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Research article

Nano-lipid particles of naproxen for topical application: In vitro / in vivo study

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Key words: Analgesic activity, Naproxen, Nano lipid particles, Osteoarthritis, Tail flick method.

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Abstract

Nano lipid particles (Nps) based Naproxen gels (Npx) have been developed as a promising topical delivery system for osteoarthritis treatment. Six formulas were developed with different polymer ratios using hot high pressure homogenization method. The characterizations of Npx-Nps for topical application were assessed for physical characteristics. Furthermore, in vitro transdermal release and the in vivo analgesic effect of the selected formula based on drug loading results (Npx-Np5) was carried out. The mean particle size was ranged between 203±10 nm and 228±30 nm for Npx1 and Npx4 respectively. Npx-Np5 showed highest drug loading (9.8±2.23%) and encapsulation capacity (98.0±3.9%). In vitro release study through rat skin showed that Npx-Nps5 had a more pronounced permeation profile compared with commercial gel. Moreover, the analgesic effect induced by Npx-Np5-carbapol gel was 1.5 times higher than that induced by Naprosyn 10%gel after 4 h. Lipid nanoparticles are carriers with good prospects of successful marketing.

Introduction

Naproxen Npx chemical name is (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid is a nonsteroidal anti-inflammatory drug (NSAID) of propionic acid class, it is a nonselective COX inhibitor [1]. Npx is widely used in rheumatic diseases and other painful inflammatory conditions such as rheumatoid arthritis and osteoarthritis [2]. Although Oral use of Npx is very effective, Npx like other NSAIDs can causes irritation and ulceration of the gastro-intestinal mucosa after oral administration especially upon frequent use. Topical use of the NSAIDs is a good alternative to oral use [3]. The difficulties occur in controlling the concentrations of drug that pass through skin layers is the main challenge in topical drug delivery formulae [4]. Topical use of drugs will provide high concentration of the drug locally if it able to penetrate stratum corneum layer [4]. Several studies showed that percutaneous absorption of Npx is low due to its chemical nature [5-6]. Various enhancers and vehicles have been investigated to overcome the transdermal penetration of naproxen through the skin [7]. Lipid based nanoparticles with controlled drug release pattern could be useful technology to improve delivery of Npx through transdermal drug delivery system. Nanolipid carriers (Nps) have both benefits of being nano sized in addition to its lipohhilic nature. Also it is considered as save drug carrier that it composed of biocompatible and biodegradable components [8-9]. Several studies have reported the advantages of using nanolipid carriers for topical purposes [10]. It is able to enhance the solubility of entrapped drug, adhere to the skin surface and facilitated the transport of drugs in a controlled manner. In addition it has ability to improve skin hydration through clogging [11]. In order to develop an alternative formulation for the topical administration of Npx**,** the present study is carried out to design a transdermal delivery system of Npx based on nanolipid carrier, it is prepared by hot high pressure homogenization technique, which embedded in carbopol gel base compared with commercial product Naprosyn 10 % Gel. Characterization of hydrogel was done by means of techniques such as SEM, size distribution and zeta potential analysis and differential scanning calorimetry in order to better understand its properties. Tail flick method was performed to assess in vivo analgesic activity of the selected Npx-Np-carbapol gel formula.

Materials and methods

Materials

Naproxen (Sigma Aldrich Ltd., Germany), Compritol® ATO 888 (glyceryl behenate NF; Gattefosse´ s.a., Lyon, France) and Miglyol® 812 (Caprylic Triglyceride, GmbH, Hamburg), Poloxamer 188 was kindly supplied by Sigma Aldrich Ltd., German, lecithin (Sigma Aldrich Ltd., German). Sodium Lauryl Sulfate (SLS) Unikem (Copenhagen, Denmark), Acetonitril; HPLC grade (Scharlau Chemie SA, Barcelona, Spain), disodium hydrogen phosphate (Koch-Light Laboratories,

Colnbrook Bucks, UK), Carbopol 971P NF (Azelis Ltd, Hungary). All other chemicals and reagents used were of analytical grade.

