American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Formulation and Evaluation of Novel Gel Containing Liquid Crystals of Naproxen.

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Date of Receipt- 15/05/2014
Date of Revision- 18/05/2014
Date of Acceptance- 21/05/2014

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ABSTRACT

Amongst various routes of administration, topical route is the most preferable and applicable route for drug delivery. Oral administration of naproxen as NSAID has clinically limited use because it causes irritation and ulceration of gastro-intestinal mucosa. Liquid crystals are phases of matter, with choice of self- associated structure, drug solubilization capacity and hence can decrease the drug degradation. Liquid crystals phase possesses high viscosity which allows controlled release of drug. In proposed work naproxen liquid crystals are prepared in combination with poloxamer 407, GMO and carbopol 940; which later incorporated in gel dosage form for efficient topical delivery. Gel formulation is preferred because it provides good bioavailability, extended contact time which is necessary to maintain therapeutic concentration in the skin as well as systemically for a longer period of time. *In-vitro* drug release study of prepared formulations were performed with 15% poloxamer with increasing concentration of GMO in F1-F3, with carbopol 940 gel base in F13 and poloxamer 407 without GMO in F14. The formulation F3 containing poloxamer 407 with increasing GMO concentration had shown 70.52% drug release at the end of 10 h. Thus, gel containing liquid crystals of naproxen was successfully formulated and evaluated; which demonstrates controlled release rate. This release pattern would prevent excessive accumulation of active agent in the epidermis thereby avoiding irritation and may also enhance safety and efficacy as compare to conventional products.

Keywords: Naproxen, Liquid Crystals, Topical Drug Delivery, Gel.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentration. The route of administration has a significant impact on the therapeutic

outcome of a drug. Drug application to the skin can be devoted to treat dermatological disorders (topical delivery), to treat deep tissues such as muscle and vein (regional delivery), and to allow the penetration of drugs to the systemic circulation (transdermal delivery). Transdermal delivery involves the application of a drug to the skin to treat systemic disease and is aimed at achieving systemically active levels of the drug. Percutaneous absorption with appreciable systemic drug accumulation is absolutely essential. Ideally, there would be no local accumulation of drug, but such accumulation is unavoidable. The transdermal route has been recognized as one of the highly potential routes of systemic drug delivery and provides the advantage of avoidance of the first-pass effect, ease of use and withdrawal (in case of side effects), and better patient compliance.[1] Most recent approach to drug delivery is to deliver drug into systemic circulation at predetermined rate which is known as controlled drug delivery system. Such a system helped to overcome side effects associated with conventional system of medication, which require multi-dose therapy. Transdermal therapeutics system defined as self contained, discrete dosage form which when applied to intact skin; deliver the drug through the skin, at the controlled rate to the systemic circulation. Thus it is anticipated that transdermal drug delivery system can be designed to input drugs at appropriate rate to maintain suitable plasma drug level for therapeutic efficacy by using skin as the port of entry of drugs.[2]

The Non Steroidal Anti-Inflammatory Drugs (NSAIDs) has prominent anti-inflammatory, analgesic and antipyretic properties. They are used in the treatment of osteo and rheumatoid arthritis. Oral therapy of NSAIDs is very effective, but the clinical use is often limited because of its potential to cause adverse effects such as irritation and ulceration of the gastro-intestinal (GI) mucosa. Patient noncompliance is also a common therapeutic problem in the management of chronic inflammatory diseases, because most NSAIDs must be administered in multiple daily doses to maintain therapeutic blood levels. Administration of these agents via the dermal route can bypass these disadvantages of the oral route and may maintain relatively consistent plasma levels for long term therapy from a single dose.[3]

Liquid crystals (LCs) are a state of matter that has properties between those of a conventional liquid and those of a solid crystal. The major classes of LCs are thermotropic, lyotropic and metallotropic LCs. Thermotropic and lyotropic LCs consist of organic molecules. Thermotropic LCs exhibit a phase transition into the LC phase as temperature is changed. Lyotropic LCs exhibit phase transitions as a function of both temperature and concentration of the LC molecules in a solvent (typically water). Metallotropic LCs are composed of both organic and inorganic molecules; their LC transition depends not only on temperature and concentration, but also on the inorganic-organic components ratio.

A lyotropic liquid crystal consists of two or more components that exhibit liquid-crystalline properties in certain concentration ranges. In the lyotropic phases, solvent molecules fill the space around the compounds to provide fluidity to the system. In contrast to thermotropic liquid crystals, these lyotropic crystals have another degree of freedom of concentration that enables them to induce a variety of different phases. A compound, which has two immiscible hydrophilic and hydrophobic parts within the same molecule, is called an amphiphilic molecule. Many amphiphilic molecules show lyotropic liquid-crystalline phase sequences depending on the volume balances between

the hydrophilic part and hydrophobic part. The content of water or other solvent molecules changes the self-assembled structures. At very low amphiphilic concentration, the molecules will be dispersed randomly without any ordering. At slightly higher (but still low) concentration, amphiphilic molecules will spontaneously assemble into micelles or vesicles. This is done so as to 'hide' the hydrophobic tail of the amphiphiles inside the micelle core, exposing a hydrophilic (water-soluble) surface to aqueous solution. These spherical objects do not order themselves in solution, however at higher concentration, the assemblies will become ordered. E.g. A typical phase is a hexagonal columnar phase, where the amphiphiles form long cylinders (again with a hydrophilic surface) that arrange themselves into a roughly hexagonal lattice. At still higher concentration, a lamellar phase may form, wherein extended sheets of amphiphiles are separated by thin layers of water. For some systems, a cubic (also called viscous isotropic) phase may exist between the hexagonal and lamellar phases.

There has been great interest in lyotropic liquid crystalline systems (LLC) as delivery systems in the cosmetic and chemical industries and also in the field of pharmacy. The reasons for this interest include the extensive similarity of these colloid systems to those in living organisms. LLC systems are characterized by the properties of both liquids and solids, i.e. they exhibit in part a structure typical of fluids and also the structured, crystalline state of solids. They are usually formed from water and one or two surfactants and possibly co-surfactants, in the definite proportions of the given components, with low energy input or by means of spontaneous structural organization; their production is therefore relatively simple and energy-saving. They are thermodynamically stable, and can be stored for long periods of time without phase separation. Depending on the concentration of the solvent (generally water or an aqueous solution) and on the polarity of the solvated mesogen, these systems can undergo various phase transformations and structural modifications, therefore their consistency, rheological properties can be changed systematically, as required.[4]

The aim of present work was to develop skin compliant lyotropic liquid crystals, with relatively various concentrations of poloxomer 407, glyceryl mono-oleate (GMO) and water content, to study the impact on structure by means of Polarized Light Microscopy (PLM), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD) and oscillation rheology measurement.

Research Envisage

Why liquid crystalline formulation?

LCs are substances that exhibit a phase of matter that possess properties between those of a conventional liquid and solid crystal which offer a number of useful properties for drug delivery. First, they allow drug solubilization, and with a proper choice of self-association structure, both water-soluble and oil-soluble drugs may be incorporated also in rather high concentrations. This, in turn, offers possibilities to increase the drug solubility, to decrease drug degradation, and to control the drug release rate. Second, liquid crystalline phases frequently display a rather high viscosity, which may also offer opportunities when the drug formulation needs to be localized, E.g., intramuscularly, on the skin or in the oral cavity.

Thermotropic LC and lyotropic LC are the two main categories of the liquid crystalline formulation. Present work is on lyotropic liquid crystal gel. As lamellar

lyotropic liquid crystalline systems are thermodynamically stable, optically isotropic or anisotropic and are formed spontaneously. They can be stored for long periods without phase separation. A lamellar phase exhibits interesting solubility properties which make it a good choice as a vehicle. Lamellar phases possess one dimensional order with hydrophobic and hydrophilic layers, so it is possible to incorporate water soluble, oil soluble as well as amphiphilic drugs within the structured lamellar layers. As lamellar liquid-crystalline systems contain a large proportion of incorporated water in the sandwich layers between hydrophilic domains, evaporation of water is less than that from traditional water-in-oil creams. Their moisture content is retained for a long time, so the transepidermal water loss is replaced by long-lasting hydration according to the needs of the skin. New possibilities for the development of drug delivery systems are inherent in these systems because of their stability and special skin-friendly structure.

