



FORMULATION AND DEVELOPMENT OF LIPID BASED NON-AQUEOUS NANO EMULSION FOR SELECTED NSAID

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ABSTRACT

Oral administration of non-steroidal anti-inflammatory drugs is avoided in patients with disease like peptic ulcer, gastro esophageal reflux (GERD), irritable bowel syndrome. Administration of drug through topical route through the skin could reduce the above side effects associated with oral administered drug formulation. Hence, the aim of present study was to develop Nonaqueous Nanoemulsion (NANEs) of Naproxen for topical administration. NANEs is a multiphase colloidal dispersion heterogeneous system consist of fine nonaqueous polar solvent in oil dispersion with surfactant. NANEs of naproxen were prepared using high energy emulsification method, which break large micro droplets into nano size droplets. Drug-excipients Compatibility was studied by FT-IR. Rheology, Flow behavior, Viscosity, pH, Globule size, Drug content, in-vitro drug release of NANE were performed. For optimization of nonaqueous nanoemulsion Box-Behnken experimental design was implemented. The variables selected are phase volume ratio, surfactant concentration and stirring time. Data analysis showed that surfactant concentration and phase volume ratio significantly affect the viscosity of the formulation. From the present study, it can be concluded that a stable non-aqueous nanoemulsion can be obtained by using glycerin as dispersed phase, mineral oil as continuous phase and glycerol monostearate as surfactant, which can be used as vehicle for the water sensitive materials. This nonaqueous nanoemulsion has improved the stability of Naproxen and overcome the oral drawback associated with it.

Keywords: Non-steroidal Anti-inflammatory Drug, Gastro Esophageal Reflux, Non-Aqueous Nanoemulsion, Nonaqueous Polar solvent, Box-Behnken Experimental Design.

1. INTRODUCTION

Naproxen is a synthetic, medium potency, non-steroidal anti-inflammatory drug used for the relief of inflammation like gout, arthritis, spondylitis, bursitis. Naproxen exerts its clinical effects by blocking the COX-1 and COX-2 enzymes resulting in decreased prostaglandin synthesis. While both enzymes contribute to the development of prostaglandin. COX-1 enzymes are constitutively active and can be found in normal tissues such as the stomach liner, whereas the COX-2 enzyme is inducible and produces prostaglandins that cause pain, fever and inflammation. The COX-2 enzyme mediates the desired antipyretic, analgesic and anti-inflammatory properties provided by Naproxen. Emulsion is one of the most convenient and advantageous formulation consist of continuous and disperse phase, from that one of the liquid phase is water, however emulsion can be formulated without an aqueous phase of

water to produce non-aqueous, anhydrous or oil-in-oil emulsions. Such type of formulations can substitute conventional emulsions, where the presence of water can be avoided. In such type of emulsions drug is dissolved in a suitable non aqueous disperse phase solvent. Lipid formulation can reduce the inherent drawback and facilitates the formation of solubilized phases through which absorption of drug occur. Unfortunately, the major difficulty in formulating non-aqueous nano emulsions arises from the lack of appropriate data on surfactant action in relevant non-aqueous media. Various non-ionic surfactants were used to improve stability and elegance of oil-in-oil emulsion formulation by converting into suitable topical delivery system. Nonaqueous Nano emulsions are thermodynamically stable transparent, isotropic systems of nonaqueous polar solvent, oil and surfactants. Their long-term stability, ease of preparation and high solubilization of drug molecules make them

promising as a drug delivery tool. They have found wide applications in oral drug delivery to enhance the solubility and bioavailability of drugs. They are also being investigated ardently for potential applications in ocular, pulmonary, nasal, vaginal and parenteral drug delivery. Recently, there has been a surge in the exploration of nonaqueous nanoemulsion for topical delivery. The objective of present study was to overcome the undesirable side effect associated with oral administration of Naproxen like peptic ulcer, gastro esophageal reflux (GERD), irritable bowel syndrome and to enhance stability of naproxen by using nonaqueous nanoemulsion system. Conventional emulsion has large droplet size thus it gives poor bioavailability, in nonaqueous nanoemulsion particle size goes on decreasing which increase in the bioavailability of the drug. Phase inversion, phase separation, flocculation, coalescence, creaming, cracking are the drawbacks of conventional emulsions but nonaqueous nanoemulsion does not show it. Decrease in particle size increase viscosity and increase in viscosity increases stability of formulation, which can be achieved in NANEs. It acts as a carrier for lipophilic drug. NANEs are nontoxic and non-irritant hence can be applied to skin and mucous membrane. They can also control the release to drug through skin for longer duration of time. Hence to achieve all above desirable properties we formulate naproxen in nonaqueous nanoemulsion [1-3].

2. MATERIAL AND METHODS

Naproxen as a drug was obtained from drug laboratory of DVVPFs college of Pharmacy, Ahmednagar (MH), India. Glycerine from Loba chemie, Mumbai, Mineral oil from Poona chemical laboratory, Pune and Glycerol monostearate from Research lab fine chemical industry, Mumbai. All other materials used in this study were of analytical grade.

2.1. Preformulation Studies

Preformulation study was carried out like IR spectrum, UV Spectra, Melting Point, Solubility, pH, Differential Scanning Calorimetry, drug excipient compatibility for its identity, purity and physicochemical characterization [4-7].

2.2. Analytical Methods Development

2.2.1. Determination of λ_{max}

Accurately weighed amount of Naproxen (100 mg) was transferred to a 100 volumetric flask. 50 mL of 7.2 phosphate buffer (containing 0.5%v/v of Tween 80) was

added to dissolve the drug and volume was made up to 100 mL. The resultant solution had the concentration of 1mg/mL which was labeled a 'Stock'. From this stock solution 10 mL was diluted to 100 mL with 7.2 phosphate buffer which has given the solution having the concentration of 100 μ g/mL. Necessary dilutions were made by using this second solution to give the different concentrations of Naproxen (1 to 12 μ g/mL) solutions. The absorbance of above solutions was recorded at λ_{max} 273 nm of the drug using double beam UV-Visible spectrophotometer. Standard graph was plotted between the concentration on X-axis and absorbance on Y-axis [8, 9].

