ORIGINAL ARTICLE

Stability and *In vitro* Comparative Study of Optimized Non-Aqueous Nano Emulsion of Naproxen with Marketed Formulations

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Abstract

Aim: The current study's objective was to evaluate stability study and compare and analyze the improved non-aqueous nanoemulsion (NANE) of naproxen with marketed gel formulations (M1 and M2). **Methods:** Utilizing a low energy emulsification technique, an optimized NANE of naproxen was prepared. The comparison research was assessed for general appearance, pH, viscosity, drug content, and *in vitro* drug release against marketed formulations. Six months of stability investigations were carried out as per ICH Q1A R2 guideline. During stability study, phase separation, creaming, cracking, phase inversion, color, pH, viscosity, and drug content were evaluated. **Results:** In comparative study, pH of NANE and marketed formulations M1 and M2 was found to be 5.5 ± 0.36, 5.8 ± 0.80, and 5.6 ± 0.08, respectively. Viscosity is a crucial characteristic of NANE; it was found that optimized NANE showed lower viscosity (54.40 ± 1.25 mPa.S) than marketed formulations (M1-78.20 ± 0.76 mPa.S, M2-80.56 ± 0.28 mPa.S). Drug content of NANE of naproxen and marketed formulations was found to be 95 ± 0.46, 98 ± 0.16, and 97 ± 0.38, respectively. From the *in vitro* drug release study, we found that optimized NANEs of naproxen showed highest percentage of drug release (96.24 ± 1.96) as compared to marketed formulation M1 (90.58 ± 1.31) and M2 (89.15 ± 1.33), respectively. No phase separation, creaming, cracking and phase inversion was seen after agitation, centrifugation, and freeze-thaw cycles. This demonstrated the NANE excellent stability in the presence of gravity. pH and drug content of NANE was stable within 6 months of stability study. Viscosity of optimized NANEs decreased from 55.80 ± 0.48 mPa.S to 51.20 ± 1.06 mPa.S within 6 months in accelerated stability study and stable in intermediate stability study. **Conclusion:** It was discovered following a stability assessment that there was no indication of instability.

Key words: Accelerated stability study, comparative study, nanes, naproxen, phase separation

INTRODUCTION

A synthetic, moderately potent non-steroidal anti-inflammatory medicine called naproxen is used to treat inflammation caused by conditions such as gout, arthritis, spondylitis, and bursitis. Naproxen works clinically by inhibiting COX-1 and COX-2 enzymes, which results in less prostaglandin formation. Although, both enzymes are involved in the production of prostaglandin. Normal tissues like the stomach lining contain COX-1 enzymes, which are constitutively active. In contrast, COX-2 enzymes are induced and create prostaglandins, which are responsible for pain, fever, and inflammation. The intended analgesic, anti-inflammatory, and antipyretic effects of naproxen are mediated by the COX-2 enzyme.[1] Emulsions can be prepared without an aqueous phase of water to generate non-aqueous, anhydrous, or oil-in-oil emulsions. Emulsions are one of the most practical and useful formulations because they have continuous and dispersion phases, one of which is a liquid phase. Such formulations, when the presence of water can be avoided, can replace conventional emulsions. A suitable non-aqueous dispersion phase solvent is used to dissolve the drug in this sort of emulsion.[2] The inherent disadvantage of poor solubility in water can be lessened by lipid

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formulation, which also makes it easier for solubilize phases to form and allow for drug absorption. Non-aqueous nano emulsions (NANE) are clear, isotropic systems of non-aqueous polar solvent, oil, and surfactants that are thermodynamically stable. They show promise as a drug administration method due to their long-term stability, simplicity in manufacturing, and excellent solubilization of drug molecules. To improve the solubility and bioavailability of pharmaceuticals, they have a wide range of uses in oral drug delivery. They are also vigorously being researched for possible uses in parenteral, ophthalmic, pulmonary, nasal, and vaginal drug administration. The study of NANE for topical distribution has increased recently. The aim of the current research work was to study stability of NANE of naproxen by adopting a NANE technology and to avoid the negative side effects of oral naproxen administration, such as peptic ulcer, gastroesophageal reflux disease, and irritable bowel syndrome.[4] The bioavailability of a drug is increased in NANE because the particle size of marketed emulsions, which have huge droplet sizes, continues to decrease. Phase inversion, phase separation, flocculation, coalescence, creaming, and cracking, are the some drawbacks may associated with emulsion that can be overcome by NANE. Particle size reduction enhances viscosity, and viscosity enhancement promotes formulation stability, which is possible with NANEs.[3,4]

MATERIALS AND METHODS

Chemicals

Naproxen as a drug was obtained from drug laboratory of DVVPFs college of Pharmacy, Ahmednagar (MH), India. Glycerine from Lobachemie, 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.