Drug selection

Naproxen [(S)-6-methoxy-alpha-methyl-2naphthaleneacetic acid] is a non-steroidal anti-inflammatory drug, used in the treatment of inflammatory and degenerative disorders of the musculoskeletal system. It has side effects when taken orally like epigastric pain and bleeding, heartburn, nausea, diarrhoea, vomiting, peptic ulcer and hepatic impairment etc. Thus, an alternative route of administration for this drug is being currently investigated. Recent studies have shown significant drug levels in deep tissues such as fascia, muscle and synovium after topical application, which is a desirable feature for the relief of local symptoms with low dose, thereby reducing systemic side effects.[5] Also peak plasma level of naproxen upon oral administration is 3-4 h after 500 mg dose and steady state levels are attained after 4-5 doses at 12 h intervals. Approximately 500-750 mg dose required twice a day by the oral route.[6] From the NSAIDs class naproxen is selected because of its potency and more tolerability as compared to other NSAIDs.[7]

Why ‘gel’ as dosage form?

Naproxen has very poor solubility characteristics in common solvents such as water and alcohol. For topical use it is only available commercially in suspension, in a semisolid aqueous cream and in tablet form. The cream provides poor bioavailability of naproxen as the discrete particles thereof do not permeate very efficiently into the skin. The cream formulation is designed to apply for a very short periods which therefore does not provide the extended contact time necessary to maintain a therapeutic adequate concentration into the skin. Therefore there is a need to develop suitable formulation containing naproxen for direct application to the skin in order to treat susceptible conditions. Gel is viscous semisolid preparation; which sticks to skin for longer time. So to treat inflammatory conditions for long time, gel is the suitable and hence selected as dosage form.

MATERIALS AND METHODS

Naproxen was procured as a gift sample from Glenmark Pharmaceuticals, Goa (India). Glyceryl mono-oleate was purchased from Mohini Organics Pvt. Ltd., Mumbai (India). Poloxamer and polyethylene glycol was purchased from BASF, Mumbai (India) and Sigma Aldrich, Mumbai (India) respectively. All other excipients used were of analytical grade.

Formulation

Optimization of GMO Composition for LC Gel System

Various compositions of GMO (60 to 80%) were prepared in water. At the end of 7th day all compositions were tested for presence of liquid crystals by cross polarized optical microscope.

Formulation Design for LC Topical Gel of Naproxen

To obtain LC gel, specified amount of the GMO was melted (60°C), and then naproxen and other oil soluble ingredients were added under constant stirring. Simultaneously, water phase containing all water soluble ingredients was warmed to 60°C. Thereafter the water phase was added to the oil phase with constant stirring. The stirring was continued till the system get cooled and the resulting formulations were allowed to rest in closed vials for 1 week at room temperature to reach equilibrium. Prepared formulations were coded as F1 to F12 (Table 1).

Formulation of Conventional Gel of Naproxen

Carbopol 940 (1%) was dispersed in 50:50 propylene glycol: water and stirred continuously at 300 rpm for 3 h. Then, the naproxen (5%) was dispersed in 14 ml of propylene glycol and then added to the carbopol mixture. The dispersion was then neutralized and made viscous by the addition of triethanolamine.[8] Prepared formulation was coded as F13 (Table 1).

Formulation of Poloxamer 407 Gel of Naproxen without GMO

These were considered as blank formulations. The gels were prepared by the cold method described by Schmolka *et al*., 1972. For the preparation of the control gel, a weighed amount of poloxamer 407 was gradually added to cold (5-10°C) sterile solutions with gentle stirring and the containers were left overnight in a refrigerator to ensure complete dissolution. As a result clear, viscous solutions formed. To these viscous solutions 5% naproxen was added with continuous stirring and raising temperature above the gelation point so as to obtain the gel. Finally volume and pH were adjusted and resulting formulations were allowed to rest in closed vials for 1 week at room temperature to reach equilibrium.[9] Various formulations with varying quantities of poloxamer 407 and other relative ingredients were prepared. The prepared formulations were coded with F14-F16 (Table 1).

RESULTS AND DISCUSSION

Characterization of Naproxen

Description

The sample of naproxen was a white amorphous, odorless powder.

Identification

Melting Point-Melting point of drug sample was observed in the range of 154-156°C (complied with the literature).

IR Spectroscopy-Figure 1 shows IR spectrum of pure naproxen; which was matched with the IR spectrum of naproxen given in literature.

Compatibility Study

Using Infrared Spectroscopy

The prepared liquid crystalline gel systems were evaluated for compatibility studies to evaluate interaction between drug and excipients. For the confirmation of interaction between drug and other excipients IR spectra of formulations were taken (Figure 2) and compared with the IR spectrum of pure

drug (Figure 1). The pure naproxen showed characteristic peaks at 3109.13 (OH stretching), 1262.69, 1388.86 (OH bending), 2967.09 (C=C stretching), 2938.14 (C-H aliphatic stretching), 1627.87 (aromatic stretch). The IR spectra of formulation showed peaks at 3008.98 (OH stretching), 1351.02(OH bending), 2955.37 (C=C stretching), 2921.65 (C-H aliphatic stretching), 1648.96 (aromatic stretch); which signified that there was no interaction between drug and excipients. All peaks of functional groups of naproxen were present in the IR spectra of formulation. So, it was concluded that naproxen is compatible with all ingredients present in liquid crystal gel.

Using Differential Scanning Calorimetry

Liquid crystalline gel of naproxen is supposed to contain lamellar gel phase with entrapped water, in addition to the bulk water phase and dispersed oil phase. A dynamic equilibrium is maintained between the water interlamellarly inserted into the hydrophilic gel phase and bulk water phase. The latter is mainly fixed mechanically by the hydrophilic gel phase. The thermal analysis of the drug, as well as the lamellar structure of the drug incorporated in liquid crystal was performed using DSC.[10] The DSC of naproxen (Figure 3) shows a single sharp endothermic peak at 155.95°C with total enthalpy change (ΔH) of -99.29J/g.

The DSC of formulation F6 (Figure 4) containing 5% drug in liquid crystals showed main transition peaks at 99.57 with total enthalpy change (ΔH) of -97.94 J/g. Mainly at 99.57^{o}C the interlamellar fixed water was released. As the DSC method was based on the principle that the fraction of drug solubilised within the matrix did not contribute to the melting endotherm associated with the dispersed drug fraction. The disappearance of the naproxen peak at 155.95°C in formulation indicates that incorporated drug may be dissolved in the molten carrier during the drug loading process.

Optimization of GMO Composition for LC Gel System

The most important aspect of the liquid crystalline system is the proper concentration of the liquid crystalline precursors.

From Figure 5, it was observed that GMO forms distinct liquid crystals in the range of 60 to 80% at room temperature without any aggregates. Hence, it was decided to use 60-80% of GMO concentration for the preparation of liquid crystal gels.

Evaluation of LC Gel

Clarity

All formulations were examined 7 days after preparation. The formulations of poloxamer 407 with GMO (F1 to F12) were found to be opaque. The formulations of poloxamer 407 without GMO (F14 to F16) were found to be white. The results are quoted in Table 2.

pH Measurement

pH of all prepared formulations were found in the range of 6.8-7.2 (Table 2), thus indicating suitability for application to the skin as closer to skin pH. Naproxen is weak acid having pKa 4.29 and almost present in ionized form at skin pH.

Drug Content

Good uniformity of drug content among the prepared gel formulations were observed within range from 92-98% (Table 2). These results indicated that the process employed to prepare gels in this study was capable of producing gels with uniform drug content and minimal content variability. Also drug contents of all formulations were found within limit (90-110%).

