DRUG DISCOVERY

# Fabrication and characterization of piroxicam loaded Nano emulsions for topical drug delivery to fight osteoarthritic pain

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## ABSTRACT

Nano systems such as microemulsions (ME) and nano emulsions (NE) offer considerable opportunities for targeted drug delivery to and via the skin. ME and NE are stable colloidal systems composed of oil and water, stabilized by a mixture of surfactants and cosurfactants, that have received particular interest as topical skin delivery systems. Nano emulsions offer major platform for local targeted drug delivery through the skin. Nano emulsions represent stable colloidal systems comprising of oil and water, achieving stability by admixture of surfactants and co-surfactants and such system offer suitable platform to mitigate the chronic pain associated with osteoarthritis. In this context, we formulate and evaluate piroxicam-nano emulsion with respect to effects of surfactants, co-surfactants and lipid phase on the physicochemical properties of finished emulsion. Multiple trial formulations were developed and characterized. Almost all the formulations were stable but 4 out of 6 formulations showed excellent stability during accelerated stability studies. Drug release was within range of 99.51% to 101.01% for different formulations which in turn gave the hypothesis that emulsion produced by this method can be used for topical delivery. Texture and spread-ability of most formulations were adequate which ranging from 3-6 cm for topical use which ensured patient compliance. Entrapment efficiency showed that drug is entrapped sufficiently and drug release is as desired. Also, SEM and zeta sizer showed a small nanoparticle having size rage within 200 nm which was the main objective to achieve. PDI value was less than 1 indicating homogenous Nano emulsion and zeta potential values ranging between -24.7 mV to -39.4mV showed formulation stability as charged particles have the tendency to repel each other and thus preventing aggregation. In conclusion, the formulation and technique used in the preparation of the emulsion is proved to be significant and can be tried for more elaborate animal studies and which in turn can be easily used for mass production of Nano emulsion for commercial purpose.

**Keywords:** Nano emulsion, piroxicam, arthritis, surfactants

DISCOVERY SCIENTIFIC SOCIETY

## 1. INTRODUCTION

The prevalence of different types of arthritis is increasing with the rise in cases of obesity, sport injuries and other incidences of joint injuries. Based on such grounds, the research for finding different techniques to treat degenerative joint diseases is surging day by day (Liu et al., 2021). Osteoarthritis is an enfeebling chronic joint disease which is degenerative in nature and is the most common type of arthritis. It is symptomized by pain and stiffness and caused by injury or abnormality in joints. In osteoarthritis degradation of articular cartilage and formation of painful osteophytes (bone spurs) occur, as a result of these factors the bones at joints are exposed to one another and rub against each other, which contributes to inflammation and pain (Cho et al., 2015).

In recent years, substantial progress has been made in nanotechnology and nanomedicine. With the help of nanotechnology targeted delivery of drugs has been improved significantly (Kovooru et al., 2021). Optimal drug dosage, extended effect, reduction of side effects and lower chances of drug resistance are the main advantages of nanotechnology (Motawea et al., 2017). These advantages are the reason for increased use of nanotechnology in the treatment of chronic pain such as osteoarthritis. The NSAIDs (Nonsteroidal anti-inflammatory drugs) used for chronic pain usually cause GIT disturbances and their pharmacokinetics parameters are also affected, these results in altered efficacy. Nanotechnological approach via local delivery helps in eradication of these issues. In case of osteoarthritis the topical nanotechnology provides targeted delivery, prolonged release of drug, increased absorption and bioavailability and decreased GIT complications related to NSAIDs (Jin, 2020).

Innovations in nanotechnology and various nanoscale platforms have also fueled growing interest in innovative medication delivery methods. Because of their nanoscale size, nano-platforms can preferentially collect and bind to the specified location with regulated release behavior. Nano carriers can be manufactured to improve efficacy while minimizing side effects by carrying the active ingredient to a specific target site (Al-Halafi, 2014). For instance, in treatment of cancer, nanoparticles can reap the benefits of tumor cells' improved susceptibility and retention effect because of their compact size and leave the systemic flow to accumulate in tumor tissues (Al-Halafi, 2014; Hanieh et al., 2022).

Nano-emulsions are a type of emulsion that has consistent and extremely small droplet size (Sonneville-Aubrun et al., 2004). They fall in range of 20-500 nm. From both a basic and applied perspective, the production of kinetically stable dispersions of these small sizes is of tremendous interest (Solans et al., 2003). Direct applications of nano-emulsions in items have been developed in recent years, primarily in pharmacy and beauty products. These recent uses have necessitated research into optimizing approaches for nano-emulsion preparation (Gutiérrez et al., 2008; Aboalnaja et al., 2016; Naseema et al., 2021).

