

Lecithin – Stabilized Microemulsion – Based Organogels for Topical Application of Ketorolac Tromethamine. II. In vitro Release Study

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Abstract

Ketorolac tromethamine (KT) containing microemulsion-based gels (MBGs) have been formulated, based on the previous phase diagram studies, using a pharmaceutically acceptable surfactant (soybean lecithin; Epikuron 200) and oil (isopropyl myristate; IPM) and the effect of formulation variables on the release profile of the drug from MBGs through intact guinea pig skin and various artificial membranes was then determined experimentally. It was observed that as the lecithin concentration increased from 40 to 50 and then 60% w/w in formulations, a significant decrease in KT release was obtained. A remarkable increase in the drug release was also observed in formulations containing 6.5% w/w of KT compared to those containing 1% w/w of the drug. Increasing the water content of the organogels also resulted in an increase in KT release. The optimized formulation of the organogel composed of 40% lecithin and 60% IPM (containing 0.6% w/w of water and 6.5% w/w of KT) showed the highest drug release rate. Moreover, the viscosity of different formulations and their rheological behavior were also determined. All formulations showed a slightly rheopectic behavior. It was found that an increase in lecithin concentration resulted in an increase in the viscosity of the organogel. Results have shown that KT could be incorporated at high concentrations into lecithin organogels and these systems could be considered as desirable drug delivery vehicles for water soluble drugs and are capable of providing an appropriate drug release rate and pattern.

Keywords: Lecithin organogels; Ketorolac tromethamine; Release rate; Microemulsion-based gels.

Introduction

Among various nonsteroidal anti-inflammatory drugs (NSAIDs), Ketorolac tromethamine (KT) has been widely used for postoperative and emergency treatment of pain. However, it accompanies adverse effects including gastrointestinal irritation when administered orally. Topical administration of KT offers the advantage of enhanced drug delivery to the affected sites with a reduced incidence of GI side effects. Although the transdermal route of administration has been recognized as one of the potential routes for both the local and systemic delivery of drugs, and also the skin is an exceptionally effective barrier to most chemicals,

very few drugs can permeate into it in amounts sufficient to deliver a therapeutic dose. Therefore, systems that make the skin locally more permeable and thereby enable transdermal delivery are of great interest.

In apolar organic solvents, soybean lecithin can form a thermoreversible, isotropic, nonbirefringent gel-like system, so-called microemulsion-based gel or organogel, characterized by considerably high viscosity and optical transparency (1, 2). Lecithin as a naturally occurring surfactant is capable of forming reverse micelle-based microemulsions in an apolar environment due to its geometrical constraints. It is believed that upon addition of a specific amount of water, the small reverse micelles tend to grow

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monodimensionally into long flexible and cylindrical giant micelles, above a critical concentration of lecithin. These giant structures then build a continuous network with a high viscosity (3, 4). Lecithin organogels have attracted much attention as a biocompatible matrix for transdermal drug delivery because a) they are thermoreversible systems and become liquid with lower viscosity at temperatures above 40°C and regain high viscosity by cooling, b) they are capable of solubilizing various guest molecules, c) they do not cause irritation, d) they possess long term stability, and e) they are transparent, allowing the use of spectroscopic methods to detect possible structural changes of the guest molecules (5, 6).

This research relates to a novel method of administration of a pharmacologically active anti-inflammatory agent, Ketorolac tromethamine, to promote the absorption and provide therapeutically effective concentration in the bloodstream. In the previous paper, partial phase behavior of systems composed of lecithin / IPM/ water or KT solutions at various lecithin/IPM weight ratios (k_m) was reported (7). To achieve the final objective, the potential of lecithin organogels for an effective delivery of KT was evaluated. In this regard, various formulations were prepared and the influence of formulation variables on the release rate and profile of the drug have been investigated.