2.2.2. Construction of Calibration Curve in Ethanol

Accurately weighed quantities of 10 mg of Naproxen were dissolved in 10 mL of Ethanol (1000 μ g/mL) from resultant solution, stock solutions of concentration (100 μ g/mL) were prepared. From this stock solution, 0-10 mL aliquots were withdrawn and diluted with up to 10 mL to obtain solutions of concentration 0-100 μ g/mL. Absorbance of solutions was measured at wavelength of maximum absorbance at λ_{max} 242 nm for Ethanol using UV-visible spectrophotometer. Calibration curve were plotted as absorbance vs. concentration, the linearity or regression equation was also obtained [8, 9].

2.2.3. Construction of Calibration Curve in Phosphate buffer pH 5.0

Accurately weighed quantities of 10 mg of Naproxen were dissolved in 10 mL of Ethanol (1000 μ g/mL) from resultant solution, stock solutions of concentration (100 μ g/mL) were prepared by diluting with phosphate buffer pH 5.0. From this stock solution 0-10 mL aliquots were withdrawn and diluted with up to 10 mL to obtain solutions of concentration 0-100 μ g/ml. Absorbance of solutions was measured at wavelength of maximum absorbance at λ_{max} 273 nm for Phosphate buffer pH 5.0 using UV-visible spectrophotometer. Calibration curves were plotted as absorbance vs. concentration, the linearity or regression equation was also obtained [8, 9].

2.2.4. Fourier Transform Infrared (FTIR) Spectrophotometer Study

The FTIR spectra of Naproxen and excipients were recorded using FTIR (cary-630 Agilent technology). The spectra were recorded over the range of wave number 4000 to 400 cm^{-1} [10, 11].

2.2.5. Differential Scanning Calorimetry

The DSC patterns were recorded on a Mettler Toledo Star System. 4.800 mg of drug was heated in crimped aluminum pans at a scanning rate of 10°C/min in an atmosphere of nitrogen gas flow 40 mL/min using the range of 40-300°C [12].

2.2.6. Determination of retention time of Naproxen by RP-HPLC

Naproxen Stock solution of 1000 ppm in Methanol was prepared and 10µl aliquot of the resulting solution was injected into a RP-HPLC system elution having flow rate of 1.3 ml/min. for 8 min. Agilent Open Lab Control having C₁₈ column with Buffer: Methanol (40:60) as a mobile phase at 230nm was use for study [13].

2.2.7. Preparation of calibration curve for Naproxen by RP-HPLC

Stock solution of 1000 ppm was prepared in methanol (HPLC grade) and further diluted with methanol as solvent to get solutions with concentration range 1, 2, 3, 4, 5, 10, 25, 50, 100µg/mL. 10µl aliquot of the resulting solution was injected into injection port of RP-HPLC and calibration curve were plotted as area vs.

concentration, the linearity and regression equation were also obtained [13].

2.2.8. Solubility determination in oils, polar solvents

The solubility of Naproxenin various oils, surfactant was determined by dissolving an excess amount of Naproxenin 5 mL of each of the solvent [14].

2.3. Formulation of Nonaqueous Nanoemulsion (NANEs)

NANEs was prepared by using different phase volume ratio, surfactant concentration and time of High-speed homogenizer. It is obvious from preliminary experiments utilising various methods of separation that 10% Naproxen as a medication, 5% Glycerol Monostearate as a surfactant, 5 mL Mineral oil as a continuous phase, and 5 mL Glycerine as a dispersed phase are all viable options. In first beaker weighed quantity of Naproxen was dissolved in mineral oil then GMS (Glycerin mono stearate) was added to second beaker i.e. Heated the glycerin to around 50-60 degrees Celsius, chilled, then added it to the second beaker and homogenised for 3 minutes using Remi's Ultraturex High Speed Homogeniser at 15000-16000 rpm.

Table 1: Formulation of Glycerin in oil NANE with Glycerol monostearate surfactant

| Emulsion Type | Surfactant conc. (%) | Phase Volume Ratio | Method of preparation and Stability of NANE Formulation | | | |
|---------------|----------------------|--------------------|---|-------------------------|-------------------------|---------------------------------------|
| | | | Trituration (1.30hr) | Magnetic Stirrer (2 hr) | Probe Sonication (2 hr) | High speed homogenization (15000 psi) |
| Drug | - | | | 10% | | |
| G/O | 1 | 1:9 | ± | ± | ± | ± |
| | | 2:8 | ± | ± | ± | ± |
| | | 3:7 | ± | ± | ± | ± |
| | 3 | 1:9 | ± | ± | ± | ± |
| | | 2:8 | ± | ± | ± | ± |
| | | 3:7 | - | ± | ± | ± |
| | 5 | 1:9 | + | - | ++ | ± |
| | | 2:8 | + | - | ++ | ± |
| | | 3:7 | +++ | + | ++ | + |
| | | 4:6 | ** | + | +++ | ** |
| | | 5:5 | *** | + | +++ | *** |
| | | | | | | |

G/O-Glycerine in Oil, ± (Unstable), - (Stable up to 0-3 days), + (Stable up to 4-8days), ++ (Stable up to 15-30days), +++ (Stable up to 30-35 days), ** (Stable up to 40-60 days), *** (Stable for more than 90 days).