There are possible advantages to applying nano emulsions instead of classic emulsions for this reason: They can significantly boost the bioavailability of lipid soluble drugs; they disperse light weakly and can therefore be integrated into optically transparent products; they could be used to attenuate product texture; and they are highly resistant to particle agglomeration and gravitational isolation (McClements, 2011). The incorporation of hydrophilic and lipophilic drugs scaled back noxious and irritant consequences when compared to micro emulsions. Drug protection from hydrolysis and oxidation, excessive drug permeation through the epidermis, more pleasant sensory characteristics and comparatively low surfactant concentrations are all advantages of using nano emulsions as a provider for topical drug delivery (Dal Mas et al., 2016).

Piroxicam is an analgesic and anti-inflammatory N-heterocyclic carboxamide of 1, 2 benzothiazine 1, 1 dioxide. Piroxicam inhibits prostaglandin synthesis in vitro and in vivo by acting as a selective reversible inhibitor of the cyclo-oxygenase step of arachidonic acid metabolism (Kochevar et al., 1986). Piroxicam is preferred as an analgesic because it inhibits phenyl quinone-induced writhing more effectively than aspirin, fenoprofen, ibuprofen, naproxen, or phenylbutazone. The drug's effectiveness is maintained without increasing the dosage and patients with osteoarthritis tolerate dosages of 20mg daily well (Brogden et al., 1981). Orally available drugs of Piroxicam, has analgesic and anti-inflammatory response but they also cause the gastrointestinal ulcers. Topically available drugs are preferred so that they can easily penetrate the skin. Hence, herein we propose to prepare the nano emulsion formulation with optimum characteristics to deliver the piroxicam topically to inflamed joints traversing the skin barriers.

## 2. MATERIALS AND METHODS

Piroxicam was used as an active pharmaceutical ingredient (API) provided by University of Central Punjab. The Tween-80 was a nonionic surfactant and emulsifier. Oleic acid was used as solution phase for the synthesis of nanoparticles, which function as a kinetic knob to control the size and morphology of nanoparticles. Paraffin was also added as thermal energy storage media.

**Spontaneous emulsification preparation method**

This technique involved preparation of nano emulsion in 2 stages. The first stage included formation of an organic solution, comprising of tween 80 oleic acid and paraffin oil by weighing accurately, vortexed for 15 mins to get a homogenized mixture

(phase A) than emulsion is formed by injecting this aqueous phase into phase A and emulsified with the aid of overhead mixture (Hiedolph, Germany) at 5000 rpm for about 15 mins. The organic solvent ethanol was then added in the third stage by stirring sufficiently until homogenized mixture is achieved. The multiple compositions of nano emulsions in percentages are mentioned in table 1.

**Table 1** Composition of formulations

| Preparations | Compositions (% w/w) | | | | | |
|---|---|---|---|---|---|---|
| | Oleic Acid | Paraffin Oil | Tween80 | Ethanol | Piroxicam | Water |
| NE1 | 6 | 6 | 4 | 4 | 0.05 | 40 |
| NE2 | 5 | 5 | 4 | 4 | 0.05 | 35 |
| NE3 | 6 | 6 | 4 | 4 | 0.05 | 35 |
| NE4 | 6 | 6 | 5 | 5 | 0.05 | 35 |
| NE5 | 6 | 6 | 6 | 6 | 0.05 | 35 |
| NE6 | 6 | 6 | 4 | 4 | 0.05 | 30 |

**Physical appearance**

The physical appearance of all batches of emulsions was visually investigated considering multiple factors: Aesthetic appearance, color, creaming, cracking phase inversion and stickiness. The organoleptic properties i.e., color, appearance and odor of nano emulsions were observed for 4 weeks.

**pH Determination**

The pH range within 5 to 6 for skin which is slightly acidic in order to prevent microbial infection Digital pH meter was used for pH determination (Patel & Joshi, 2012).

**Spread-ability study**

The Spread-ability of the emulsion is useful as it describes the behavior of the emulsion when oozing out of the tube and also show how smoothly it will be applied on the affected part. As for inflamed skin the smooth spreading of formulation is important. The spread-ability was designed to decide 48 hrs after formulation preparation by evaluating the spreading of the nano emulsion between two glass plates after 1 minute. An aliquot of 500 mg of nano emulsion was placed in a hover at the distance of 1 cm from the already marked glass plate on which second glass plate was placed. The widening in bread thasan outcome of weights added prompting spreading of emulsion was noted (Arora et al., 2014).