Experimental

Materials

Soybean lecithin (Epikuron 200; E200), isopropyl myristate (IPM) and Ketorolac Tromethamine (KT) were provided by Lucas Meyer Company (Germany), Ruger Chemical Company Inc. (USA), and Lemmon Company (USA), respectively. HPLC grade acetonitrile and methanol, o-phosphoric acid 85%, sodium chloride, sodium phosphate dibasic and potassium phosphate monoacid were supplied by Fisher Scientific (USA). Cellulose acetate membrane (MWCO 3500) and silicone Elastomer sheeting (0.005") were purchased from Spectrum Laboratories Inc. (USA) and Advanced Biotechnologies Inc. (USA), respectively. All reagents were used as received.

Methods

Preparation of KT – loaded organogels

Based on the phase diagrams constructed, lecithin solutions were prepared by first

dissolving lecithin in the organic solvent (isopropyl myristate [IPM]) with the aid of a magnetic stirrer and then while still stirring, the necessary amount of aqueous solution of KT was added into the mixture to obtain a clear gel (8). Formation of clear, homogenous and nonbirefringent gels, after the addition of KT solutions by a micropipette syringe, took place within 30 seconds.

New method of preparation

In this method, the drug-containing gels were prepared by first dissolving KT into the solution of lecithin in IPM and then adding water to induce gelation. To facilitate the dissolution and obtain a homogenous mixture of dissolved components, the mixtures were heated for a very short time with constant stirring until solubilization of the drug was completed. Agitation was then stopped and the samples allowed cooling and setting to obtain clear, homogenous and nonbirefringent gels at room temperature.

Construction of calibration curves

Two series of different concentrations: 8, 16, 24, 32, and 40 µg/ml and 1, 5, 10, 15, 20, 25; as well as a 50 µg/ml solution of KT in phosphate buffer were prepared to construct the calibration curves. Solutions were analyzed spectrophotometrically (at a wavelength of 322 nm) and by using an HPLC method, respectively. Linear regression analysis of the corresponding plots showed a correlation coefficient of 0.9999 and 0.9997 for UV spectrophotometric and HPLC methods, respectively.

Preparation of synthetic membrane and guinea pig skin

Cellulose acetate and silicone elastomer membranes were soaked in distilled water for 24 h. Full thickness hairless guinea pig abdominal skin samples were prepared and cut into small pieces of circular sections with a 3 cm diameter. All the pieces were washed and then transferred onto an aluminum foil, dried and stored in a freezer until use.

Release / permeation studies

The release/permeation of KT from the lecithin gels through various selected membranes was determined using Franz-diffusion cells, having a diameter of 9 mm and a volume of 5.1 ml. Permeation studies were performed using guinea pig skin, cellulose acetate and silicone elastomer as synthetic membranes. In these cells, the skin or

artificial membrane was placed between the donor and receptor compartments of the cells. In case of guinea pig skin, the membrane was carefully mounted onto the diffusion cell with the stratum corneum side facing the donor compartment. The effective area of membrane available for diffusion was 0.64 cm². In all experiments, 0.3 g of each drug containing formulation was placed over the membrane, and then covered with paraffin. The receptor compartment was filled with 5.1 ml of degassed phosphate buffer solution (pH=7.4). The cells were thermostated at 32°C in an incubator, and the receptor solution was stirred with a magnetic stirrer at 200 rpm throughout the experiment. The receptor phase was withdrawn at predetermined intervals up to 12 and 24 h for synthetic membranes and guinea pig skin, of fresh phosphate buffer equilibrated at 32°C. Drug concentration within each receptor solution was determined either using a spectrophotometer (in case of artificial membranes) or an HPLC method (in case of guinea pig skin).

