0.982 | 0.101 | 0.954 | 20.843 | 0.990 | 0.760 | 10.767 | 0.991 | 0.028 | Hixson crowel |
| NNE 5 | 0.871 | 11.291 | 0.981 | 0.260 | 0.996 | 30.454 | 0.985 | 0.583 | 26.554 | 0.987 | 0.062 | Higuchi |
| NNE 6 | 0.963 | 8.390 | 0.981 | 0.163 | 0.971 | 24.223 | 0.993 | 0.736 | 15.213 | 0.994 | 0.042 | Hixson crowel |
| NNE 7 | 0.981 | 6.811 | 0.981 | 0.109 | 0.961 | 19.506 | 0.993 | 0.736 | 11.897 | 0.992 | 0.030 | Peppas |
| NNE 8 | 0.941 | 9.581 | 0.974 | 0.243 | 0.980 | 27.91 | 0.973 | 0.880 | 13.325 | 0.996 | 0.055 | Hixson crowel |
| NNE 9 | 0.990 | 7.490 | 0.982 | 0.132 | 0.931 | 21.213 | 0.961 | 1.530 | 2.772 | 0.992 | 0.035 | Hixson crowel |

**Table 9: Stability data of optimized formulation of nanosuspension**

| Time period | Particle size | Zeta potential |
|---|---|---|
| Initial | 159.7 | 53.5 |
| After storage | | |
| 1 Month | 159.7 | 53.7 |
| 2 Month | 159.95 | 53.94 |
| 3 Month | 160.14 | 54.12 |

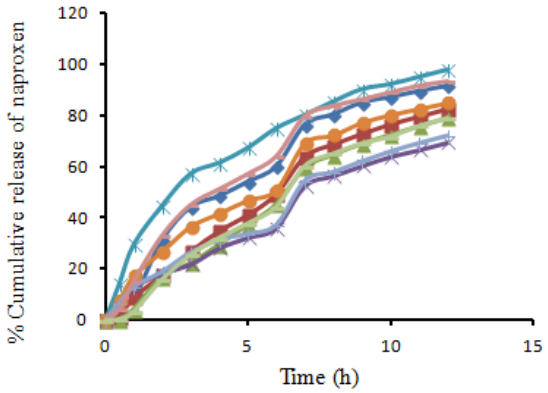



**Fig. 6: Cumulative release of naproxen from nanosuspension formulations: % Cumulative release of NNE1, % Cumulative release of NNE2, % Cumulative release of NNE3, % Cumulative release of NNE4, % Cumulative release of NNE5, % Cumulative release of NNE6, % Cumulative release of NNE7, % Cumulative release of NNE8, % Cumulative release of NNE9**

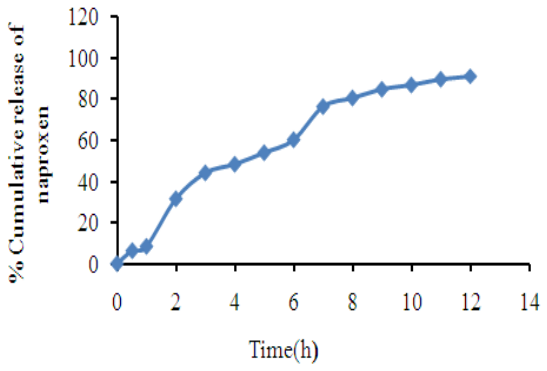



**Fig. 7: Dissolution profile of optimized formulation**

## CONCLUSION

The nanosuspension of poorly water soluble drug like naproxen is proved to be better and a cost effective alternative. Naproxen loaded Eudragit RS 100 nanosuspension can be administrated as intra-muscular or as eye drop for inflammatory ocular diseases. The formulations were optimized by using design of experiments by employing a $3^2$ factorial statistical design. The nanosuspensions were studied for particle size, zeta potential and entrapment

efficiency, formulation NNE1 was selected as an optimum formulation with desired properties. Drug: polymer ratio had significant influence on particle size, Zeta potential and entrapment efficiency. The model equations, contour plot and surface plots for selected independent variables were also generated. Moreover, the utilization of statistical design of experiment in the optimization process significantly reduced the requirement of the number of experiment and time.

**CONFLICT OF INTERESTS**

Declared None

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