Method

Preparation of Npx-Nps

Optimization of a design was done, to minimize the cost and to maximize the efficiency of formula. The main design variable was polymer ratio (Compritol® ATO 888: Miglyol® 812 ® ratio) which studied to achieve optimum formula conditions. Six formulations of Npx- Nps have been prepared using a hot high pressure homogenization method. Method selection based on the flexibility in the temperature selection, suitability for many application, and the small particle size (Nano range) could be obtained. Different ratios of Compritol® ATO 888 and Miglyol® 812 were tested as shown in table 1 based on many pre-study trials the mentioned percentages and combinations were selected. Lipid components in each formula and lecithin (0.5%) were melted in a water bath at 75 to 80°C then weigh of Npx equivalent to 10% was dispersed. Lipid melt containing Npx was dispersed under continuous stirring in 20 ml aqueous solution of mixture of 1% Poloxamer 188 and 0.2% SLS (w/v) which was preheated to 80°C using high shear homogenizer for one cycle (10,000 rpm, 5 min). The primary emulsion obtained was ultra- sonic for 10 min, then it was cooled down. To obtain adequate transdermal formulae, 1 % (w/v) of carbopol 971P was added to the Npx- Nps dispersion and left to hydrate for 24 hours during gentle agitation. The dispersion of carbopol was later neutralized with triethanolamine.

Physicochemical characterization of naproxen nanoparticles

Surface morphology

Scanning Electron Microscope (Metler Toledo, Tokyo, Japan) was used for examining the surface morphology of prepared Npx- Nps. Samples were mounted with double-sided conductive adhesive on the surface of the observation desks and coated uniformly with gold.

Particle size, particle size distribution and Zeta potential

Zetasizer 300 (Malvern Instruments, UK) was used for the evaluation of particle size distribution and zeta potential analysis of Npx-Nps in cabapol gel at 25°C. The gel was diluted with deionized water and the particle size was evaluated by volume distribution. To evaluate the stability of the prepared systems, samples were stored at 25±1°C and measurements were carried out on the first day and after two months. Each value was measured in triplicate.

Differential scanning calorimetry (DSC) measurements

Npx inclusion within Nps, was confirmed by differential scanning calorimetry (DSC) measurements using Differential scanning calorimeter (Metler Toledo, Japan). Samples were hermetically sealed in perforated aluminum pans and heated at a temperature range of 25–300°C at a constant rate of 10°C min^{-1}. The system was purged with 100 ml min^{-1} nitrogen gas to maintain an inert atmosphere thermogram compared with thermograms for each substance alone, blank Nps and Npx- Nps.

Drug loading and encapsulation efficiency measurements

The percentage of drug loading and encapsulation efficiency was assessed in the external aqueous phase by measuring the amount of free drug concentration [12]. Npx- Nps samples were suspended in 0,5 ml deionized water in the upper chamber of the Vivaspin filter tubes(Vivaspin, Sigma Alderich, Germany) with a 50 KDa molecular weight cut- off filter membrane. Tube that has been centrifuged for 30 min at 20,000 rpm (Minispin, Eppendorf, Hamburg, Germany).The filtrate was then diluted with methanol, and the amount of Npx un-entrapped was injected into validated HPLC system. Percent drug loading and encapsulation efficiency were calculated according to the following equations:

DL% = (wt of the drug - wt of free drug) / wt of lipid ×100 …… (1)

EE%= (initial wt of drug – wt of free drug) / initial wt of drug…..(2)

Where *W* is the weight in milligrams.

HPLC analysis of Npx was done as mentioned by published assay study of Bilal Y., *et al.*, 2014 [13]. Briefly, the analysis was conducted on a Waters Acquity HPLC™ (Waters Corp., Milford, MA, USA), and Ibuprofen as internal standard (IS). The separation was achieved by means of an isocratic mobile phase consisting of 20 mM phosphate buffer (pH 7) containing 0.1 percent trifluoroacetic acid (TFA)–acetonitrile (65:35 v/v). Flow rate was adjusted at 1.0 ml·min^{-1}, and the volume of the injection was 20 µL with 255 nm wavelength UV detection. All samples were filtered before injection by an aqueous 0.2 µm pore size membrane filter. All data were collected and analyzed using Lynx TMV 4.1 software (Waters Corp.). The data have been collected and analyzed using the Waters Corp software Lynx TMV 4.1. According to a previously published test study [13], the assay method was validated for selectivity, linearity, precision, accuracy,

transportation, recovery and stability; shortly before the start of this study.