High performance liquid chromatography Assay

A Waters liquid chromatograph equipped with two Waters 151 HPLC pumps, a Waters 746 data module, a Waters 717 plus autosampler, and a Waters Lambda – Max model 480 LC spectrophotometer were used for analysis. A C18 column (3.9 mm i.d. × 30 mm) was eluted at 37°C with a mobile phase consisting of acetonitrile–phosphoric acid solution (1.3 mM, pH 3.02) with a ratio of 34:66 (v/v) at a flow rate of 1.5 ml/min and an injection volume of 20 µl (9). Concentration of KT was determined using the corresponding calibration curve constructed by the peak area. The cumulative amount of KT permeated through guinea pig skin or synthetic membranes was plotted as a function of time. The slope of the linear portion of the plot was derived by regression analysis and considered as the release rate or flux (µg/cm²/h).

Drug release data analysis

The amount of KT released into the receptor medium was determined either spectrophotometrically or using an HPLC method. For those samples analyzed by a spectrophotometer, the UV detector was set at the wavelength of 322 nm and the concentrations were determined from the calibration curve constructed with known amounts of drug under identical conditions. An HPLC method was

utilized when guinea pig skin was used as the membrane for permeation studies. Permeation curves were constructed by plotting the cumulative amount of drug penetrated through a unit area of the membrane versus time. The steady state flux was determined by regression analysis of the linear portion of the plot. The permeation study results are the average of six replicates. The time was also determined by extrapolating the linear portion of the cumulative amount – time curve to abscissa.

Viscosity measurements

The viscosity of lecithin/IPM/water systems depends on the amount of lecithin and water incorporated into the organogel. An attempt was therefore made to observe the effect of added water and lecithin on the viscosity of the system. Viscosity of each sample was measured, using both cylindrical and cone & plate viscometers at a controlled temperature of 25°C.

Statistical analysis

A one-way ANOVA was used to examine the statistical difference in the release profile between organogels of various compositions. Multiple comparisons within the formulations were also determined. Differences were assumed to be significant at p<0.05.

Results and Discussion

In general, the release rate of drug from organogel systems depends on the drug partition coefficient, drug solubility in the oil and aqueous phases, dispersed droplet size, phase volume ratio, viscosity and specific drug–excipient interactions. Small droplet size speeds up drug release and has superior shelf stability (10, 11). Delivery of a drug from an organogel is also directly proportional to the concentration of the drug. The intensity of drug partitioning into stratum corneum depends mainly on the lipophilicity of the drug used. Usually the drug present within the external phase is released on the surface of membrane. Then, the drug present in the internal phase partitions into the external phase to maintain the equilibrium. Therefore, drug transport may be controlled by either a partitioning process between the internal and external phases of the organogel, or between the external phase of the organogel and the skin. The thermodynamic driving force for release will reflect the relative activities of the drug in different phases (12).

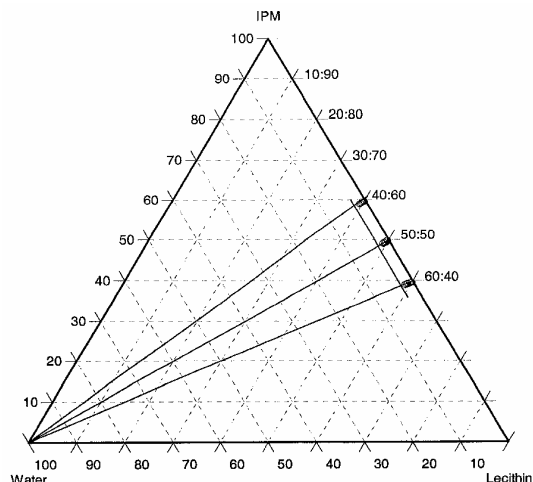


Figure 1. Phase diagram of a system containing lecithin, IPM and water, along with 6.5% w/w of KT in a gel formulation (new method).

The phase diagram resulting from the new method of preparation showed the existence of a smaller area of organogel compared to those from usual method (Figure 1). However, by using the new method of preparation, it is possible to incorporate a higher amount of drug into the organogel. In this case, for each of the organogel samples, up to 6.5% w/w of KT could be dissolved compared to 1% w/w in case of the usual method (7). It seems that the presence of lecithin in the organic solution brings about an increase in the solubility with respect to that observed in either water or IPM. This finding is in conformity with Williman and his coworkers who reported a considerably high solubility of several drugs in lecithin gel than in water or IPM (8).