Polarized Light Microscopy (PLM)

The first step in development of dosage form was to determine the emulsifier and water ratio where the liquid crystalline structure formed. Polarized light microscopy of these multi-component systems, containing various proportions of nonionic surfactants, GMO, water; revealed a lamellar liquid crystal pattern with a characteristic ribbon structure and fan texture in polarized light.

Formulation F1-F3 (Figure 6- A, B, C) showed characteristic concentric lamellar mesophases showing oily streaks with inserted Maltese cross rearrangement of plane layers. In the formulation liquid crystals were also observed around the oil globules (Figure 6-C). Oily streaks may be present because of low concentration of poloxamer 407. The concentration of gelling agent poloxamer 407 in formulations F1-F3 was 15%. The formulations F4-F6 (Figure 6-D, E, F) showed characteristic fan texture and typical lamellar-type birefringency; which revealed the formation of lamellar phase. The concentration of gelling agent poloxamer 407 in formulations F4-F6 was 18%.

The concentration of Poloxamer 407 was increased (21%) in formulations F7-F9 (Figure 6-G, H, I) for which we observed the clear plane parallel shaped structures indicating lamellar phase. The GMO concentration has also played important role in the phase behavior of the liquid crystals. The formulation containing high amount of GMO, showed less oily globules. The fan texture and typical lamellar type birefringency observed in the formulations (possessing poloxamer 407 concentration 18% and 21%) may be because of characteristic solubilizing and water retaining property of poloxamer 407. As the concentration of poloxamer 407 increased from 21% to 24% aggregation was observed as seen in case of formulations F10-F12 (Figure 6-J, K, L). In these formulations disappearance of lamellar phase was observed. This may be because of the high concentration and gelling property of poloxamer 407.

In-vitro Drug Release Study

An essential parameter in the evaluation of drug delivery is the rate at which the drug is released from the carrier. Release of naproxen from various gel formulations were studied using a modified Keshary-Chien diffusion cell.[11] The initial drug release study was carried out on the cellophane membrane having pore size 0.45 µ (area 1.70 cm^2) for all samples. Cellophane membrane was fixed between the donor and receptor compartments of the cell. Receptor compartment was filled with receptor fluid (PBS pH 7.4, 40 ml) in such a way that, the membrane surface was just flushed to the surface of fluid (PBS pH 7.4). Receptor medium was maintained at 37±1˚C and stirred continuously on a magnetic stirrer at 100 rpm. 0.5 mg of gel was placed in the donor compartment.[12] Sink condition was maintained throughout the study and readings were recorded in triplicate. The release data was analyzed with the various mathematical models to know release kinetics.[13]

The *in-vitro* drug release study was carried out for all formulations. Drug release data of liquid crystalline naproxen gel formulations F1-F3, F13 and F14 is quoted in Figure 7. Formulations F1-F3 contains 15% of poloxamer along with increasing concentrations of GMO whereas formulation F13 contains Carbopol 940 as a gel base. Formulation F14 was of 15% poloxamer 407 without GMO.

Conventional gel prepared with carbopol (F13) showed 35.5% drug release, whereas poloxamer 407 gel (F14) showed 50.28% drug release at the end of 10 h. This may be attributed to solubilizing property of poloxamer 407. The lower drug release of the formulation F13 attributed to low solubility of naproxen in carbopol gel. As with Carbapol, solvents like glycerin and propylene glycol

can modify hydrogen bonding characteristics between water, solvent and polymer, thereby affecting the swelling, viscosity property and so thereby affects release of the drug.

In presence of GMO (F1-F3) drug release was found to be increased as compare to plane poloxamer (F14) and carbopol gel (F13). It was observed that the liquid crystalline formulation F3 showed significantly ($P < 0.05$) higher amount of drug release (70.52%) at the end of 10 h as compared to F13. This may be due to enhanced solubilizing property of GMO as the concentration increased from 60 to 80%. The lamellar phase structures exhibit interesting solubility properties, in that the lamellar lipophilic bilayers structure alternate with hydrophilic layers that contains inter-lamellar water making them suitable for incorporating water-soluble, oil-soluble and amphiphilic drugs.

As the concentration of GMO was increased the drug release was found to be increase. This may be because of more amount of drug partitioned into lipophilic GMO phase as drug naproxen is lipophilic in nature.

The formulations F4-F6 contain increased concentration of poloxamer 407 (18%) as compared to F1-F3 and also increased concentration of GMO from 60-80%. As the concentration of GMO in formulations was increased drug release was found to be increased (Figure 8). When the release data was compared with formulations containing 15% of poloxamer, drug release rate was found to be increased. This may due to the solubility enhancement property of Poloxamer 407.

In formulations (F7-F9) concentration of poloxamer 407 was increased to 21% (w/w) along with increase in concentration of GMO from 60 to 80%. Drug release was found to be retard for formulation containing 21% of poloxamer 407 as compared to formulations with 15% and 18% poloxamer 407 (Figure 9). This may be due to the saturation of drug in the receptor medium, high viscosity of gels and gelling behavior of poloxamer 407.

From the Figure 10, it was concluded that formulation containing 80% of GMO exhibits highest drug release as compared to 70% and 60 % of GMO. The formulation containing GMO: water (80:20) with 15% poloxamer showed 70.52% drug release, 18% poloxamer showed 85.36% drug release and 21% poloxamer showed 49.28% drug release at the end of 10 h. As concentration of poloxamer was increased from 18% to 21% drug release was found to be decreased. This may be due to the higher amount of poloxamer and its gelling behaviour.

The formulation containing 18% of poloxamer with GMO: water concentration 80:20 showed higher amount of drug release as compared to formulations containing 15% and 21% poloxamer this may due to the higher amount of formation of lamellar phase by GMO in the 18% poloxamer concentration, which favors solubilizing properties of poloxamer 407 as in poloxamer 407 based formulations solubility and viscosity plays key role in the drug release.

Analysis of Drug Release

A sample of dried skin was rehydrated by immersing in phosphate buffer solution pH 7.4 at room temperature for 15 min before being mounted on diffusion cell. The exposed skin surface area was 1.70 cm^2 and the receptor volume was 40 ml. The receptor compartment was filled with PBS pH 7.4 which was stirred and kept at 37°C during experiment. A sample of 0.5 gm of 5% naproxen gel was administered on stratum corneum side of the skin mounted on the chamber. The receptor chamber is sampled at predetermined time intervals and the concentration was determined with the help of UV spectrophotometer at 271 nm. Sink condition was maintained throughout the

study and readings were recorded in triplicate.[14] The drug release data of all formulations was fitted in various kinetic models and the obtained results are summarized in Table 3.

There are possibly three steps which govern the drug release from the matrix these are penetration of the dissolution medium into the matrix, dissolution of dispersed drug particles and diffusion of the dissolved drug through the matrix. The slowest step will control the overall drug release rate. If the diffusion of the drug is the rate-limiting step, the drug release will likely to follow Higuchi square root kinetics and Korsmeyer Peppas. However, if the dissolution of the drug is the rate-limiting step, the drug release follows zero-order kinetics.

Peppas (1985) used *n* value in order to characterize different release mechanisms, concluding values were *n*=0.5 for Fickian diffusion and higher values of *n* between 0.5 and 1.0, or *n*=1.0, for mass transfer following a non-Fickian model. When n value greater than one (n >1) system follows super case II transport mechanism.

From the release kinetics study (Table 3) it was observed that formulation F1 to F16 mostly followed the Korsmeyer Peppas model as best fit model because of the higher value of correlation coefficient as compared to other models. For all formulations value of n, release exponent were found to be in the range of 0.57 to 0.71 which indicates that drug was released by non-Fickian mechanism. From the result it was also observed that formulations without GMO (F13-F16) showed zero order drug release indicating that dissolution of drug is the rate limiting step for these formulations. Formulations containing 80:20 GMO: Water concentrations were continued for further evaluation along with control formulation F13.

