RESEARCH ARTICLE

A Nanotechnological Approach for Enhancing the Topical Drug Delivery by Newly Developed Liquid Crystal Formulations

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ABSTRACT

Transdermal drug administration is a great substitute to oral medication administration. Inadequate penetration of active pharmaceuticals via the skin, on the other hand, is a common phenomenon. As a result, we investigated the ability of liquid crystal (LC) topical formulations to improve skin penetration in the current study. LC-forming lipids are a significant class of biocompatible amphiphiles with applications in cosmetic, dietary, and medicinal technologies. Just a few experiments have looked into how the concentration of LC-forming lipids affects the ability of drugs to reach the skin following topical application. We initially prepared LC formulations of p-aminobenzoic acid (PABA) as a hydrophilic drug mode (The homogeneity and viscosity of the prepared formulations is determined). Additionally, we used a Zetasizer to determine the zeta potential and particle size of LC formulations. The dialysis procedure was used to determine the liberation of drugs from LC formulations. In-vitro skin penetration tests were conducted to decide if LC formulations could increase skin penetration and concentration. By understanding the influence of the LC-forming lipid concentration used in the LC preparation, researchers might establish LC formulation methods that increase drug skin penetration.

Keywords: LC-forming lipids, Liquid crystal, Penetration of skin, Topical drugs.

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INTRODUCTION

Transdermal administration allows patients to easily and painlessly self-administer drugs. It reduces the need for repeated dosing and the rises and drops of plasma levels associated with oral administration and injections, enabling the reliable delivery of medications with a limited half-life. However, transdermal drug delivery often encounters difficulties as a result of inadequate or absent penetration of the drug product via the skin. The stratum corneum, or the outermost layer of tissue, serves as the main obstacle to fluid penetration in and out of the skin. As a consequence, it is important to address the stratum corneum barrier while developing transdermal drug delivery systems. Chemical processes such as penetration enhancers[1] and physical methods such as iontophoresis,[2] phonophoresis,[3] and electroporation[4,5] have been tested to improve the skin penetration of various medications. Physical approaches frequently have a number of disadvantages, such as the need for advanced implementation apparatus and a high cost. As a result, this analysis aimed to put to the test a chemical or formulation solution capable of increasing skin penetration by using a LC formulation.

Because of their exceptional structural flexibility and effectiveness for pharmaceutical applications, LC formulations such as cubosomes and hexosomes have gained increased attention in recent years. LC-forming lipids are a significant class of biocompatible amphiphiles with applications in cosmetics, dietary supplements, and pharmaceutical technologies.[6] It has been shown that the LC phases can include biologically active molecules, including enzymes, vitamins, and some proteins.[7-9] This capacity broadens the range of therapeutic uses.

Recent experiments have shown the possibility of designing LC formulations for use as a transdermal drug delivery mechanism.[10-12] However, methods for developing and refining

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transdermal LC formulations have received little attention. Additionally, relatively few experiments have examined the impact of LC-forming lipids concentration on the capacity of drugs to penetrate the skin after topical application. Thus, the objective of this report was to establish strategies for developing and optimizing transdermal LC formulations using a variety of different LC-forming lipid concentrations.

This study aimed to see how a well-known LC-forming lipid, glycerol monooleate (GMO), affected skin penetration in Figure 1. Previous studies have shown the effectiveness of GMO in the transdermal drug delivery system,[11,13] No studies have investigated the effect of its concentration on the skin penetration-enhancing ability of drugs to our knowledge.

As a hydrophilic drug model, we initially prepared LC comprising PABA. We tested the homogeneity and viscosity of the prepared substance. A Zetasizer was also used to assess the zeta potential and particle size of LC formulations. The sample release from LC was evaluated by the dialysis release method. In vitro, skin penetration studies were conducted to determine the potential of LC formulations to enhance skin penetration and concentration.

MATERIALS AND METHODS

Materials

GMO purity ≥97%, PABA, and a surfactant named Pluronic® F127 Sigma-Aldrich provided them all. Some of the reagents were used as-is due to their excellent accuracy or HPLC designation.

Composition of Liquid Crystal (LC)

The topical LC used in this analysis are mentioned in Table 1. To create these formulas, the concentration of GMO absorbed in PABA was differed (before the use of GMO it was melted at 70°C). The mixture was spread by homogenizer As discussed later in the results section, the TP10 and TP50 formulations were refused.

Calculations of Zeta Ptential and Particle Size

A dynamic light scattering Zetasizer was used to determine the zeta potential and particle size of LC. Prior to measurement, LC compounds were submerged in H_2O and mixed with a vortex mixer. Particle size study of the LC, the model's "Size" parameter was changed to "Zeta", Each zeta potential and particle size value was repeated three times.

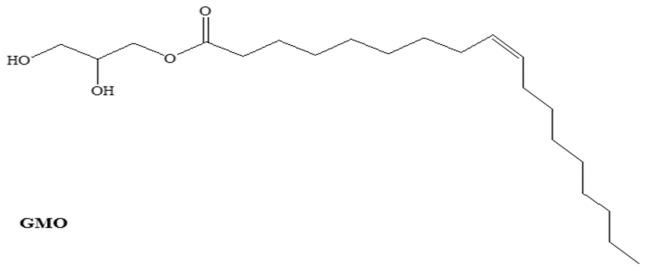

Figure 1: Chemical structures of glyceryl monooleate (GMO).