**Viscosity study**

The viscosity study was done using Brookfield Viscometer at 5, 10 and 20 and 50 rpm. The viscosity at 5 rpm was selected with the spindle no 4. Viscosity is an important physical property. Generally, an increase in the viscosity of formulation would result in a more rigid structure with a consequent decrease of drug release rate (Alliod et al., 2019).

**Drug entrapment efficacy**

The mini column centrifugation technique was utilized to check entrapment efficiency. In 20ml of ethanol solution, 0.1 g of formulation was added and dispersed completely. 100ml (0.1%w/v) of final volume was prepared and centrifuged at 15,000 RPM. These columns were then eluted and the absence of crystals was checked under microscope. These eluted columns were further diluted with ethanol and concentration of piroxicam was determined by using a UV-visible spectrophotometer at ƛmax335 nm (Garg et al., 2017). % Drug Entrapment = Amount of drug Entrapped/ total amount of drug*100.

**Droplet size, Polydispersity index and Zeta-potential**

The small size of particle is important for emulsion to remain stable. This is because that Brownian movement overpowers the gravity. Droplet size and zeta potential are the most common and effective tests for determining the stability, as particle size is important to reduce the flocculation and coalescence (Rai et al., 2018). The particle size and PDI of nano emulsions were analyzed employing photon correlation spectroscopy (PCS) using Malvern Zetasizer, which monitors the variation in light scattering because of Brownian motion of particles as function of time (Ribeiro et al., 2015a).

**Scanning electron microscopy**

The morphological examination of the formulated nano-emulsions was investigated using an electron microscope (Model JSM 5910, Joel, Japan). The samples were sprayed with platinum in a sputter coating machine prior to scanning. Coating is done to facilitate conduction of electrons through the samples for imaging.

**Stability studies**

Stability studies are performed for assessing stability of the drug substance under the influence of a various environmental factors like temperature, humidity and light (De Oca-Ávalos et al., 2017). The stability studies of nano-emulsion were carried out after storing the formulation as per International Conference on Harmonisation guidelines. The storage conditions followed are ambient (25±2°/60±5 % RH), refrigeration (5±3°) and freeze (–20±5°). The requisite volume of nano-emulsion was stored in glass bottles and were tightly sealed. For all the formulations stability study was done at $20^0$C and $40^0$C for about 6 weeks these formulations were monitored carefully to observe any issue of the instability or abnormality (Ali et al., 2014).

**Skin irritation test**

Skin irritation test was conducted to see any lesions or side effects of prepared formulation. 3 volunteers were chosen and approximately 1 g of formulation was applied topically on the handover the area of 3 inch and monitored for any irritation, redness, or any other side effect.

## 3. RESULTS AND DISCUSSIONS

This present study was carried to formulate nano-emulsions by the spontaneous emulsification method. To develop the formulation, the essential task was to select the appropriate excipient with good compatibility. Test formulations were developed with specified amounts of tween 80, ethanol, oleic acid and paraffin oil. Tween 80 was used to (Patel et al., 2012) reduce the interfacial tension between the continuous and aqueous phase while ethanol was used to increase the permeation of tween 80. For oil phase both oleic acid and liquid paraffin were used. They found to have liquid transparent form with no change in colour, appearance or odor within 4 weeks. The pH of all preparations ranged from 5 to 6 and found to be stable during pH testing. The pH test is shown in the Table 2. The topical formulation must be compatible with skin surface and hence the formulations are acidic, they can be used. All the preparations were stable and no major changes in pH were seen as the range was nearly neutral.

The spread-ability values ranged from 3 cm to 6 cm (shown in Table 2). The spread-ability values of NE3 and NE6 is the highest while NE5 have the lowest value. This phenomenon can be explained by direct inverse relationship with the amount of present surfactant is present i.e., with the increase in the concentration of surfactant there is decrease in the spread-ability. All formulations except NE4 & NE5 exhibited tremendous spread-ability which highlights the easy applicability to skin. Moreover, good spread-ability also hinted the good extrusion behavior from the container which contributes to patients' acceptance and application.

It was observed that with the increase in the oil content there was an increase in the viscosity. The viscosity of formulation NE2 was lower than other formulations which can be due to lesser oil content. Overall low viscosity was analyzed which is expected for nano-emulsion and is shown in Table 2 (Ali et al., 2014). Moreover, pseudo plastic behavior was observed as the nano-emulsion formulation remained slightly viscous under static conditions and exhibited less viscous properties after application resulting in easy spread-ability which is also mentioned above in the spread-ability results section, highlighting the enhanced drug permeation after topical application.