2.3.1. Box-Behnken Experimental Design

Systematic optimization procedures are carried out by choosing an objective function, finding the most important or contributing factors and using the so-called

response surface technique to examine the relationship between responses and factors. DESIGN-EXPERT, Version 11 Software, Stat Ease, Minneapolis, MN was used for the study [15, 16].

2.4. Characterizations of Optimized Nonaqueous Nanoemulsion

Non-aqueous nano emulsion was evaluated for organoleptic properties. The pH values of optimized nonaqueous nanoemulsion were measured by using digital pH meter [17].

2.4.1. Rheology

Rheological measurements are very useful to characterize the flow properties of emulsion systems and to predict their behavior during manufacturing, packaging or final use. All rheological tests were carried out by using Brookfield R/S-CPS+ Rheometer [16, 18]. Generally, emulsions exhibit non-Newtonian flow behavior. The non-Newtonian flow type was confirmed by plotting the viscosity curve of the non-aqueous nanoemulsion [18].

The flow type was determined using increased shear rate (1-100 sec⁻¹) linearly for 150 seconds. The measured viscosity vs. shear rate curve indicates the flow type of NANEs [18].

The viscosity of NANEs was measured at changing shear rates from 1-100 sec⁻¹ and 100-1 sec⁻¹ with equal stray [19].

The dynamic viscosity of nonaqueous nano emulsion was studied to resolute the thixotropic behavior of formulation. The process parameters embrace the increased and decreased shear rate from 1-100 sec⁻¹ and 100-1 sec⁻¹ for 150 seconds [19].

2.4.2. Drug content

For Drug content of the non-aqueous nanoemulsion 1 ml of NANEs dissolved in 100 ml of PBS of pH 5 and drug content was determined by UV spectroscopy at 273 nm [20-21].

2.4.3. Globule Size

The size and size distribution analysis were performed on the selected formulation by using Nicomp 1800 size analyzer [22].

2.4.4. Invitro Drug Release

In-vitro drug release study of non-aqueous nanoemulsion was performed by using Franz diffusion cell with a cellulose membrane (Artificial membrane 0.1µm) [23-24].

3. RESULTS AND DISCUSSION

3.1. Preformulation studies

3.1.1. Identification tests

All identification tests were carried out as per Indian

Pharmacopoeia specifications, volume-III, 2018. Identification tests were specific for the drug. The test results and physicochemical characterization are mentioned in Table 2.

Table 2: Identification tests for Naproxen

| Test | Result | Standard[26] |
|---------------|----------|-----------------------|
| Color | White | White to light yellow |
| Odor | Odorless | Odorless |
| Taste | Acidic | Acidic |
| Melting point | 153°C | 154-158°C |
| pH | 5.5 | 5.2-5.5 |

3.1.2. Determination of λ_{max}

In the preformulation study, the standard curve of Naproxen was plotted in standard solvent and in drug release media which shows well linearity as mentioned in Table 3. It was found that the estimation of Naproxen by UV spectrophotometrically method at λ_{max} 273nm in phosphate buffer solution pH 5.0 had good reproducibility and this method was used in the study. The correlation coefficient for the standard curve in ethanol and Phosphate Buffer Solution pH 5.0 was found to be 0.996 which is closer to 1, the regression equation produced was further used for drug content, solubility determination and for *In vitro* drug release study.

Table 3: Calibration curve of Naproxenin different solvents

| Parameter | Regression analysis with different solvent | |
|-------------------------|--|------------|
| | Ethanol | PBS PH 5.0 |
| UV-VIS Range (nm) | 200-400 | 200-400 |
| λ_{max} | 242 | 273 |
| Linearity range (µg/mL) | 0-10 | 0-10 |
| Intercept | 0.015 | -0.005 |
| Slope | 0.057 | 0.131 |
| Regression Coefficient | 0.996 | 0.996 |

3.1.3. FTIR analysis

The FTIR spectrum of Naproxen is shown in Fig.1. Principal peaks were found in the range corresponding to functional groups. Appearance of the principle peaks in spectrum confirms the drug sample is Naproxen and is pure.

3.1.4. Differential scanning Calorimetry (DSC)

DSC of the Naproxen showed sharp peak at 160.45°C that is analogous to the melting point temperature of Naproxen.