Effect of membrane on KT release from organogels

Release studies were performed using both cellulose acetate and silicone elastomer in order to evaluate the influence of various artificial membranes on the release rate. A significant decrease ($p < 0.05$) in KT release was obtained when using silicone as a synthetic membrane. The release rate with cellulose acetate membrane was 3 times higher ($22.746 \mu\text{g}/\text{cm}^2/\text{h}$) than that observed with silicone membrane ($7.678 \mu\text{g}/\text{cm}^2/\text{h}$). This may be due to the differences in molecular weight cut-offs (MWCO) between cellulose acetate (3500 Dalton) and silicone elastomer membrane. As the drug molecular weight approaches the MWCO, diffusion through membranes slows dramatically. The effect of membrane on the release rate of KT from an organogel composed of 0.1% w/w water and 1%

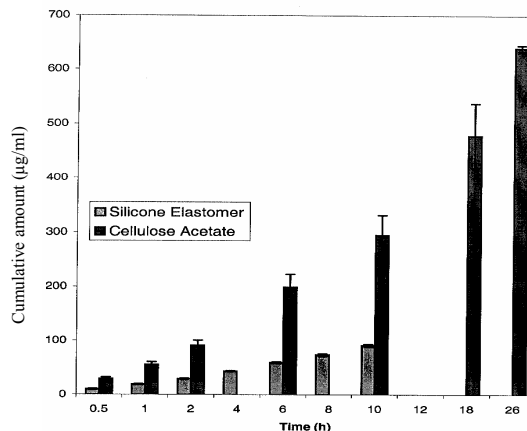


Figure 2. Effect of membrane on KT release from an organogel composed of 0.1% w/w water and 1% w/w of KT at a k_m of 40:60 ($n=6$).

w/w of KT at the k_m of 40:60 is shown in Figure 2. It should be noted that when silicone elastomer was used as the membrane, the experiment was run up to 10 h.

Effect of KT concentration on the release rate across cellulose acetate membrane

The effect of drug concentration on the release rate from an organogel composed of 0.1% w/w water and both 1% and 6.5% w/w of KT at the k_m of 40:60 was evaluated. A significant increase ($p < 0.05$) in the drug release was obtained in formulations containing 6.5% of KT compared to those containing 1% of the drug. The release rate of formulation composed of 6.5% KT was about 10 times ($223.12 \mu\text{g}/\text{cm}^2/\text{h}$) higher than that obtained from the formulation prepared with 1% of the drug ($22.746 \mu\text{g}/\text{cm}^2/\text{h}$). The effect of KT concentration on the release rate from organogels composed of 0.1% w/w water and both 1% and 6.5% w/w of KT at the k_m of 40:60 is shown in Figure 3.

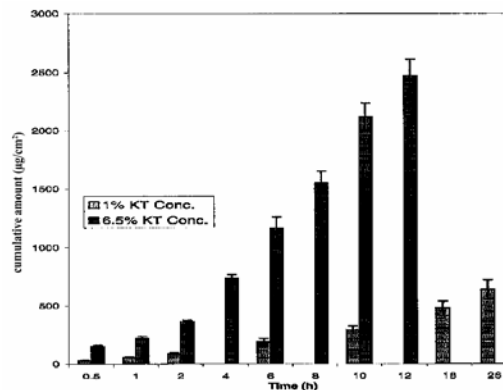


Figure 3. Effect of KT concentration on the release rate across cellulose acetate membrane from an organogel composed of 0.1% w/w water and both 1% and 6.5% w/w of KT at a k_m of 40:60 ($n = 6$).