Formulation F6 showed higher amount of drug diffused as compared to plane carbopol gel formulation. Formulation F6 contains GMO: water in the ratio of 80:20 with 18% of poloxamer 407 as gelling agent. It was observed that concentration of poloxamer 407 affects the solubility and viscosity of formulations. Formulation containing low concentration of poloxamer 407 (15%) showed average permeability while high concentration of poloxamer 407 retards drug permeation. From the Figure 11, it was concluded that the formulation F6 has good skin permeability than other formulations. This may be because of as monoolein promoting ceramide extraction and enhancement of lipid fluidity in the stratum corneum, monoolein is a penetration enhancer by itself and it has been proven that GMO/solvent systems are effective penetration enhancers for lipophilic drugs and highly polar compounds. Presence of lamellar layer and their special skin similarly structure and the proper amount of gelling agent affects the permeation. Absence of GMO affects the permeability of drug and the lamellar structural phase has showed enhanced permeation over the hexagonal and cubic phase. This might be the reason for the maximum drug diffusion from F6.

Flux and Permeability

Data analysis was done using Stress Rheologic Basic software, version 5.0. A cone and plate geometry was used with 25 mm diameter and cone of 1.0°. Fresh samples were used for every test and all measurements were carried out at 5°c, 10°c, 20°c and 37°c in triplicate.[15]

Flux, permeability and enhancement ratio for the formulations is given in Figure 12. Significant augmentation in the skin permeation of liquid crystalline naproxen gel has been observed. Formulation contains nonionic surfactant poloxamer 407 in the concentration range of 15-21% w/w to stabilize liquid crystals formed by GMO: Water system and to maintain gelling

behavior of formulations. In the formulation F6 permeability and flux was found to be highest and the increment was significant ($P < 0.001$) as compared to formulations F3, F9 and F13. This may be due to permeation enhancing effect shown by monoolein. Formulation F9 contains high amount of poloxamer 407 (21%) which retards drug permeation this may because of high viscosity of gels and gelling behavior of poloxamer 407. In the formulations F9 and F13 permeability and flux was found to be comparatively low due to high concentration of poloxamer and absence of GMO respectively.

From the results of drug diffusion study, it was observed that concentration of poloxamer 407 and GMO significantly affects drug permeation.

Generally, a drug permeating through a lamellar gel network may follow an inter-lamellar or trans-lamellar route, depending on local rates of diffusion and partition. Extremely lipophilic drugs are likely to be trapped inside the lipophilic bilayers, while extremely hydrophilic drugs will permeate through the hydrophilic regions between the lamellae, and amphiphilic drugs may move both between and across the lamellae.

Skin Deposition Study

In systemic disorders, improving the efficacy demands high drug levels in the systemic circulation. The lamellar liquid crystalline phase present in gel enhances the drug penetration into skin and because lamellar structures demonstrates the greatest similarity to the intercellular lipid membrane of the skin are primarily recommended for the development of a transdermal drug delivery system.

Naproxen in liquid crystalline gel was well penetrated through the rat skin membrane which was observed in methanolic extract from rat skin after diffusion study. High amount of drug was permeated through rat skin from formulation F6 (72.33%) as compared to conventional carbopol based naproxen gel (33.06%). Only 14.84% of drug was retained in the rat skin from formulation F6 which is very less as compared to other formulations. Hence, with use of liquid crystalline system a greater amount of drug gets permeated into systemic circulation with lesser amount of drug deposition in the skin. The drug deposition of liquid crystalline formulations in rat skin was found to be very less as compared to carbopol gel formulation shown in Table 4. This indicates that, GMO/Water/ poloxamer 407 liquid crystalline topical drug delivery promotes drug permeability in the rat skin, thus has potential for transdermal drug delivery.

Rheological Study

Effect of Temperature

The sol-gel transition temperatures for 15%, 18% and 21% Poloxamer 407 solutions with GMO: water in the ratio of 80:20 are shown in Table5. It was observed that, at the beginning, G' was low but increased drastically with increasing temperature as a result of sol to gel transition process. At the end of the sol-gel transition, G' becomes independent of the temperature. The transition temperature was found to be decrease with increased concentration of poloxamer 407. The gels showed a sol-gel transition temperature below 20°C which is the characteristic property of poloxamer.

Each Poloxamer 407 formulation was viscous liquid at the storage temperature (4°C), formed a semisolid gel at the experimental temperature (37°C, above the gel-sol transition temperature), and returned to the liquid state below the gel-sol transition temperature. The Formulations F3, F6 and F9 showed Newtonian behavior below the gelation temperature and non Newtonian behavior above this temperature.

Complex Moduli (G*)

Table 6 shows the elastic and loss moduli of the formulation F6 as a function of frequency. The measurements of the elastic modulus (*G*') and of the loss modulus (*G*") for formulation F6 were made at 37°C. It was observed that Poloxamer 407 gels are viscoelastic fluids, having *G*' larger than *G*", that is their elastic components are larger than their viscous components. For the all gel systems, G' is higher than G". From the Figure it was observed that formulation F6 has good elasticity property.

Cryo-Transmission Electron Microscopy (Cryo-TEM)

A micropipette was used to load 5 µL solutions onto a TEM copper grid, which was blotted with two pieces of filter paper, resulting in the formation of thin films suspended on the mesh holes. After waiting for about 5 s, the samples were quickly plunged into a reservoir of liquid ethane (cooled by the nitrogen) at -165^0C. The vitrified samples were then stored in the liquid nitrogen until they were transferred to a cryogenic sample holder (Gatan 626) and examined with a JEOL JEM-1400 TEM (120 kV) at about -174^0C.[16]

Figure 13[a] and 13[b] shows TEM of formulation F6. The oily globules were present in very small size and less number. Formulation F6 contains GMO: water in the ratio of 80:20 with 18% of poloxamer. The GMO oil phase separation was not analyzed in the formulation. No macroscopic phase separation was observed for dispersions with GMO: water ratio 8:2. As seen in Figure the presence of dense striated structures 13[b], which most probably represents lamellar phase, was characteristic at this composition. It reveals that the cryo-TEM analysis indicated the presence of lamellar phase accordingly several small liposomes can be seen in the background 13[a]. Also network of disordered lamellar threads was demonstrated by cryo-TEM analysis in the Figure 13[b]. The lamellar structure observed can be seen in micrographs.

Small Angle X-ray Scattering (SAXS)

SAXS is the most appropriate technique for the exact determination of the distances of interlayer spacings of liquid crystalline systems. X-ray diffractograms were recorded at room temperature (25^0C) in the inclination angle range $2\theta = 1\text{-}5^0$. The d values reflecting the extent of the structure were determined via the Bragg's equation.[17]

Optimized formulation F6 was examined by means of small angle X-ray scattering in a temperature scan range 20-70^0C. The formulation showed X-ray diffraction lines with a relative ratio of (39.2183A^o/18.2604A^o) d-spacing corresponding to a lamellar structure and the lamellar structure of the lipid phase was not affected by presence of the drug. The d spacing of the GMO-water phase containing 4% w/w naproxen is given in Table 7.

Analgesic Activity of LC Gel

Rats were placed on aluminium hot plate kept at a temperature of 62 ± 0.5^0C for a maximum time of 30 s. The temperature of the plate was monitored at all times.[18]

Analgesic activity was carried out on Eddie's hot plate for the optimized formulation F6 and conventional formulation F13. Results were compared with control group. Liquid crystal gel (F6) and carbopol gel (F13) exhibited significant increase in the latency time compared to latency time observed prior application of drug treatment. One-way analysis of variance showed significant difference in the analgesic activity ($P<0.05$) among the liquid crystal gel (F6) and carbopol gel (F13) treatment.

Liquid crystalline gel formulation showed significant difference in reaction time as compared to Carbapol gel ($P < 0.01$) and control ($P < 0.001$). The maximum latency

time of the formulation F6 observed at 60 min. From the Figure 14, it was concluded that formulation F6 has optimum analgesic activity.

Anti-inflammatory Activity of LC Gel

The effects of carbopol gel (F13) and liquid crystal gel (F6) on paw edema induced by Carrageenan are shown in Table 8. Treatment with carbopol gel (F13) and liquid crystal gel (F6) produced a diminished inflammation in rat hind paw when challenged with Carrageenan. Percentage inhibition observed in liquid crystal gel treated group was higher than that observed with carbopol gel treated group. Liquid crystal gel (F6) (500 mg topical) produced very significant (P <0.001) change in Percent inhibition of inflammation (Table 9). After 60 min liquid crystalline formulation F6 showed 92.21% inhibition while formulation F13 showed only 50.24% inhibition. Differences between paw volume of control and treated group were assessed by one way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test, P < 0.05 was considered significant.