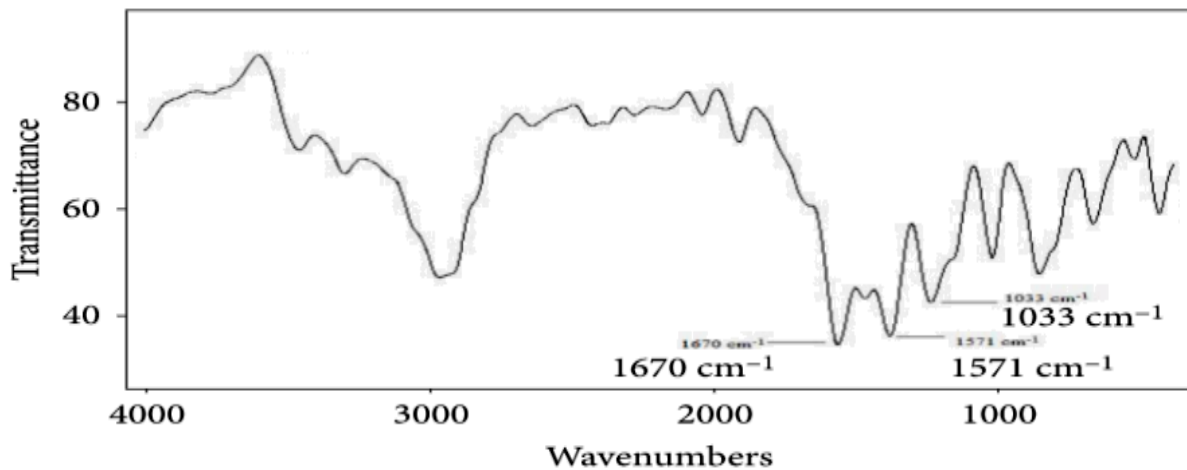


Fig. 1: FTIR spectrum of Naproxen

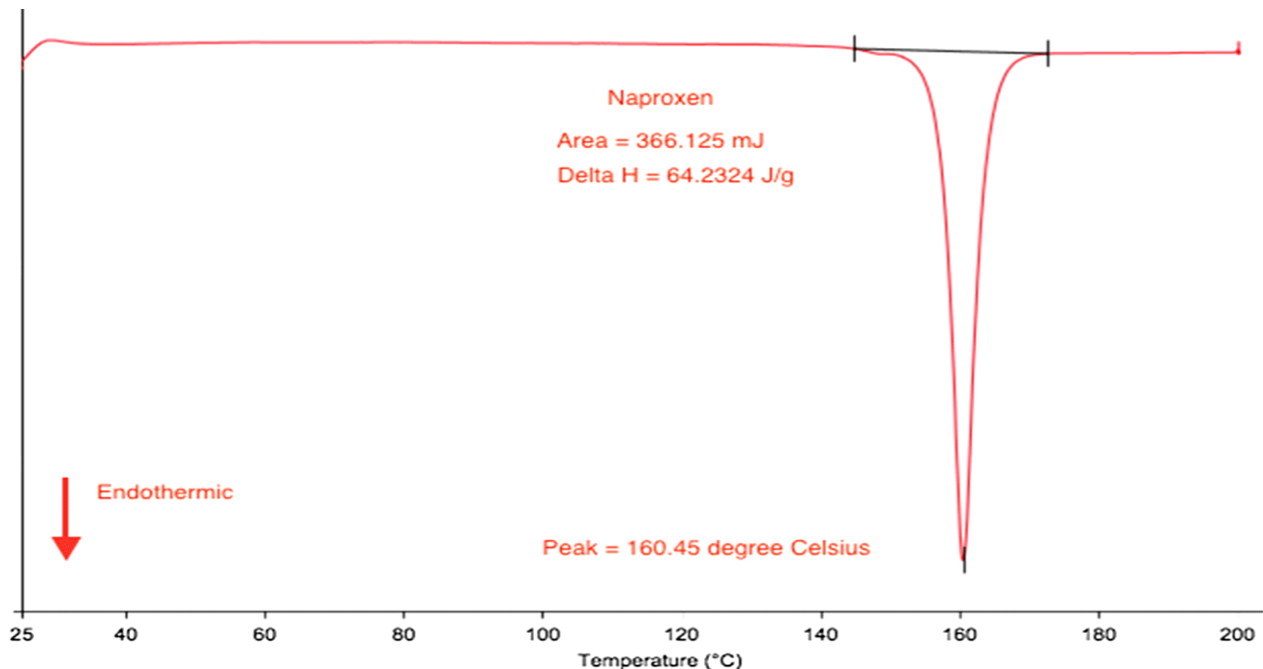


Fig. 2: DSC of Naproxen

3.1.5. RP-High Performance Liquid Chromatography

RP-High Performance Liquid Chromatography was highly sensitive technique announced for determination correlation coefficient and drug content of Naproxen was determined.

3.1.6. Solubility of Naproxen

Table 5 shows that Naproxen is freely soluble in these fluids. Also, it has solubility about 52.34 ± 0.19 (SD) $\mu\text{g/ml}$ in glycerin; therefore, glycerin can be used as dispersed phase in the non-aqueous nanoemulsion.

Table 4: Calibration Curve of Naproxen by RP-HPLC

| Parameter | Regression Analysis |
|----------------------------------|---|
| Column | C ₁₈ column (150mm×4.6mm, 5 μm) |
| λ_{max} | 230nm |
| Mobile phase | Buffer: Methanol (40:60) |
| Flow rate | 1.3 ml/min |
| Retention Time | 8.330 min. |
| Linearity Range $\mu\text{g/mL}$ | 0-100 |
| Intercept | -3996. |
| Slope | 14165 |
| Regression Coefficient | 0.999 |

Table 5: Saturated solubility of Naproxen indifferent solvents

| S. No. | Solvent | Solubility ($\mu\text{g/ml}$) (\pm SD) |
|--------|----------------------------------|--|
| 1 | Mineral oil | 123.35 ± 0.23 |
| 2 | Glycerin | 52.34 ± 0.19 |
| 3 | Ethanol | 50 ± 0.13 |
| 4 | Phosphate Buffer Solution (pH 5) | 0.60 ± 0.007 |

n=3

3.1.7. Drug excipient compatibility study

Interactions between the active substance and excipients can influence the pharmacological properties and behavior of drugs in biological systems. The IR spectral studies of pure Naproxen and formulations containing Naproxen and other excipients were carried out to study the interaction between the drug and excipients used. O-H stretching, C=O stretching of carbonyl, C=C stretching and C-O stretching of lactone of pure Naproxen and Naproxen formulations containing higher proportion of excipients were almost in the same region. It showed that IR spectra of pure Naproxen and Naproxen containing non-aqueous nanoemulsion were similar fundamental peaks and patterns.

3.1.8. Box-Behnken Experimental Design

3.1.8.1. Data Analysis

A Box-Behnken statistical design with 3 factors, 3 levels and 17 runs were selected to study the effects on dependent variables. All the batches prepared within the experimental design yielded glycerin-in-oil nonaqueous nanoemulsion and these were evaluated for viscosity (Y1) and stability (Y2). The all selected dependent variables obtained at various levels of the 3 independent variables (X1, X2 and X3 i.e., Phase Volume Ratio,

Surfactant Concentration and Homogenization Time Respectively).