In vitro transdermal penetration

In this study hairless skin of white albino rat was used. The hair of rat's skin was removed by using an electric razor, and treated as mentioned in previous published studies by Okur N., *et al.,* 2014, and Beetge E., *et al.,* 2000 [14]. The skin thickness was 630± 20 µm and the surface area was 0.850 cm^2, the skin surfaces of the rat were pulled upwards to the supply room and the reception room with the stratum corium side facing upwards into the donor compartment and the dermal side facing down into the receptor compartment. One ml of the selected Npx- Nps gel or Naprosyn 10 percent gel formulation was added to the supply chamber and 10 ml of 0.1 M saline buffered phosphate (pH= 7.4) was added to the reception room. The Stirring speed was 500 rpm and the temperature at 32.0 ± 0.5°C was maintained. After 15 minutes of equilibrium, 10 ml of receiving fluid was removed at 15, 30 minutes and every hour until 8 hours; and the same dissolution medium volume was added. 10 µl of the supernatant was filtered by a membrane filter of 0.2 µm Nylon, Millipore and injected into the HPLC system after centrifugation at 10,000 rpm for 10 min. Conditions of analysis are described in the previous section.

Analgesic effect study

The analgesic effect of Npx in formulated Npx-Nps carbapol gel was investigated. It was assessed by tail flick model. Mice were placed with a simple restraint device to fix the animal for testing on tail flick devices. The tail was placed in a sensing groove and underneath the groove was a photo sensor. The radiant heat stimulus beam focused on the distal part of the tail and the strength of the radiant heat lamp was kept constant, the time taken by mouse to remove the tail was measured [15]. Three groups of male and female Swiss- albino mice weighing 30–40 g were used; each group comprises six mice. Group I (– ve control) did not receive any treatment, 1 gm of the selected formula of Npx-Nps gel was applied to the tail of mice in group II, while 1 gm of Naprosyn 10 % gel was used for group III (+ve control). All groups were maintained in the standard lab diet and tap water and the animals were kept at room temperature [16]. All experiments have been conducted in accordance with the ethical guidelines established and approved by our university's committee on the use and care of laboratory animals.

Mechanism and kinetics of drug release

Data drug release were analyzed using zero- order, first-order, Higuchi, Hixon– Crowell, Peppas, and Weibull kinetic equations[17] DDSolver, a Microsoft Excel add-in program for modeling and comparing drug release profiles was used. The model with the highest determination coefficient (R^2) was considered to be the best fitting one.

Statistical analysis

Software SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used to analyze data. Applying one-way analysis of variance (ANOVA) and paired Student's t-test. Different values between formulations at $P<0.05$ were considered significant.

Results and discussion

Six Npx-Np formulations were prepared by hot high pressure homogenization method. To improve drug loading, penetration and controlling drug release, different concentration from lipid component were used in different ratios based on design optimization done in pre-studies. The details of the formulations are depicted in table 1.

Surface morphology analysis

SEM technique was used to performed surface analysis and investigate the morphological characteristics of different formulations. SEM results showed that Npx-Np formulations were of spherical to oval shape with smooth surface morphology with very fine cracks; Figure 1.

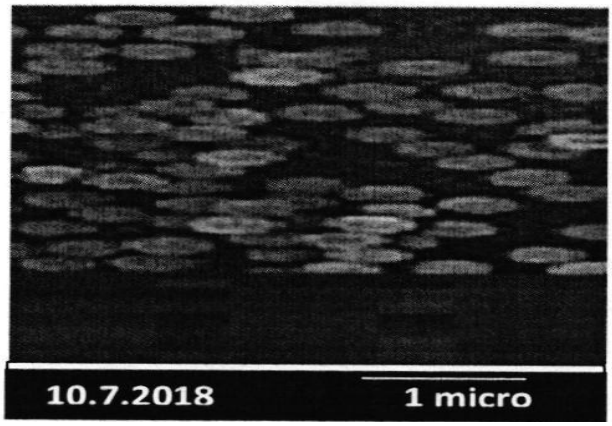

Figure 1. Scanning electron micrographs of nanolipid particles of Naproxen.