From results it was observed that the optimized formulation (F6) showed minor amount of change in paw volume and reflected significant % inhibition in inflammation as compared to control (P<0.001) and carbopol gel (F13). Hence these results demonstrated that formulation F6 has optimum analgesic and anti-inflammatory activity.

CONCLUSIONS

In the present study, the liquid crystalline gel of naproxen was successfully formulated, evaluated and effect of GMO and poloxamer 407 was studied on the permeation of naproxen from liquid crystalline gel. Formulation F1-F9 contained 15%, 18% and 21% of poloxamer. All the formulation showed lamellar structure which was revealed by polarized light microscopy. Formulation F10-F12 contained 24% of poloxamer and shown aggregated structures. In the release study it was observed that formulations containing higher amount of GMO had greater release and permeation. Among the formulations formulation F6 showed privileged release data which contains 18% poloxamer. From the result it was inferred that higher GMO concentration with specific amount of poloxamer concentration had superior results; whereas formulations without GMO (F13-F16) had shown underprivileged release data. Formulation F6 had shown preeminent analgesic and anti-inflammatory activity as compared to formulation F13 which was the carbopol gel based formulation.

Proposed work also reflects that formulation of liquid crystals gel containing 5% naproxen (F1-F9) exhibited good results as novel transdermal drug delivery system when compared to formulations F10-F16 which were without GMO. Thus, novel liquid crystal gels are proven to be a better formulation in all aspects than conventional transdermal creams and gels.

ACKNOWLEDGEMENTS

The authors are thankful to Glenmark Pharmaceuticals, Goa (India) and Mohini Organics Pvt. Ltd., Mumbai (India) for providing the gift samples of naproxen and glyceryl mono-oleate respectively.

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Table 1. Formulation design for liquid crystalline topical gel of naproxen

Ingredients	Formulations															
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
Naproxen	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GMO: water	60.40	70.30	80.20	60.40	70.30	80.20	60.40	70.30	80.20	60.40	70.30	80.20	0.47	0.100	0.100	0.100
Poloxamer 407	15	15	15	18	18	18	21	21	21	24	24	24	-	15	18	21
PEG 400	2	2	2	2	2	2	2	2	2	2	2	2	-	2	2	2
Carbopol 940	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Propylene Glycol	-	-	-	-	-	-	-	-	-	-	-	-	47	-	-	-
Methyl Paraben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Propyl Paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Table 2. Evaluation of gel formulations

Formulation	Clarity	pH	Drug content
F1	Opaque	6.8± 0.01	95±0.33
F2	Opaque	7.1± 0.03	98±0.45
F3	Opaque	7.0± 0.08	98±1.02
F4	Opaque	6.9± 0.10	96±0.69
F5	Opaque	6.8± 0.07	96±0.80
F6	Opaque	6.9± 0.02	97±1.38
F7	Opaque	7.1± 0.04	92±0.61
F8	Opaque	7.2± 0.01	94±0.44
F9	Opaque	6.8± 0.05	97±0.25
F10	Opaque	7.2± 0.19	92±0.78
F11	Opaque	6.9± 0.08	95±0.68
F12	Opaque	6.9± 0.14	97±1.45
F13	White	7.1± 0.12	96±0.56
F14	White	7.2± 0.09	92±1.34
F15	White	6.9± 0.07	95±1.09
F16	White	6.8± 0.11	98±1.01

*The values are expressed as mean ±SD, n=3

Table 3. Kinetics of drug release data (F1-F9 and F13-F16)

Batch Code	Zero order	First order	Higuchi	Peppas	Korsmeyer-Peppas parameters		Best fitting model
					N	k	
F1	0.967	0.721	0.971	0.942	0.69	8.74	Higuchi
F2	0.890	0.613	0.968	0.984	0.69	9.63	Peppas
F3	0.911	0.634	0.973	0.969	0.71	9.31	Peppas
F4	0.972	0.644	0.981	0.995	0.69	9.88	Peppas
F5	0.954	0.615	0.984	0.996	0.69	9.90	Peppas
F6	0.953	0.627	0.983	0.992	0.72	9.81	Peppas
F7	0.983	0.703	0.974	0.992	0.58	9.81	Peppas
F8	0.977	0.693	0.976	0.985	0.60	9.66	Peppas
F9	0.953	0.659	0.971	0.980	0.62	9.54	Peppas
F13	0.993	0.767	0.955	0.975	0.57	9.44	Zero order
F14	0.994	0.800	0.918	0.961	0.62	9.14	Zero order
F15	0.986	0.731	0.972	0.981	0.59	9.57	Zero order
F16	0.993	0.810	0.951	0.816	0.61	6.54	Zero order

*k- release rate constant, R- coefficient of correlation, n- release exponent

Table 4. Skin deposition study of formulation F3, F6, F9 and F13

Test formulation	Drug in receptor compartment (%) after 10 h (n=3)	Drug retained in rat skin (%) after 10 h (n=3)	Drug remained in gel (%) after 10 h (n=3)
F3	60.05	25.06	14.89
F6	72.33	14.84	12.87
F9	42.96	30.11	26.92
F13	33.06	34.42	32.52

Table 5. Elasticity module (G') at different temperature

Temperature (°C)	G' Pa		
	F3	F6	F9
1	101	105	205
5	111	113	290
10	117	2781	5951
15	2081	6882	9210
20	6442	7586	9268
25	6845	7620	9380
30	7002	7712	9396

Table 6. Complex moduli (G' and G") of the formulation F6

Frequency (rad/sec)	0.1	1	10	20	30	40	50	60	70	80	90	100
G' (Pa)	0.001	0.21	14.3	21	174.7	2900	3530	5224	8114	9560	9720	9797
G" (Pa)	0.01	0.14	4.58	90	570	2075	2028	2215	2365	2592	3200	3400

Table 7. SAXS evaluation of formulation F6

Formulation	q($Å^{-1}$)	Structure	d (angstrom)	Δ/d
F6	0.15657, **0.35575**	Lamellar	39.2183	0.0126
	0.16369, **0.1708**, 0.17792		18.2604	0.032

*Bold numbers indicate the main scattering peak

Table 8. Effects of Carbopol gel and LC gel on paw edema in rats

Treatment	Paw Volume (ml) Mean ± S.E.M.						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	0.01±0.02	0.48±0.06	0.57±0.09	0.69±0.15	0.79±0.11	0.84±0.14	0.84±0.15
Carbopol gel	0.02±0.02	0.34±0.09***	0.28±0.06***	0.20±0.14***	0.27±0.14	0.40±0.14***	0.38±0.19
Liquid Crystal gel	0.01±0.02	0.07±0.03***	0.04±0.03***	0.08±0.03***	0.12±0.04***	0.15±0.05***	0.2±0.03***

Data expressed as mean ± S.E.M. for six rats in each group. ***P*<0.01; ****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test.