3.1.8.2. Effect of formulation variables

The results clearly indicate that the stability and viscosity value is strongly affected by the variables selected for the study.

3.1.8.3. Analysis of Variance (ANOVA), pure error and lack of fit

Regression analysis was carried out to determine the regression coefficients. All the independent variables were found to be significant for all response variables. The linear as well as quadratic model was found to be significant for X and linear model for Y. So above result indicates that both the factors play an important role in the formulation of Glycerin-in-oil nanoemulsion containing Naproxen. For lack of fit P values, we obtained 0.1904 for response Y, P value not showed for response X and hence the current model provided a satisfactory fit to the data and had no lack of fit. Table 6 and 7 showed the ANOVA studies for X and Y.

3.1.8.4. Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are presented in Figs. 3 to 5, which are very useful to study the interaction effects of the factors on the responses. These types of plots are useful in study of the effects of two factors on the response at one time. In all the presented Figs.3 to 4, the third factor was kept at a constant level.

From 3D response surface plot in fig. 5, it was observed that the major effect on stability and viscosity was dependent on two factors as surfactant concentration and phase volume ratio.

Table 6: Analysis of Variance (ANOVA) of Viscosity

| ANOVA Table | | | |
|------------------------|-------------------|---------------|-------------|
| Source of Variation | | F Ratio | P Value |
| Model | | 283.95 | <0.0001 |
| A: Phase volume ratio | | 1.17 | 0.3159 |
| B: Surfactant conc. | | 1866.67 | <0.0001 |
| C: Homogenization time | | 10.50 | 0.0142 |
| Source of variation | Degree of freedom | Sum of square | Mean Square |
| Residual | 7 | 3.00 | 0.43 |
| Lac of fit | 3 | 3.00 | 1.00 |
| Pure Error | 4 | 0.000 | 0.000 |
| Cor total | 16 | 1098.24 | - |

Table 7: Analysis of Variance (ANOVA) of Stability

| ANOVA Table | | | |
|------------------------|-------------------|----------------|--------------|
| Source of Variation | | F Ratio | P Value |
| Model | | 21.56 | 0.0003 |
| A: Phase volume ratio | | 0.28 | 0.6103 |
| B: Surfactant conc. | | 135.87 | <0.0001 |
| C: Homogenization time | | 0.84 | 0.3910 |
| Source of variation | Degree of squares | Sum of squares | Mean Squares |
| Residual | 7 | 150.75 | 21.56 |
| Lack of Fit | 3 | 150.75 | 50.25 |
| Pure Error | 4 | 0.000 | 0.000 |
| Cor Total | 16 | 4329.06 | - |

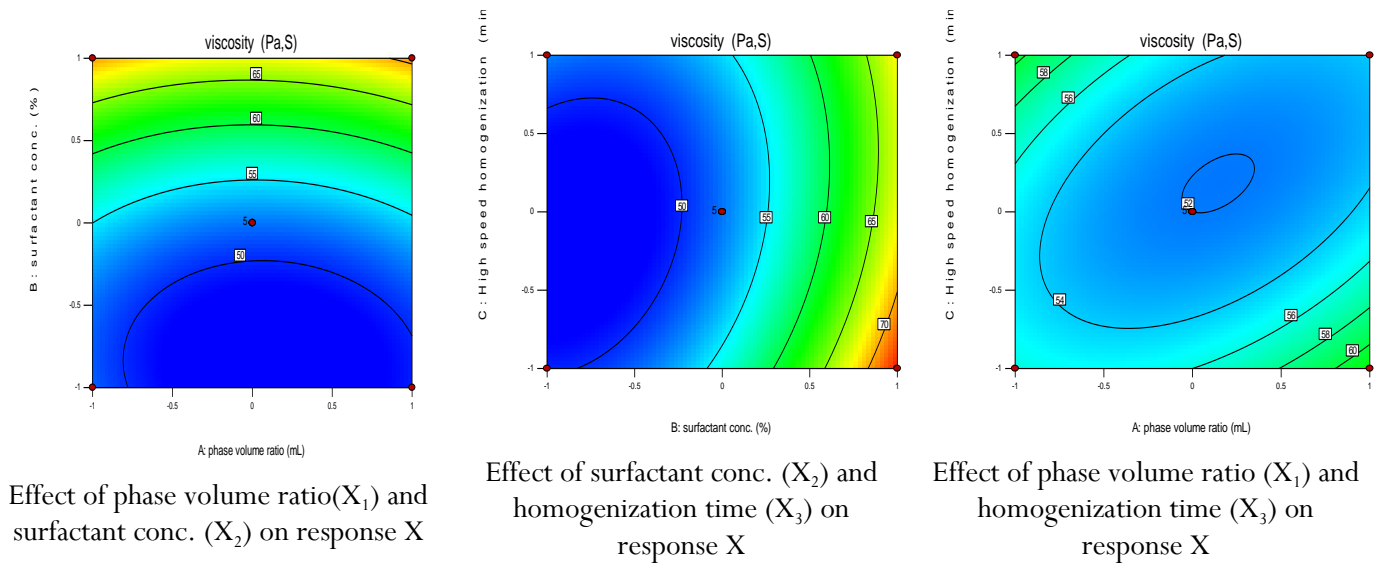


Fig. 3: Counterplots showing effects of phase volume ratio, surfactant concentration and homogenization time on viscosity