Particle size analysis distribution and zeta potential analysis

The results of table 1 showed the distribution of particulate matter and zeta potential analysis of Npx- Np after one day and two months of incorporation into carpabol hydrogel. It revealed that the particle size was vary from 203 ±10nm for Npx-Np1 formula to 228±30 nm for Npx-Np6 formula ($P < 0.01$) after one day and the particle size was ranged between 207±22nm and 235±19 nm for the same two formula after two months. Results revealed that the size of the formulations was directly dependent on the formulation lipid level but not on the

lipid type. The successive increase in bead size from Npx-Nps1 to Np-Nps6 can be attributed to the increase in lipid content. Mulla *et al.* and others reported the same observations, and their studies showed that lipid level has a significant influence upon particle size [18]. After 2 months of storage, a slight increase was observed with no particle aggregation as demonstrated by the results of the particle size. It can be concluded that the carbapol gel network was formed before naproxen particles were added and after incorporation it was not disturbed. The desired stability of the gel was achieved that particles were not aggregated and kept separate in the gel during the experiment period. After their entry into the gel network, the zeta potential of all Naproxen nano lipid formulations remained negatively charged and the zeta potential increased after two months of storage. The increase in nanoparticles zeta potential upon storage could be interpreted on the base of adsorption of more negative charges from carbapol molecules on its surface, which depicts the good stability for all formulations.

Table 1. Component, Particle size and Zeta potential of Npx-Np. After 1 day and Two Months and Drug loading (DL %) and Entrapment Efficiency (EE %).

Formula	Npx	Compritol® ATO 888 : Miglyol® 812 ® 818	Particle size after 1 day (mean ± SD)	Particle size after 2 months (mean ± SD)	Zeta potential after 1 day (mean ± SD)	Zeta potential after 2 months (mean ± SD)	DL%	EE%
Npx-Np1	10%	0.5:0.5	203±10	207±22	−27.12± 3.10	−30.2± 11.1	6.90±4.11	69.01±3.90
Npx-Np2	10%	0.5:1	209±21	211±20	−24.41± 0.85	−29.2± 2.12	7.21±1.02	72.15±2.8
Npx-Np3	10%	1:1	213±13	218±28	−25.12± 1.9	−27.5± 3.30	8.54±2.81	85.42±1.57
Npx-Np4	10%	1:0.5	220±23	228±18	−22.54± 2.02	−25.11± 4.12	7.92±0.28	79.21±2.11
Npx-Np5	10%	2:1	226±13	232±20	−25.2± 9.0	−29.2± 5.22	9.80±2.23	98.0±3.90
Npx-Np6	10%	1:2	228±30	235±19	−26.32± 5.43	−28.43± 2.56	9.13±1.28	91.32±2.32

The data are The Mean and Standard deviation of Three Determinations (n = 3).

Measurement of drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency estimation were done for all formulations in triplicate and the corresponding values are shown in table 1. Results revealed that The lowest lipid drug loading and encapsulation efficiency percentage belonged to the formulation Npx-Np1 containing lowest concentration of lipid, furthermore, increasing lipid concentration improve drug entrapment in nanolipid particles. It has been reported that the assimilation of liquid lipids to solid lipids facilitates imperfections in crystal lattice of the lipid mixture, leaving sufficient space to house more drug molecules which improve drug loading and encapsulation efficiency [5]. On the basis of drug loading and encapsulation study results; Npx-Nps5 composed of Compritol® ATO 888 and Miglyol 812 ® in ratio 2:1 was selected to evaluate the in vitro release and analgesic activity in comparison with commercial Naprosyn gel 10%.

Differential scanning calorimetry (DSC)

Figure 2 shows the compatibility of Npx with Compitrol and Mygolol using DSC technique. Naproxen showed an endothermic melting peak at about 155.5◦C conforming its melting point, DSC thermograms of Npx , and other aforementioned excipients showed no shift in Npx peak in case its physical mixture with all polymers used.