Table 9. Effects of Carbopol gel and LC gel on % inhibition of inflammation

Treatment	% Inhibition of Inflammation Mean ± S.E.M.					
	30 Min	60 Min	90 Min	120 Min	150 Min	180 Min
Carbopol gel	28.50±7.32	50.24±5.87	69.50±5.87	65.83±8.24	52.05±8.00	54.90±10.0
Liquid Crystal gel	85.57±3.58***	92.21±2.43***	88.77±1.16	85.06±1.85	81.80±2.52**	75.81±1.98

Data expressed as mean ± S.E.M. for six Rats in each group, ***P*<0.01; ****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

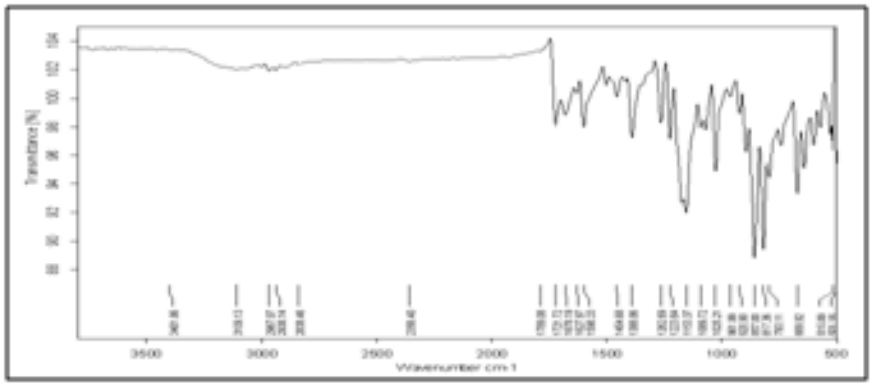

Figure 1. IR spectrum of naproxen

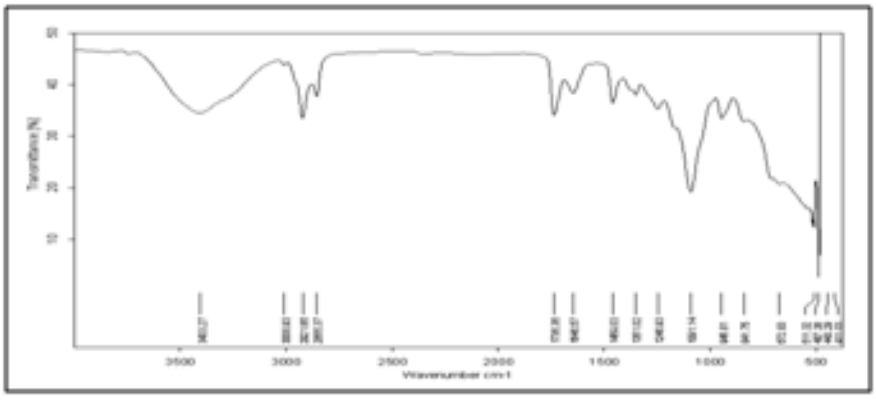

Figure 2. IR spectrum of formulation

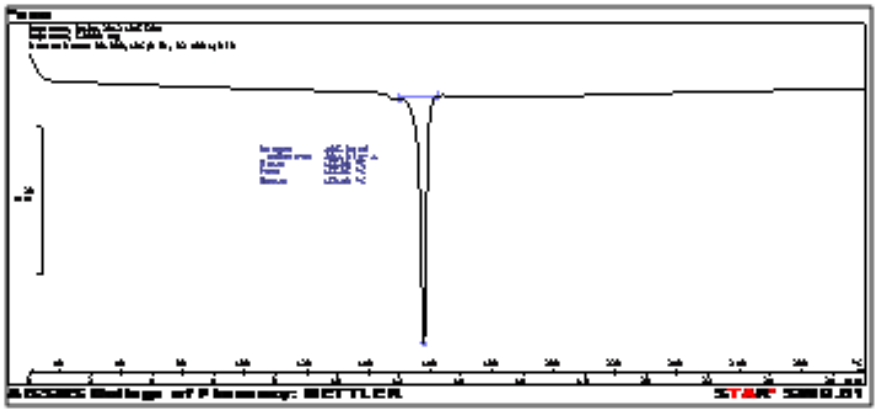

Figure 3. DSC thermogram of naproxen

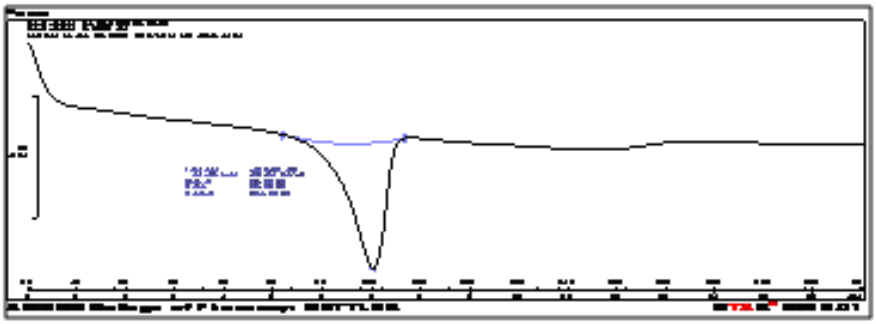

Figure 4. DSC thermogram of formulation F6

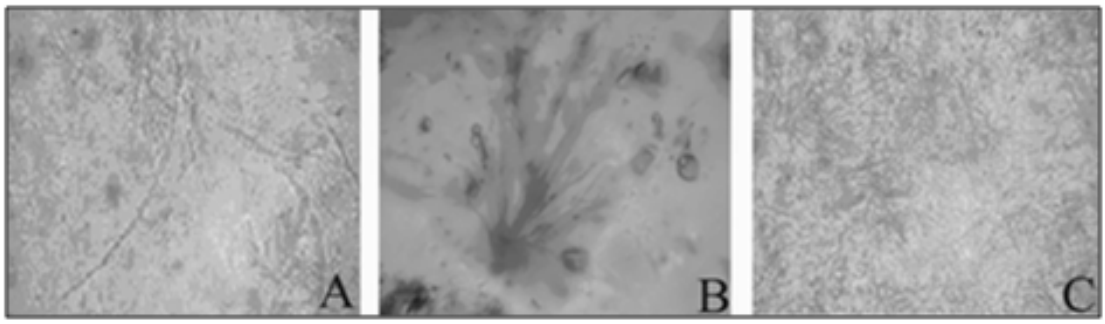

Figure 5. Polarized light microscopic images of GMO: Water compositions (A-60:40, B-70:30, C-80:20)

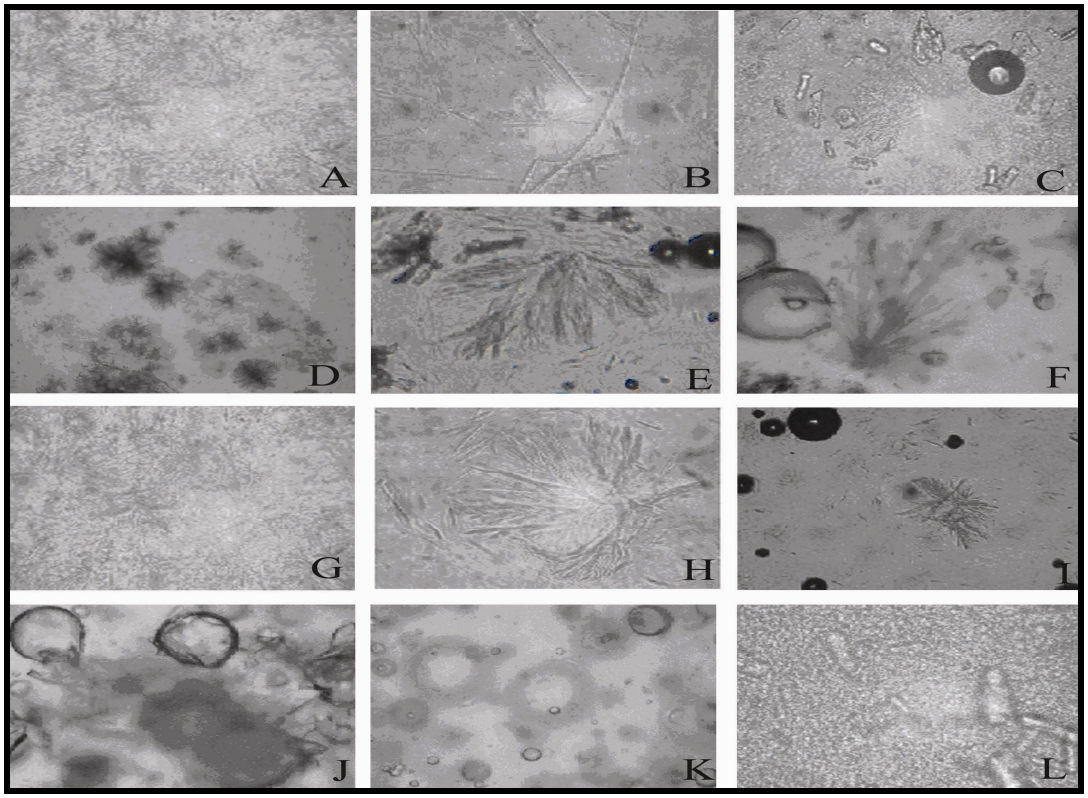

Figure 6. Polarized light microscopic images of formulations (F1-F12)