Preparation of lipid based NANE[4,5]

From the preliminary studies using different method of preparation of NANEs, it is clear that 10% Naproxen as a drug, 5% Glycerol Monostearate as a surfactant, 5 ml Mineral oil as a continuous phase and 5 ml of Glycerine as a dispersed phase are used to prepared NANE of Naproxen. A weighed amount of Naproxen was dissolved in mineral oil in the first beaker, and then Glycerin Mono Stearate, and Glycerin was heated to around 50–60°C in second beaker, cooled. Naproxen in mineral oil solution was added to the second beaker, and homogenized for 10 min at 15000–16000 rpm using Remi's ultraturex high speed homogenizer.

Comparative study of optimized NANE with marketed formulations (M1 and M2)

The optimized NANE of naproxen compared with marketed formulations M1 and M2 regarding some evaluation parameters such as general appearance, pH, viscosity, drug content, and *in vitro* drug release.

General appearance

NANE of naproxen and marketed formulations (M1 and M2) was evaluated for physical appearance.

pH

The pH values of optimized NANE and marketed formulations (M1 and M2) were measured using digital pH meter.[6,7]

Viscosity

With the use of a Brookfield viscometer, the viscosity of a nanoemulsion is measured. A 25 ml beaker is filled with 20 ml of nanoemulsion, and marketed formulation M1 and M2 separately and the viscosity is determined by spinning spindle number 6 at 10 rpm.[2,8]

Drug content

For drug content of the NANE 1 ml of NANEs dissolve in 100 ml of PBS of pH 5 and for drug content of marketed formulations M1, M2, 1 gm of respected formulations dissolve in 100 ml of PBS of pH 5 and drug content of NANE and marketed formulations was determined by UV spectroscopy at 273 nm.[6,9]

In vitro drug release

In vitro drug release study of optimized NANE and marketed formulations of naproxen was performed using Franz diffusion cell with a cellulose membrane. The dissolution medium, that is, phosphate buffer pH 5 was prepared. Cellophane membrane was previously soaked overnight in the dissolution medium. Cellophane membrane was placed between receptor and donor compartments of Franz diffusion cell. 2 ml of optimized NANE and 2 g marketed formulations of naproxen for was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 5 (32 ml) separately. Dissolution medium was kept at 37 ± 0.5°C with the membrane barely touching the surface of the receptor medium. Using a magnetic stirrer, the dissolving liquid was agitated at a speed of 100 rpm. For 8 h, aliquots of 2 ml each were periodically taken out and replaced with an equivalent volume of the receptor media at intervals of 1 h. The aliquot was appropriately diluted with receptor media before being subjected to UV-Vis analysis at 273 nm with phosphate buffer serving as a blank.[10,11]

Stability study[8,12-14]

The physical and chemical stability of optimized NANE was subjected for agitation test, centrifugation test, freeze-thaw cycle and accelerated and intermediate stability study as per ICH Q1A R2 guidelines.

Agitation test

Accurately about 5 ml of NANE was transfer over reciprocating shaker at about 60 cycles/min for 24 h. Any signs of phase separation were observed after 24 h.

Centrifugation test

Accurately about 5 ml of NANE was transfer for centrifugation at about 3500 rpm for 30 min. Any signs of phase separation were observed after 24 h.

Freeze-thaw cycles

The NANE was kept at -10°C, room temperature and 45°C for 24 h each. Any signs of phase separation were observed after three freeze-thaw cycles.

Accelerated and intermediate stability study

Stability study was conducted as per international conference on harmonization ICH Q1A R2 guideline. Accelerated and intermediate stability study was design to increase the rate of chemical degradation or physical change of the optimized NANEs of naproxen using exaggerated storage condition as a part of the formal stability study.