The entrapment efficiency was noted to show excellent results. The percentage of drug entrapped ranged from 99.51-101.01 which means that method is feasible since greater than 60% entrapment is required to formulate nano emulsions. The use of surfactants is also very important, as drug permeability and entrapment efficiency are majorly dependent on Tween 80 and ethanol.

**Table 2** Spread-ability, pH, viscosity, UV reading, drug percentage entrapment efficacy of the Nano emulsion formulations

| Sr. No. | Formulation | Spread-ability (cm) | pH | Viscosity (Cp) | UV readings | Entrapped Drug % |
|---|---|---|---|---|---|---|
| 1 | NE1 | 5.5 | 5.79 | 41.33 ± 1.27 | 0.594 | 100.1 |
| 2 | NE2 | 5 | 5.51 | 38.45 ± 1.62 | 0.653 | 99.24 |
| 3 | NE3 | 6 | 5.67 | 40.39 ± 1.74 | 0.746 | 95.51 |
| 4 | NE4 | 4 | 5.97 | 42.81 ± 1.83 | 0.992 | 98.01 |
| 5 | NE5 | 3 | 5.70 | 39.44 ± 1.56 | 0.998 | 98.81 |
| 6 | NE6 | 6 | 6.01 | 43.75 ± 1.37 | 1.064 | 95.94 |

Under the light of all above results, it was noted that formulation NE3 and NE6 showcased the most optimum and desirable results. So, these formulations were further subjected to the determination of droplet size determination, PDI and zeta potential. Results revealed that NE3 and NE6 particles are within the range which exhibits emulsion stability (Shown in Table 3). PDI is used to determine whether the particle size is homogenously dispersed or not, both the droplet sizes are less than 1 exhibiting homogeneity of the formulation. The value of Zeta potential creates energetic barriers between the particle size to overcome the instability phenomenon (Ribeiro et al., 2015b). The zeta potential between -24.7mV to -39.4mV shows that a large zeta potential makes formulation more stable since charged particle size have tendency to strongly repel each other and hence preventing the aggregation (Priya et al., 2015).

Further upon the results of size, PDI and zeta potential, NE3 stands superior to NE6, when set in comparison. Both formulations demonstrated desirable results. Therefore, for surface morphology, NE3 & NE6 were further subjected to SEM. Different magnifications were gained at appropriate accelerating voltage i.e., mostly 20 kV and SEM provided a decent study of surface morphology of the dispersed phase. The SEM analysis of above figures showed that the particles are smooth, dispersed homogenously in nano emulsion and are within the required size range of 200-400nm.

**Table 3** Particle size, PDI and zeta values of NE3 & NE6 nano emulsions

| Formulation | Particle size (nm) | Standard deviation (mV) | PDI | Zeta Potential (mV) | Electrical conductivity (mS/cm) |
|---|---|---|---|---|---|
| NE3 | 122.9 | 6.57 | 0.276 | -24.7 | 0.0219 |
| NE6 | 278.1 | 8.16 | 0.832 | -39.4 | 0.029 |