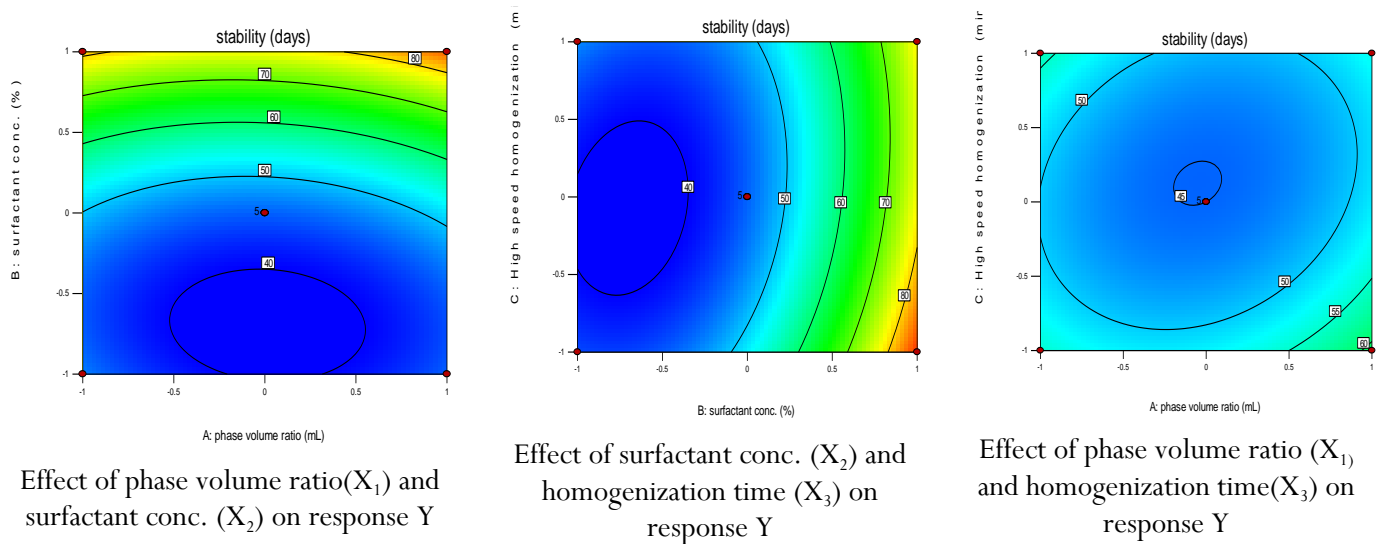
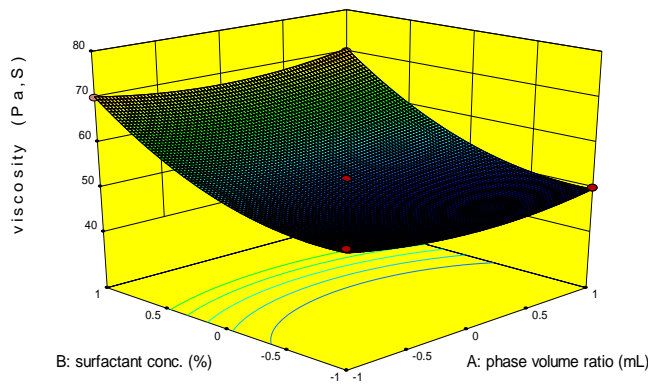
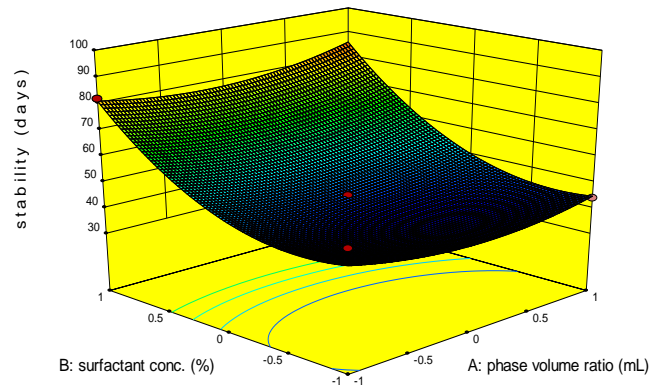


Fig. 4: Counterplots showing effects of phase volume ratio, surfactant concentration and homogenization time on stability



Effect of phase volume ratio and surfactant concentration on response X



Effect of phase volume ratio and surfactant concentration on response Y

Fig. 5: Response surface plot (3D) showing the effect of phase volume ratio and surfactant concentration on response X and Y

3.2. Characterization of Optimized Non-aqueous Nanoemulsion

3.2.1. General appearance

Formulation was soft, white semisolid, free from grittiness. pH of the freshly prepared non-aqueous nano emulsion was found to be in the range 5.0-5.5 which is similar to normal skin pH.

3.2.2. Rheology

Rheological measurements are very useful to characterize the flow properties of emulsion systems. Emulsions exhibit non-Newtonian flow behavior. The non-Newtonian flow type was confirmed by plotting the viscosity curve of the non-aqueous nanoemulsion. When shear rate was increased from 1-100 sec⁻¹, there is decrease in the viscosity of the formulation. This shear thinning effect proves that non-aqueous nanoemulsion exhibits pseudo plastic flow.

It was observed that material becomes less viscous as the rate of shear is increased is referred to as pseudo plastic.

The viscosity of optimized Glycerin in oil nonaqueous nanoemulsion formulation was found to be 52.22 m. Pa.S. The area between two curves (hysteresis area) defines the extent of the time dependent flow behavior. The smaller hysteresis area in Figure 8 shows less time is required for the regaining the original viscosity.