It should be noted that when the formulation containing 6.5% w/w of KT was evaluated, experiment was run up to 12 h. The data revealed that there is a positive correlation between drug concentration and release rate due to the increase in thermodynamic activity. In this case, thermodynamic activity of the drug increases with concentration until it reaches the limiting value which is the value of the saturated solution. This finding is in agreement with the results of Santoyo and his coworkers who reported a direct relationship between piroxicam concentration and its release rate from propylene glycol gel (13). Henmi and his coworkers also reported that the release rate of indomethacin from an oily gel formed by hydrogenated soybean phospholipids was proportional to drug concentration in the vehicle (14).

Similar results were obtained using guinea pig skin with the same formulation. There was a significant ($p < 0.05$) increase in KT release from organogels containing 6.5% w/w of KT compared to those with 1% w/w drug. The permeation profile of KT through guinea pig skin from an organogel composed of 0.1% w/w water and 6.5% w/w of KT at a k_m of 40:60 is shown in Figure 4.

Effect of lecithin concentration on the release rate across cellulose acetate membrane

The effect of lecithin concentration on the release rate of KT from organogels composed of 0.25% w/w water and 6.5% w/w of KT at k_m s of 40:60, 50:50 and 60:40 was evaluated. A significant decrease ($p < 0.05$) in KT release was obtained as the lecithin concentration was increased from 40 to 50 and then 60% w/w in formulation. The release rates of KT were 229.27, 104.15 and 84.987 $\mu\text{g}/\text{cm}^2/\text{h}$ for formulations composed of 40, 50 and 60% lecithin, respectively. This effect may be due to a decreased

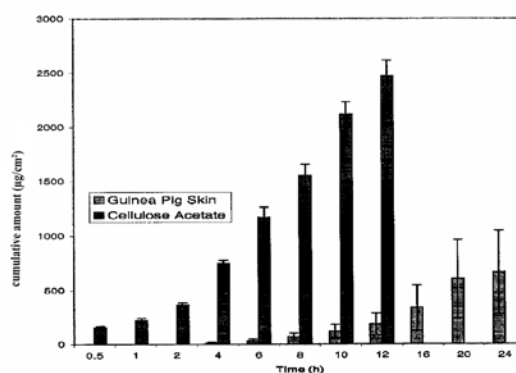


Figure 4. Comparison of the effect of cellulose acetate membrane with guinea pig skin on release rate from an organogel composed of 0.1% w/w water and both 1% and 6.5% w/w of KT at a k_m of 40:60 ($n = 6$).

thermodynamic activity of drug in the organogel at a higher concentration of lecithin. At higher lecithin concentrations, there is more extensive entanglement of long cylindrical micelles with each other, forming a network-like structure with a very high viscosity. The entrapment of the drug within this network lowers the amount of free drug available for release, causing a decrease in the release rate of KT across the membrane (15). The effect of lecithin concentration on the release rate from organogels composed of 0.25% w/w water and 6.5% w/w of KT at k_m s of 40:60, 50:50 and 60:40 is depicted in Figure 5.

Effect of water concentration on the release rate across cellulose acetate membrane

The effect of water concentration on the release rate of KT from different formulations containing 6.5% w/w of KT was also determined. A significant decrease ($p < 0.05$) in release rate from an organogel composed of 0.5% w/w water compared to those containing higher amounts of water was observed. A significant decrease ($p < 0.05$) in release rate of KT from organogels containing 0.25% w/w of water at k_m s of 50:50 and 60:40 compared to those composed of higher amounts of water was also obtained. The fluxes for systems prepared with k_m s of 40:60, 50:50 and 60:40 were calculated as 180.39, 104.15 and 84.987 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively (data is not presented graphically).