Table 1: List of marketed product of naproxen

S. No	Product name
01	Marketed gel formulation M1
02	Marketed gel formulation M2

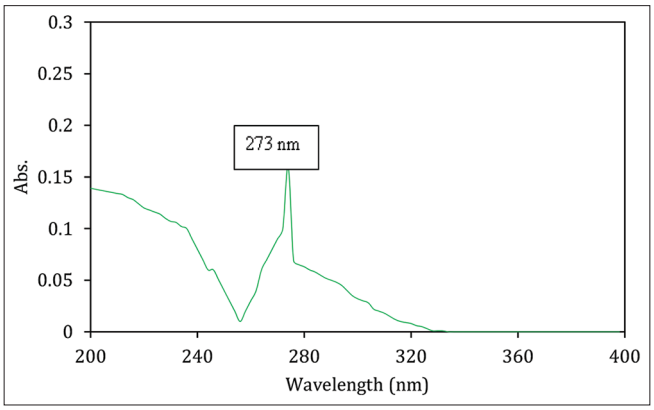

Figure 1: UV-spectra of naproxen in phosphate buffer solution ph 5.0

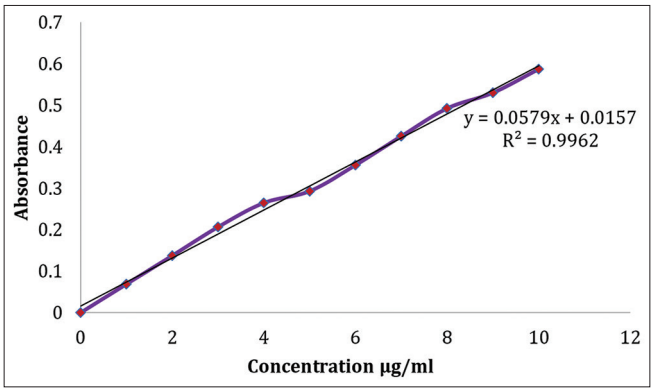

Figure 2: Calibration curve of naproxen in phosphate buffer solution ph 5.0 at 273 nm

Method

The optimized NANEs of naproxen were subjected to elevated temperature and relative humidity, that is, at 40°C ± 2°C, relative humidity (RH) 75% ± 5% (Accelerated study) and 30°C ± 2°C, RH65% ± 5% (Intermediate study). The sampling was done at 0 month, 3 months and 6 months and formulation was observed physically. The optimized NANEs of naproxen were evaluated for color, pH, viscosity, and drug content at various intervals of 0 month, 3 months, and 6 months.

RESULTS AND DISCUSSION

Comparative study of optimized NANE with marketed formulations (M1 and M2)

The standard curve of naproxen was plotted in phosphate buffer solution pH 5, [Figures 1 and 2] and it exhibits good linearity. It was discovered that the UV spectrophotometric method for estimating naproxen at max 273 nm in phosphate buffer solution pH 5.0 had acceptable consistency; hence, this method was employed in the further investigation.

Optimized NANEs of naproxen was compared with marketed formulation (M1 and M2) regarding some evaluation parameters such as appearance, pH, Viscosity, and drug content and in-vitro drug release. Table 1 contain list of marketed formulations used for comparative study with optimized NANE of naproxenAll the results of comparative study are shown in Table 2.

In vitro drug release (Comparative in vitro drug release of optimized NANE and marketed formulations M1 and M2)

Figure 3 indicate *in vitro* drug release of optimized NANEs of naproxen and marketed formulations (M1 and M2). *In vitro* drug release study was carried out for 8 h.

From the *in vitro* drug release study, we conclude that optimized NANEs of naproxen shows highest percentage of drug release (96.24 ± 1.96) as compared to marketed formulation M1 (90.58 ± 1.31) and M2 (89.15 ± 1.33), respectively.

Stability study

Agitation

No phase separation of NANE was observed after agitation on a reciprocating shaker for 24 h, which indicates nanoemulsion have good stability and formulation can with stand mechanical forces during handling and transportation

Centrifugation

Stock's low said that creaming is a function of gravity and increasing in gravity enhance phase separation. Shelf life of NANEs during normal storage condition can be assessed by phase separation due to creaming when NANEs exposed to centrifugation. Gravity of NANE can be examined by centrifugation test. No phase separation was observed after centrifugation for 30 min at 3500 rpm. This shows that NANE shows good stability under gravitation forces

Freeze-thaw cycles

It is conducted by exposing the NANEs formulation for freezing temperature at about −10°C for 24 h, then allow to thaw at room temperature for 24 h. The NANEs is then kept at higher temperature at about 45°C for 24 h and again kept at room temperature for 24 h. Then NANEs evaluated for significant changes in phase separation. This indicates completion of one cycle. NANEs was repeated this test in through three cycles. Results of study are reported in Table 3.

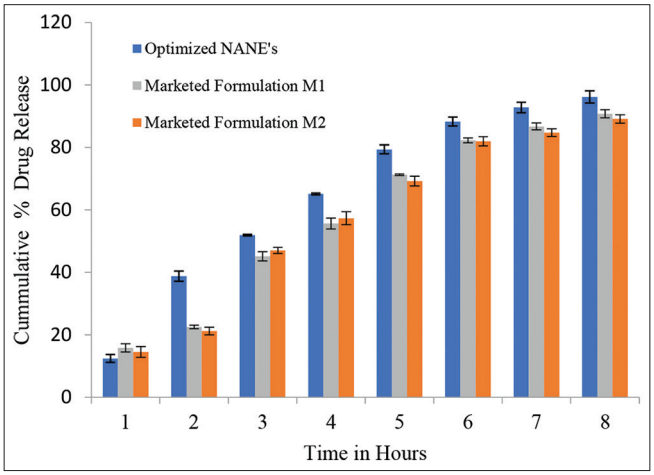

Figure 3: *In vitro* % drug release of optimized NANE and marketed formulations M1and M2