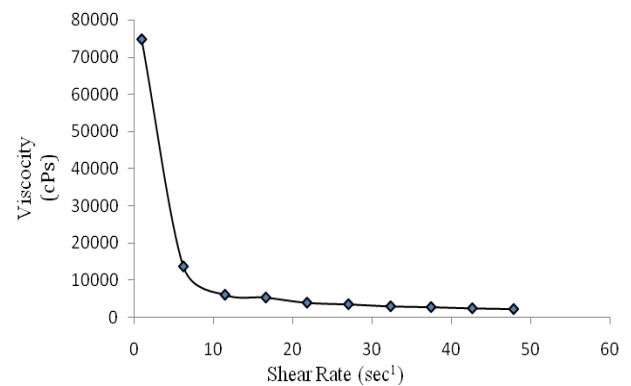


Fig. 6: Viscosity curve of non-aqueous nano-emulsion showing pseudoplastic flow

Table 8: Parameters of optimized formulation

| S. No. | Parameter | Minimum | Maximum | Average |
|--------|---|---------|---------|---------|
| 1 | Viscosity (mPa.S) | 52.22 | 74.89 | 63.98 |
| 2 | Torque (mNm) | 0.1073 | 0.8831 | 0.5770 |
| 3 | Speed (1/min) | 0.33 | 33.33 | 16.83 |
| 4 | Shear Stress (Pa) | 0.97 | 7.95 | 5.22 |
| 5 | Shear rate(1/s) | 0.99 | 99.99 | 50.49 |
| 6 | Kinematic Viscosity (m ² /S) | 0.0001 | 0.0078 | 0.0004 |
| 7 | Density g/cm ³ | 1.00 | 1.00 | 1.00 |
| 8 | Angular Velocity (1/S) | 0.00 | 0.00 | 0.00 |

3.2.3. Drug content

Drug content of three different samples from the same formulation is shown in Table 9. The results show that drug was uniformly distributed in the non-aqueous nanoemulsion.

3.2.4. Globule Size

A graphical representation of particle size distribution of freshly Glycerin in oil NANE is given in Fig. 9. Mean globule size was found to be 191.4 nm.

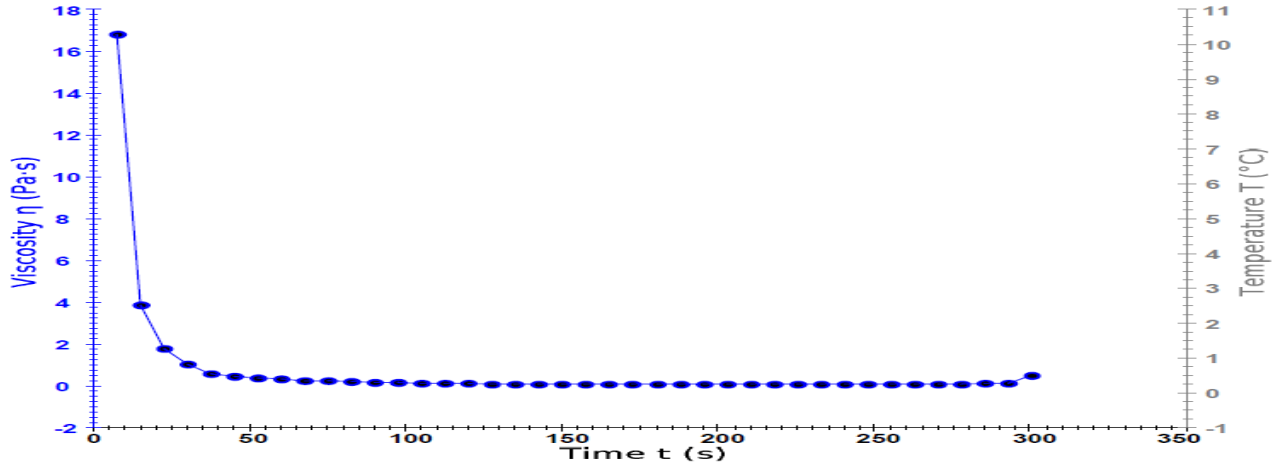


Fig. 7: Consistency curve showing flow behavior of Glycerin in oil nonaqueous nanoemulsion