The data revealed that increasing the water content of organogel (in the system with a k_m of 40:60) from 0.1% to 0.25% w/w and then 0.5% w/w resulted in a decrease in KT release. With increasing the amount of water present within the system, initially spherical reverse micelles transform into cylindrical micelles and then into long tubular and flexible micelles with the ability to entangle and build up a three-dimensional

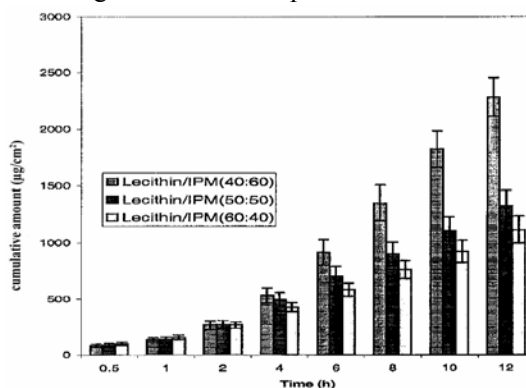


Figure 5. Effect of lecithin concentration on the release rate across cellulose acetate membrane from organogels composed of 0.25% w/w water and 6.5% w/w of KT at k_m s of 40:60, 50:50 and 60:40 ($n = 6$).

network with a high viscosity. This network is responsible for the entrapment and unavailability of drug molecules for their release from the organogel system, causing a significant decrease in release rate in systems with 0.5% w/w of water. It seems that the increase in the amount of water from 0.5% to 0.6% w/w could make the additional water and therefore KT available within the system for partitioning into the membrane. A decrease in drug release rate at higher water concentrations (0.7% and 0.8%) was observed, which suggests that at these concentrations, the three dimensional network shrinks and the organogel region ends. The same result was found for k_{ms} of 50:50 and 60:40, except for the lowest release rate of KT, happened at 0.25% w/w of water content. This finding is confirmed by Osborne and his coworkers who reported a high dependency of the release of glucose as a model hydrophilic drug across human skin from microemulsions containing different concentrations of water (16). The effect of water concentration (0.1, 0.25, 0.5, 0.6, 0.7 and 0.8%) on the 12h cumulative release across cellulose acetate membrane from organogels composed of 6.5% w/w of KT at various k_{ms} is depicted in (Figure 6).

Rheological measurements

An increase in lecithin concentration produced a significant increase ($p < 0.05$) in the viscosity of the organogel. The average viscosities were 418.80 ± 114.15 , 951.76 ± 25.70 and 1832.70 ± 358.93 poise for organogels composed of 0.25% w/w of water and 6.5% w/w of KT at k_{ms} of 40:60, 50:50, and 60:40, respectively. It should be noted that as the viscosity of the organogels increased,

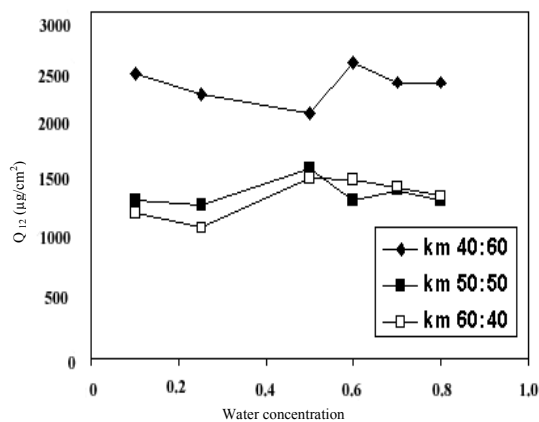


Figure 6. Effect of water concentrations (0.1, 0.25, 0.5, 0.6, 0.7 and 0.8%) on the 12 h cumulative release across cellulose acetate membrane from organogels composed of 6.5% w/w of KT at k_{ms} of 40:60, 50:50 and 60:40 (n=6).

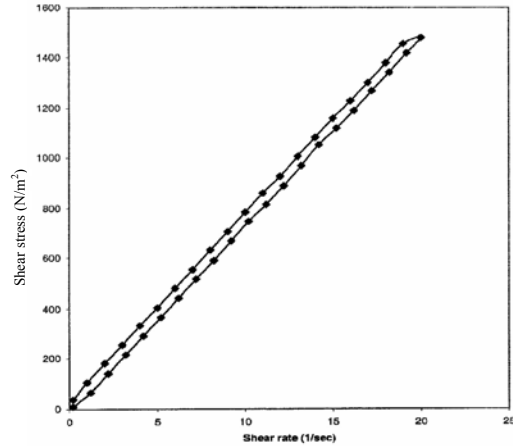


Figure 7. Rheogram showing the rheopectic behavior of an organogel composed of 0.25% w/w water and 6.5% w/w KT at a k_m of 40:60 (constructed by cone & plate viscometer) (n=6).