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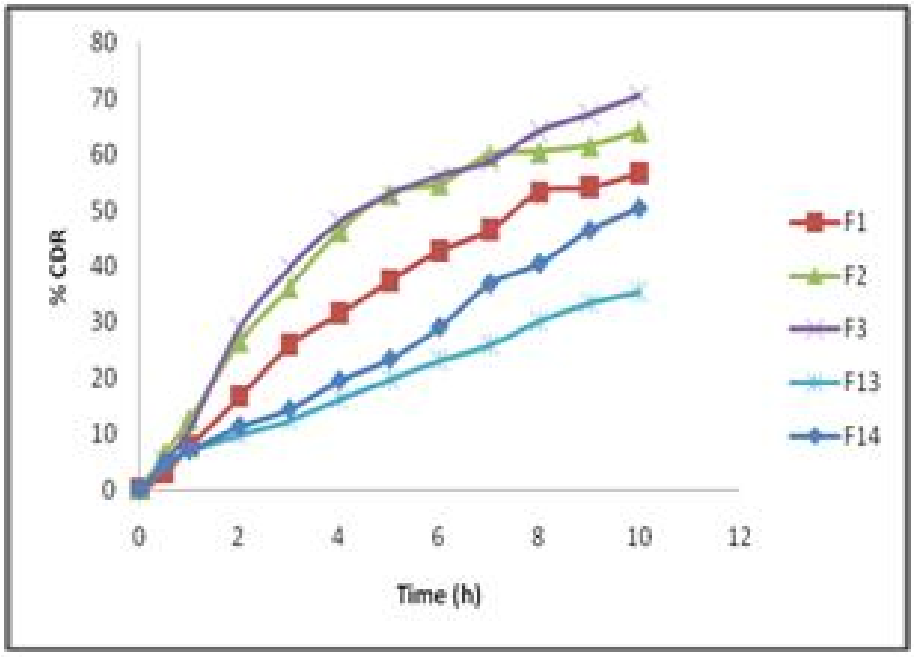

Figure 7. *In-vitro* drug release of formulations (F1-F3, F13 and F14)

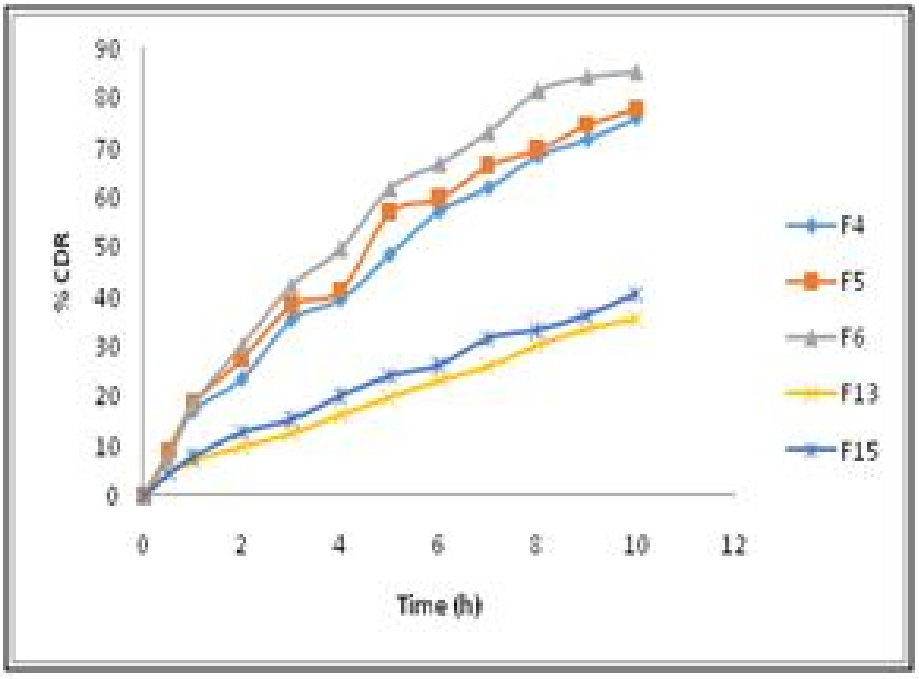

Figure 8. *In-vitro* drug release of formulations (F4-F6, F13 and F15)

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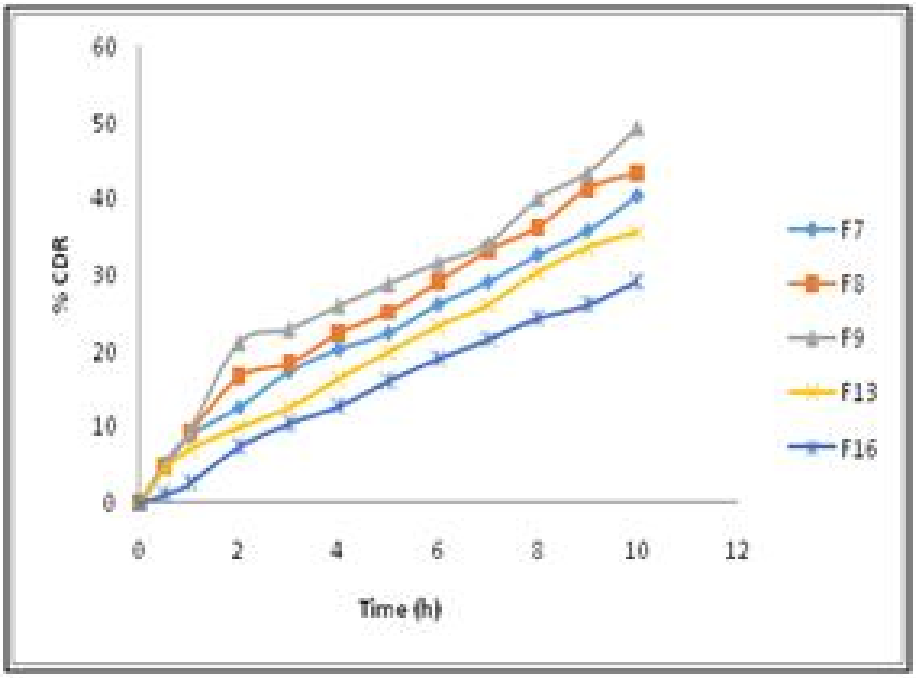

Figure 9. *In-vitro* drug release of formulations (F7-F9, F13 and F16)

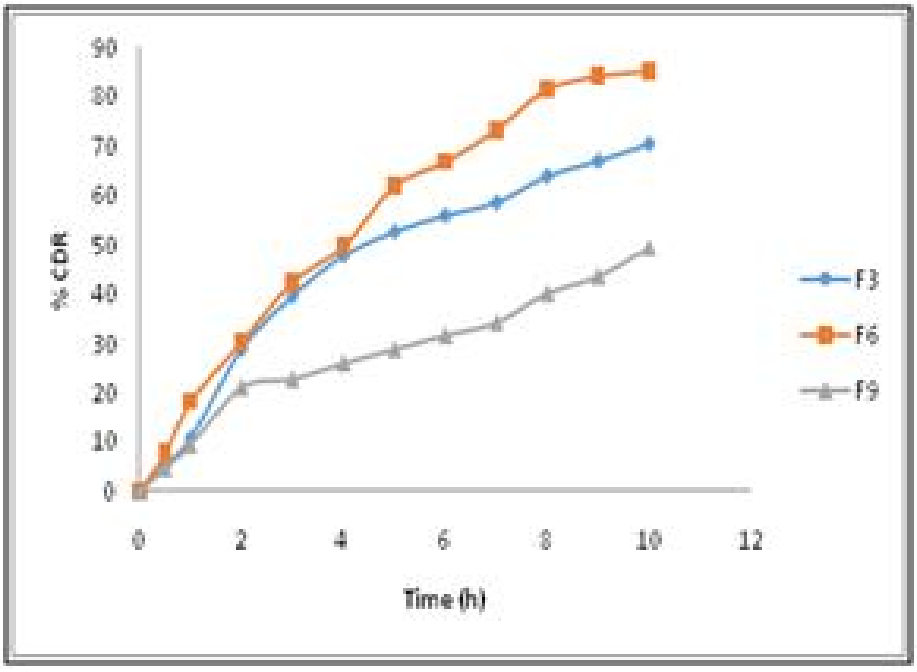

Figure 10. *In-vitro* drug release of formulations (F3, F6 and F9)