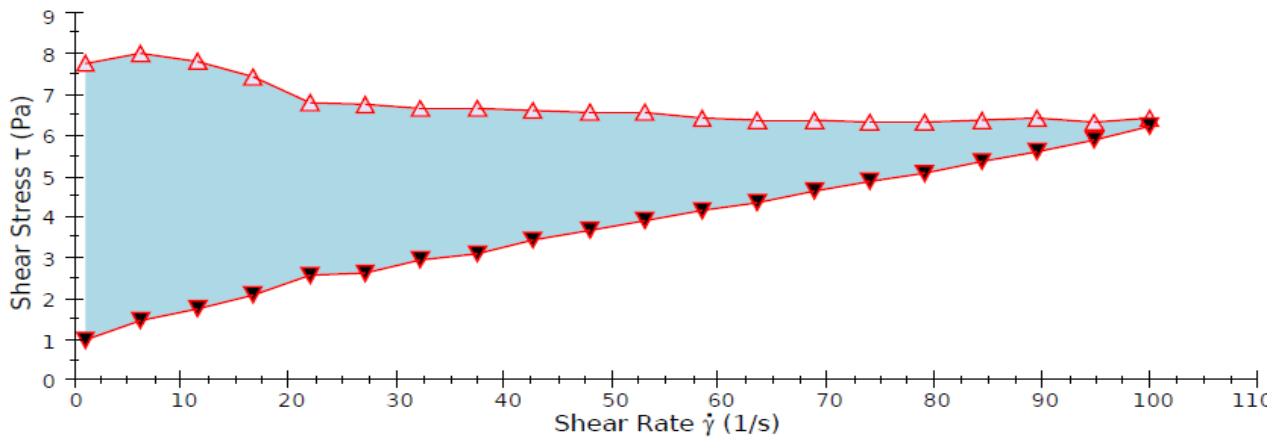


Fig. 8: Flow curve of non-aqueous nanoemulsion showing thixotropy

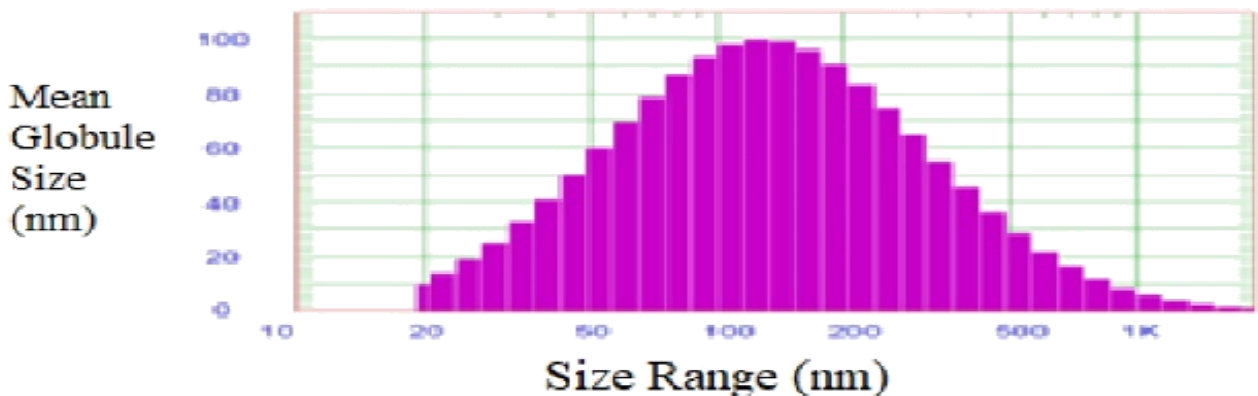


Fig. 9: Globule size distribution of optimized non-aqueous nanoemulsion

Table 9: Drug Content uniformity of optimized non-aqueous nanoemulsion

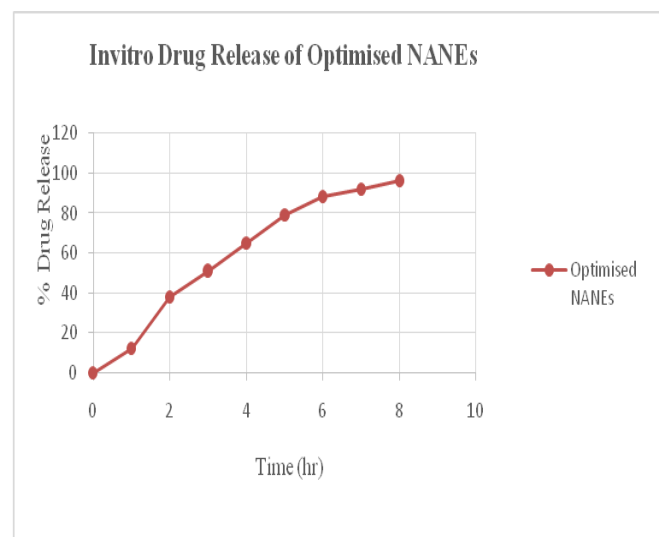
| Sr. No | Sample Number | Theoretical Drug (mg/ml) | Practically Drug content (mg/ml) | Average Drug content % w/v |
|--------|---------------|--------------------------|----------------------------------|----------------------------|
| 1 | 1 | 100 | 92 | 92 |
| 2 | 2 | 100 | 94 | |
| 3 | 3 | 100 | 90 | |

3.2.5. *In vitro* Drug Release

It followed zero order drug release kinetics.

Table 10: *In-vitro* Drug release parameters of G/O NANEs

| S. No | Parameter | Dissolution/ Permeation medium |
|-------|--|--------------------------------|
| 1 | Amount of % drug release | 96.81 % |
| 2 | Steady-state flux J _{ss} (µg/cm ² h) | 5.18 |
| 3 | Permeability coefficient K _p (cm/h) | 0.004 |
| 1 | Amount of % drug release | 96.81 % |

**Fig. 10: *In-vitro* drug release study of optimized formulation of NANEs**

4. CONCLUSION

The objective of the present study was to formulate a stable non-aqueous nanoemulsion for Naproxen by using pharmaceutically approved ingredients. Drug complies with all the identification tests carried out as per Indian Pharmacopoeia. Non-aqueous nanoemulsion

was obtained by using glycerin as a disperse phase and mineral oil as a continuous phase and glycerol monostearate as a surfactant. During formulation, it was found that emulsification was achieved when single surfactant was used, but not with surfactant combination. Only glycerol monostearate gave glycerin-in-oil nonaqueous nano-emulsion with cream like consistency. For optimization of nonaqueous nanoemulsion Box-Behnken experimental design was implemented. Data analysis showed that surfactant concentration and phase volume ratio significantly affect the viscosity of the formulation. Both variables had positive effect on viscosity. But stability was strongly affected by only one independent variable that is surfactant concentration. An optimized formulation was obtained by increasing stability while keeping constrains on viscosity. An optimized formulation had 5% glycerol monostearate as a surfactant, 50:50 phase volume ratio (Glycerine: Mineral Oil) and trituration for 90 min. Optimized non-aqueous nanoemulsion was characterized by pH, viscosity, drug content, globule size analysis and *in vitro* drug release. pH of NANEs was in a range 5.0-5.5 which is similar to normal skin pH. The viscosity of optimized formulation was found to be 52.22 m.Pa.S. Drug content of the formulation was 92 % and drug was uniformly distributed in the NANEs. Mean globule size was found to be 191.4nm. *In-vitro* release studies showed a slow release of Naproxen from non-aqueous nanoemulsion. From the present study, it can be concluded that a stable non-aqueous nanoemulsion can be obtained has improved the stability of Naproxen and overcome the oral drawback associated with it.

5. ACKNOWLEDGEMENTS

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Conflict of interest

None declared

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