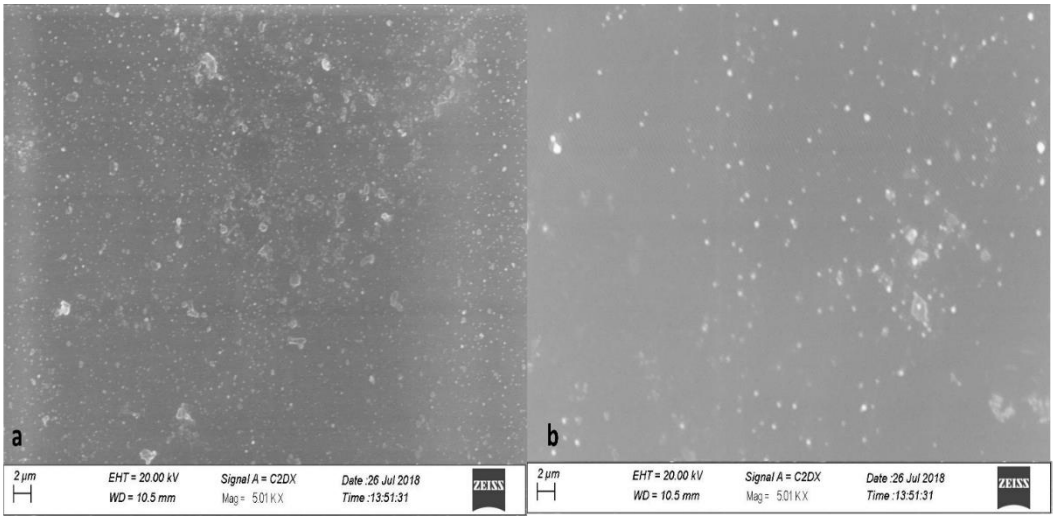


**Figure 1** SEM images of sample (a) NE3 (b) NE6

Stability studies were carried out at 20℃ and at 40 °C. At 20℃, all the formulations were homogenous, smooth and had no sign of instability. However, after 6 weeks NE1 and NE5 showed phase separation because there was a change of color from transparent to yellow and appearance of oily phase was observed (shown in Table 4). At 40°C all formulations except NE5 exhibit stability and was homogenized. But after 6 weeks instability was observed in both A1 and A5 as there was visible change of color and phase separation (Table 4). The main reason of instability of emulsion would be in sufficient forces due to which micelles are held in their ultimate form. Additionally, raised temperature studies at 40 ℃ are done for accelerated stability studies to test the formulated product for more turbulent conditions than the one commercially available (Mat Hadzir et al., 2013).

Accelerated studies are only performed to check the stability at hostile condition however this does not necessarily mean that the trial preparation is not suitable for local storage conditions. In the most interesting aspect, it was noted that subjects to whom nano-emulsion formulations were applied, reported no unpleasant experience whatsoever. It is noteworthy that along with visual inspection, subjects feeling of sensation was also inquired to note any irritation or unpleasant sensation which may be unobservable to visual inspection and no such reports were observed.

**Table 4** Stability at 20 °C and 40°C

| | Stability at 20°C | | | | Stability at 40°C | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 Weeks | | 6 Weeks | | 4 Weeks | | 6 Weeks | |
| Preparation code | Instability indication | Appearance | Instability indication | Appearance | Instability Indication | Appearance | Instability indication | Appearance |
| NE1 | nil | Homogenous | Oily appearance | Phase separation | Nil | Homogenous | Pleasant odor | Phase Separation |
| NE2 | -do- | -do- | nil | Homogenous | -do- | -do- | nil | Homogenous |
| NE3 | -do- | -do- | -do- | Homogenous | -do- | -do- | -do- | Homogenous |
| NE4 | -do- | -do- | -do- | Homogenous | -do- | -do- | -do- | Homogenous |
| NE5 | -do- | -do- | Change of color | Phase separation | Unpleasant odor | Phase separation | Unpleasant odor | Phase separation |
| NE6 | -do- | -do- | -do- | Homogenous | nil | Homogenous | nil | Homogenous |

## 4. CONCLUSION

To prepare efficient nano emulsion, the used components must be compatible with each other, and their screening is required. This study confirms that Tween 80, ethanol, oleic acid and paraffin along with piroxicam yield a stable nano emulsion which was the main objective of this study. All the characterization studies showed that nano emulsion prepared with these components and with this method can work as the base to formulate a commercial nano emulsion. Since piroxicam has increase side effects when used orally, this study serves as a technique to prepare nano emulsions topically to address the side effects which will help in the patient compliance.

The pH study showed formulation remains within the range of 5-7 as skin has slightly acidic pH thus to prevent infection while low viscosity is observed which is generally a characteristic of nano emulsions and spread-ability ranging from 3-6cm was in range of patient compliance At ambient temperature these formulations can be sable up to 6 months while zeta potential ranging between -24.7 to -39.4 showed that the formulations are stable as charged particles have the tendency to repel each other so stability is increased the with particle size in the required range less than 200 nm and PDI value showed that the droplet size is dispersed homogenously. Therefore, more attention should be given to preparation of nano emulsions by spontaneous emulsification method and more studies should be done to yield homogenous and stable topical formulations with decreased side effects. Further, this study set the course for future project to be carried out on the in vivo testing of formulated nano emulsions.

**Ethical approval**

The ethical guidelines are followed for human subjects in the study. The experimental investigations were carried-out following the guideline of Declaration of Helsinki of 1975 of revised rule of 2013-version.

**Informed consent**

Written informed consent was obtained from the participants.

**Conflicts of interests:**

The authors declare that there are no conflicts of interests.

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**Data and materials availability**

All data associated with this study are present in the paper.

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