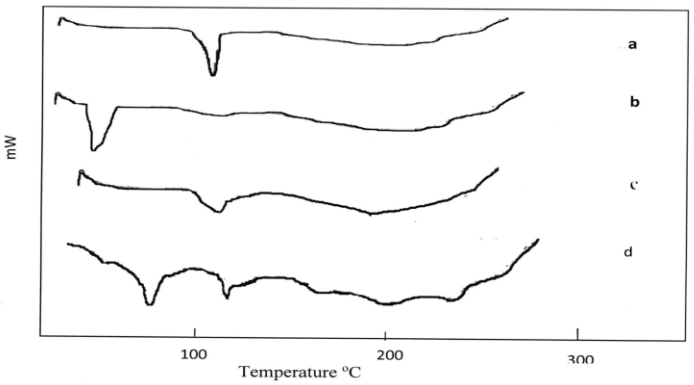

Figure 2. Differential scanning calorimetric thermogram of (a) Naproxen, (b) Compritol ATO 888, (c) Miglyol® 812 ® 818 and (d) physical mixture of MbH: Compritol ATO 888: Miglyol® 812 ® 818 in 1:1:1.

In vitro drug release studies

The transdermal in vitro penetration profiles of Npx from Npx- Nps carbapol- gel and 10% gel from Naprosyn are shown in Figure 3. The cumulative penetrated concentration of Npx from Npx-Nps carbapol-gel and

Naprosyn 10% gel were 1.8 mg/cm² and 1.3 mg /cm² after 8 hours, respectively. During the first hour, the penetration rate of Npx was rapid from both tested formula although it was faster from Npx-Nps5 carbapol-gel. This may due to rapid hydration of skin layer by hydrophilic gel in both formulae, in addition to free drug adsorbed on gel was rapidly released. Significant increase in drug release ($P < 0.05$) after 2 h from Npx-Np5 carbapol gel as compared to Naprosyn 10% was observed which could be elucidated on the base of hydrophobic nature of fatty polymers that the drug was embedded within it and thus elaborate the absorption of Npx through stratum corneum lipids of the skin [19-20]. Furthermore, the Npx penetration from Npx-Nps5 carbapol-gel was obviously higher than Naprosyn gel after 8 hours, it was about 1.38 times higher. This may due to the smaller size of carrier particles which has higher adhesion ability to skin that may improve the absorption of Npx. The regression coefficient R^2 values obtained for drug release showed that the release from carpabol Npx-Nps5 gel followed higuchi model of release, n was more than 1 suggests that super case II transport was the mechanism of release. Jafar A., *et al.*, study revealed that nano lipid formulation enhance drug absorption through layers of the skin compared to the drug solution formulation [21]. Study done by Argemı´ A., *et al.*, suggested also the higher ability of particles to increase the permeation and the accumulation of the drug in the horny layer [22].

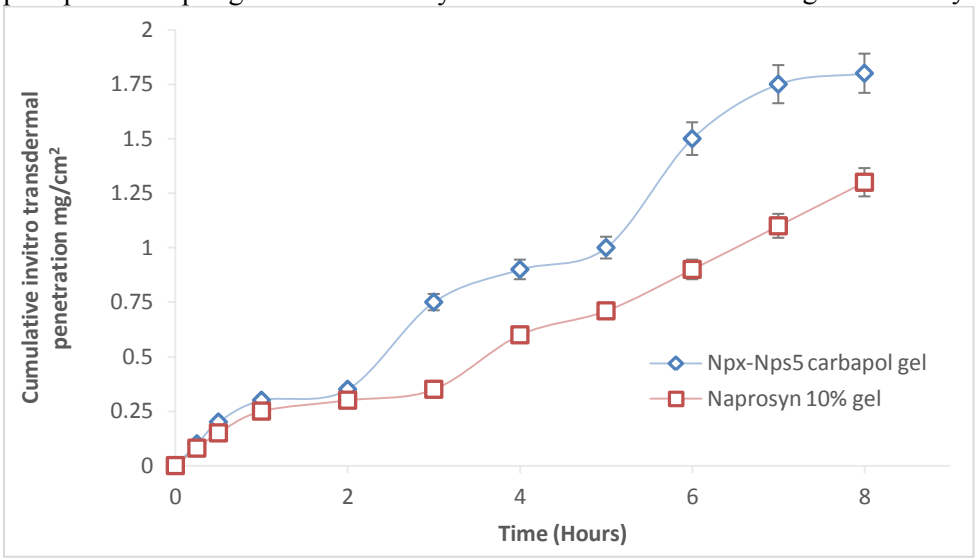

Figure 3. Cumulative amount of naproxen permeated across rat skin (data is mean and standard deviation of three determinations, n = 3; ANOVA test followed by Tukey's test showed that the effect of time and formulation on naproxen permeation was significant $p < 0.05$).