Accelerated and intermediate stability study

The Optimized NANE was subjected for stability studies to analyses its physical and chemical stability. The optimized NANEs of naproxen were subjected to elevated temperature and relative humidity, that is, at 40°C ± 2°C, RH75% ± 5% (Accelerated Study) and 30°C ± 2°C, RH65% ± 5% (Intermediate Study). NANE was monitored for changes in color, pH, viscosity, and drug content at an interval of 0 month, 3 months, and 6 months for the period of 6 months.

(a) Stability study at 40°C±2°C, RH75%±5% (Accelerated study)

Optimized NANEs when evaluated for drug content and found that, it was constant at initial, after 3 months and after 6 months. The chemical composition of formulation not altered during study. Slightly viscosity of formulation was changes, initially viscosity of formulation was found to be 55.80 ± 0.48 (mPa.S); then it was decreases up to 51.20 ± 1.06 (mPa.S). Due to gelation of glyceryl monostearate initially viscosity of formulation increases. No any changes in color and pH of formulation were observed. Hence, it concludes that NANE was stable at accelerated study.

(b) Stability study at 30°C±2°C, RH 65%±5% (Intermediate study)

Drug content was slight decreases from 95 ± 0.66 to 94 ± 0.74 within 3 months. There was initial increase in viscosity followed by slightly decreasing viscosity was observed. pH of formulation slightly decreases.

Table 2: Evaluation parameters of optimized non-aqueous nanoemulsion of naproxen and marketed formulations

Evaluation parameters	Optimized non-aqueous nanoemulsions of naproxen	Marketed formulation-M1	Marketed formulation-M2
Appearance	Soft, white semiliquid	Soft, Semisolid	White, Semisolid
pH	5.5±0.36	5.8±0.80	5.6±0.08
Viscosity (mPa.S)	54.40±1.25	78.20±0.76	80.56±0.28
Drug content (mg/ml)	95±0.46	98±0.16	97±0.38

The data are represented as Mean value±SD (*n*=3), SD: Standard deviation

Table 3: Stability study analysis

Test	Conditions	Duration	Observations			
			Phase separation	Creaming	Cracking	Phase inversion
Agitation	On rotary shaker 60 cycles/min	24 h	No	No	No	No
Centrifugation	3500 rpm	30 min	No	No	No	No
Freeze-thaw cycles	Three cycles: Refrigerator temperature-room temperature-45°C	9 days	No	No	No	No

Table 4: Results of accelerated and intermediate stability study of non-aqueous nanoemulsions

Temperature (°C) and RH (%)	Duration in months	Stability	Color	pH	Viscosity (mPa.S)	Drug Content (mg/ml)
Accelerated study 40°C±2°C, RH 75%±5%	0	Stable	White	5.8±0.30	55.80±0.48	95±0.84
	3	Stable	White	5.8±0.08	54.68±0.94	95±0.93
	6	Stable	White	5.8±0.36	51.20±1.06	95±0.76
Intermediate study 30°C±2°C, RH 65%±5%	0	Stable	White	5.7±0.64	58.40±0.52	95±0.66
	3	Stable	White	5.4±0.16	57.92±0.78	94±0.74
	6	Stable	White	5.4±0.84	56.70±0.44	94±0.82

The data are represented as mean value±SD (n=3) SD: Standard deviation

From the stability study, it was concluded that optimized NANE was stable at 40°C ± 2°C, RH 75% ± 5% (Accelerated Study), and at 30°C ± 2°C, RH 65% ± 5% (Intermediate Study). Table 4 indicates results of accelerated and intermediate stability study.

SUMMARY AND CONCLUSION

A comparison of optimized NANE of naproxen with marketed formulations revealed that the appearance, pH, viscosity, drug content, and *in vitro* drug release was similar to marketed formulations. To evaluate the stability of optimized NANE of naproxen, agitation, centrifugation, freeze-thaw cycling and an accelerated and intermediate stability study were used. After conducting a stability evaluation, it was found that there was no sign of instability. In addition, stability tests lasting 6 months were carried out at two different temperatures, 40°C ± 2°C, RH 75% ± 5% and 30°C ± 2°C, RH 65% ± 5% (accelerated study and intermediate study, respectively). Over the course of the investigation, there was no phase separation and NANEs remained stable at all temperatures. During the comparative and stability study pH, viscosity, and drug content were not dramatically impacted. This leads us to the conclusion that the optimized NANEs of naproxen was stable and superior to marketed formulations.

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