the release rate decreased. Viscosity measurements were determined by applying increasing and decreasing values of shear rate, in order to reveal any possible thixotropic behavior. All formulations surprisingly gave a slightly rheopectic rheogram. This is essentially the opposite of thixotropic behavior, in that the viscosity of the gel increased with an increase in the shear rate. A plot of shear rate versus shear stress was constructed as the shear rate was increased up to a certain value and then immediately decreased to the starting point. As shown in Figure 7, the up and down curves do not coincide and a hysteresis loop is formed due to the increase in viscosity of the organogel with increasing shearing time. It was found that there was a significant increase ($p < 0.05$) in viscosity in the presence of 0.5% w/w of water (k_m of 40:60) compared to the other concentrations (Figure 8).

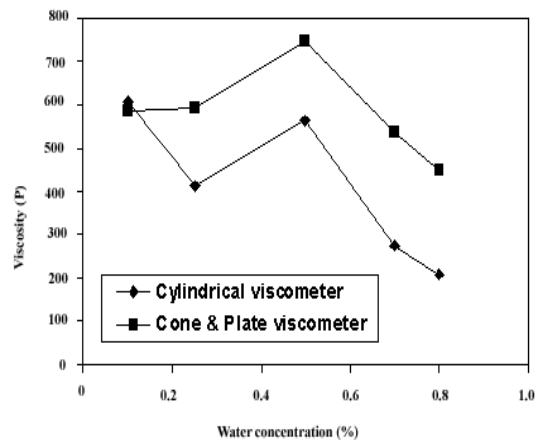


Figure 8. Effect of water concentration on the viscosity of an organogel containing 6.5% w/w of KT at k_m of 40:60 (n=6).

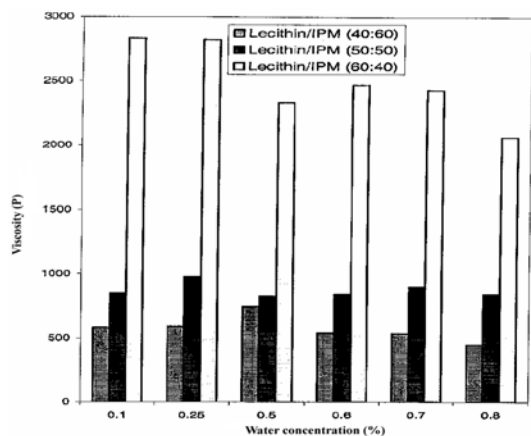


Figure 9. Viscosity of organogel samples with different compositions, measured by cone and plate viscometer (n=6).

This result explained the significant decrease in the release rate of KT from this formulation. Therefore, at this specific concentration of water, long tubular micelles could be entangled and form a three dimensional network with a very high viscosity which affects the release rate of system. As KT release decreased with an increase in lecithin content, an inverse correlation existed between the release rate and the gel viscosity values. These data are confirmed by Santoyo and his coworkers who reported that drug release rate is inversely related to the viscosity of the continuous phase (13). Viscosity profile of the organogel samples with various compositions is indicated in (Figure 9). Systems with k_m of 60:40 showed the highest viscosity compared to the corresponding systems prepared with the other k_m s.

Conclusion

Among all the different microemulsion-based gels investigated, the formulation containing 0.6% w/w of water and 6.5% w/w of KT with a lecithin/IPM weight ratio of 40:60 showed the highest release profile. Our study demonstrates that lecithin organogels are promising vehicles for topical application of KT. We revealed that due to the skin penetration enhancing potential of lecithin organogels, these vehicles could promote transdermal absorption of KT.

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