Tail flick method

Tail flick method was performed to assess analgesic activity of Npx-Np-carbapol gel. The analgesic activity was performed in white albino mice using Naprosyn 10% gel as + ve control and –ve control was third group that did not receive any medication. The time taken until mice withdrew their tail from the radiant heat source was used as the test end point. Ten seconds was used as a time off to prevent tail injuries [23]. Figure 4 shows the results of analgesic activity using tail flick method. Results show that Npx- NpS5-carbapol gel caused a significant increase in the analgesic effect ($p < 0.05$) in mice after 0.25 h (7.1 ±0.19 s.) and a maximum analgesic effect time of 3 hours. All measurements from 0.25 h to 7 h showed a significant increase in the analgesic effect ($p<0.05$) compared to the – ve control group. Naprosyn 10% gel showed an analgesic effect of 5 h in mice. Analgesic effect began after 0.25 h (5.10± 0.09 s.) and the maximum analgesic effect time was observed after 3 h. At 7 h Naprosyn 10 percent gel, the measurement showed a non- significant analgesic effect ($p<0.05$) compared to the – ve control group. The results showed that the analgesic effect of Npx- Nps5 carbapol gel was 1.5 times higher than that of Naprosyn 10 % gel after 4 hours and the duration was approximately 1.4 times higher. These results from the tail flick test showed good agreement with the in vitro release test results.

Conclusion

This study has shown that Naproxen nanoparticles can be formulated using a hot high pressure homogenization method. Npx- Nps in carbapol gel has been shown to improve the in- vitro release rate. The results of the tail flick method showed good agreement with the results of the in vitro release. Improved activity can be attributed to nano- sized particles, better wettability and a larger surface area. Lipid nanoparticles are therefore carrier systems with good prospects for successful marketing.

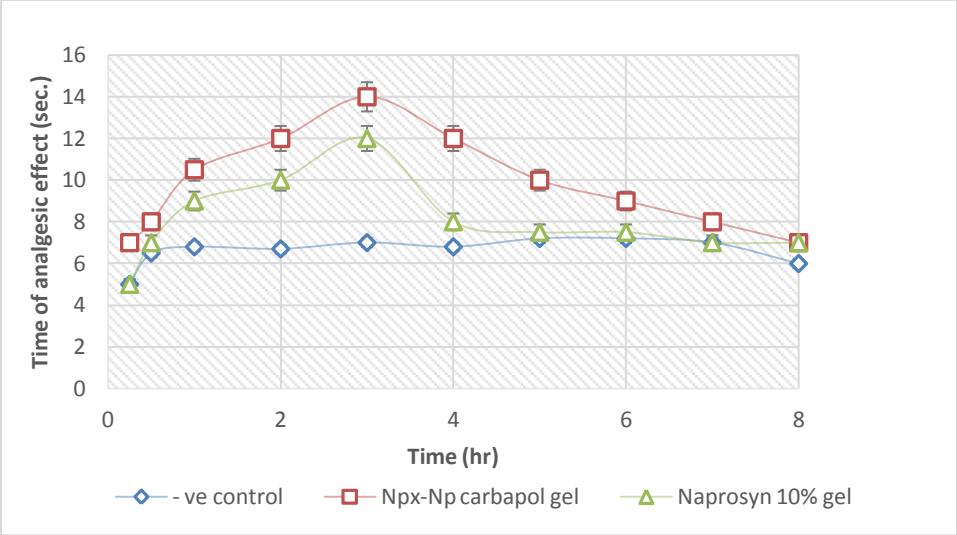

Figure 4. Analgesic effect of Npx-Np cabapol gel in mice compared with Naprosyn 10% gel using tail flick method. All values are means ± SE of six determinations.

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