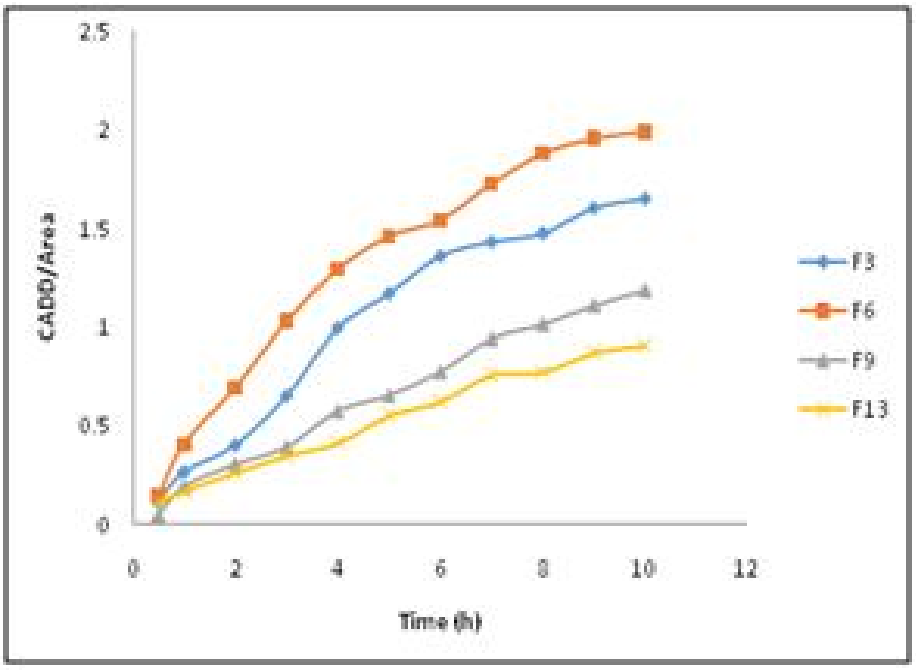

Figure 11. *In-vitro* drug diffusion of formulations (F3, F6, F9 and F13)

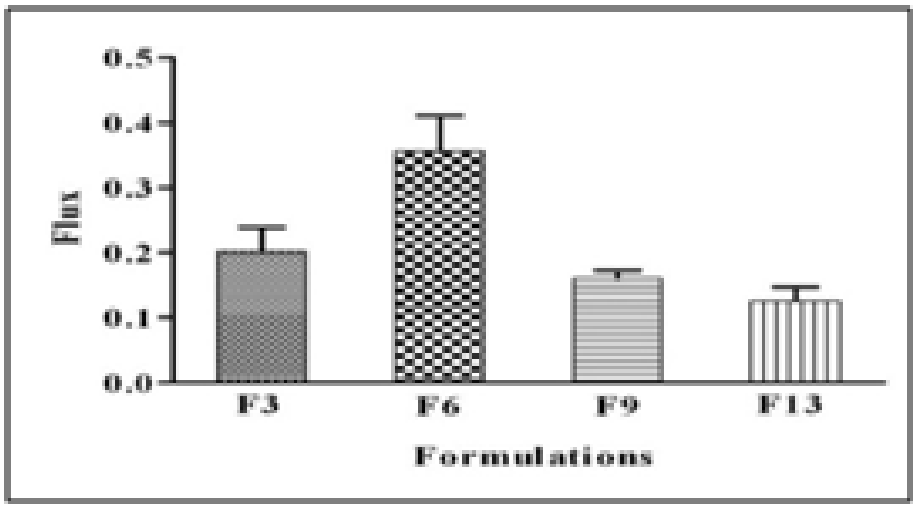

Figure 12. Permeability flux of optimized formulations

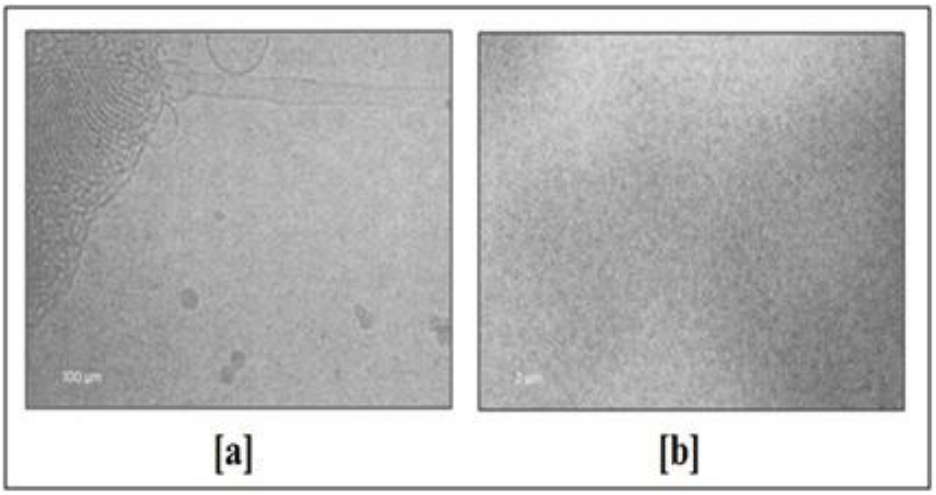

Figure 13. Cryo-TEM micrographs of formulation F6

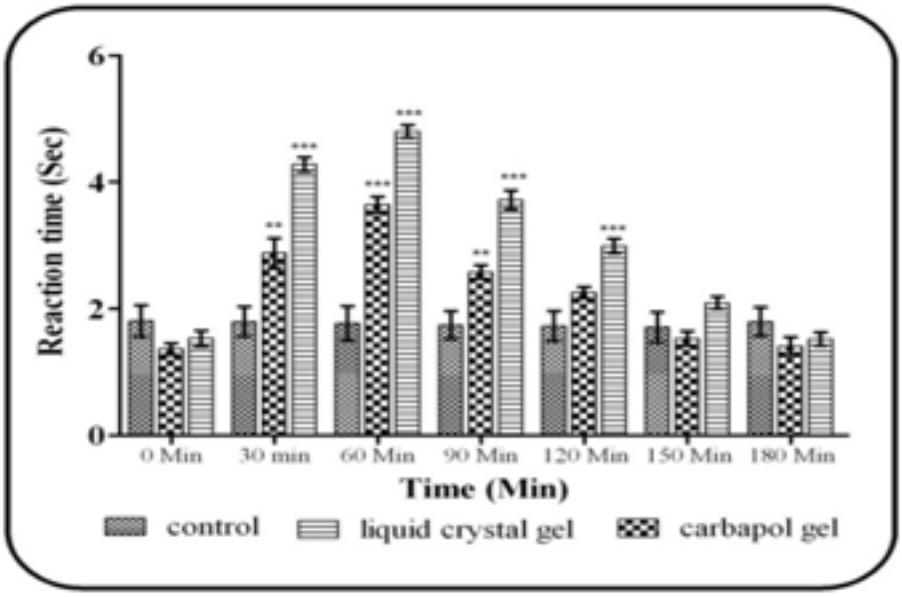

Figure 14. Latency time shown by mice