Viscosity Measurement

By viscometer (Alpha Analytical, MCR 301), which provided a sensitive viscosity calculation (relative error of 1% across the range 0.3–10,000 mPas.)

Experiment with Release

A dialysis membrane dialysis (molecular weight cutoff; 10 kDa, Thermo Fisher Scientific) was performed in a vertical-type diffusion cell (effective diffusion area: 0.95 cm^2) with the receiver chamber maintained at 32°C. The receiver chamber was filled with phosphate-buffered saline (PBS; pH 7.4). To initiate the release assay, PABA solution (1.0 mL) or its LC mixture (1.0 mL) was added to the donor cell. To maintain a steady rate, a 500-L aliquot was removed from the receiver chamber and replaced with the same volume of PBS. The volume of PABA produced was calculated using high performance liquid chromatography (HPLC) (Shimadzu Ltd., Japan). The average proportion of compound release against time was plotted using Higuchi's plot.[14]

Animals

Male hairless rats were allowed unlimited food and water, placed in (25°C) rooms with a 12-hour light-dark cycle (07:00–1900 hours).

HPLC Operating Conditions

- The in vitro or in vivo test sample (50 L) was mixed with the same volume of acetonitrile (to precipitate plasma proteins)
- centrifuged for 5 minutes at 4ºC with methylparaben (10 g/mL) as an internal control.
- The collected supernatant (20 L) was placed in a Shimadzu HPLC (Japan) unit, which included a machine controller (CBM-20A), a pump (LC-20AD), an autosampler (SIL-20AC), a column oven (CTO-20A), a UV detector (SPD-M20A), and analysis equipment (LC Solution).
- The column was a GL Sciences Inc. ODS-3 (5 m, 4.6 250 mm) (Nihon Waters K.K., Japan) that was held at 40ºC. 8: 52 (0–4 minutes), 35: 65 (4–14 minutes), and 8: 92 (14–20

Table 1: Composition of topical LC nanoparticle

Formulation code	*Composition of topical LC nanoparticle formulations*
TP10*	PABA solution containing 5% Pluronic® F127 and 10% GMO
TP20	PABA solution containing 5% Pluronic® F127 and 20% GMO
TP30	PABA solution containing 5% Pluronic® F127 and 30% GMO
TP40	PABA solution containing 5% Pluronic® F127 and 40% GMO
TP50*	PABA solution containing 5% Pluronic® F127 and 50% GMO

The concentration of PABA was 10 mM
Formulation code: T = Topical formulation; P = PABA; Number = Percentage of GMO.
*: Rejected topical formulations.

minutes) acetonitrile: 0.1% phosphoric acid Increase the flow rate to 1.0 mL/min.
- PABA has been reported at the UV 280 nm wavelength.

Experiment with *In Vitro* Skin Penetration

A hairless rat skin from the abdomen was excised under anesthesia using a pentobarbital (50 mg/kg) i.p. injection. Extra fat was collected from skin samples placed vertically in diffusion cells with the epidermis approaching the donor compartment. As with the release trial, the receiver chamber was filled with PBS and maintained at 32°C. These skin penetration tests were performed after a 60 minutes hydration period with PBS. To initiate the in vitro skin penetration assay. The donor cell was treated with a PABA solution (1.0 mL) or LC-formulation (1.0 mL). A 500-L aliquot was withdrawn from the receiver chamber and substituted with the same quantity of PBS to keep the volume constant.

Determination of the Skin's Concentration

- The skin extract (0.1 g) was cut with scissors and homogenized for 5 minutes at 4°C in a homogenizer with water (0.9 mL) (Polytron PT-MR 3000; Kinematica Inc., Littau, Switzerland).
- After diluting the homogenate 1:1 with acetonitrile, it was fixed for 15 minutes.
- After 5 minutes of centrifugation at 4°C, the supernatant (50 L) was combined with the same amount of acetonitrile comprising methylparaben (10 g/mL) and centrifuged for a further 5 minutes at 4°C.
- The collected supernatant (20 L) was inserted into an HPLC unit, and the same conditions as previously mentioned were used for the calculation.

RESULTS

Homogeneity and Visibility

Visible appearance and homogeneity of the prepared LC nanoparticle was determined. The resulting formulations were an invisible white mixture lacking clear aggregates (TP20, TP30, TP40), while refused formulations exhibited a non-uniform dispersion (TP50). With a large proportion of LC-forming lipids (> 40%), Since non-uniform aggregate mixtures were found, no further analysis with TP50 was carried out.

Determination of Particle Size and Zeta Potential

Figure 2 illustrates the zeta potential and particle size values of topical LC nanoparticles. The results revealed that particle size decreased when the GMO content rose, and the negative value of zeta potential becomes greater. Low-GMO formulations, such as TP10, maintained a negative surface charge and a large particle size. Since the low surface charge means that the 10% GMO structures would be unstable, no further research was carried out.

Measurement of Viscosity

A viscometer was used to determine the viscosity of LC formulations. The obtained viscosity values are shown in Figure 3. The dosage of GMO used in the formulation has a significant effect on these values. As the GMO content was improved, the viscosity of the LC formulations improved. Before being used in formulation preparation, GMOs must be melted at 70°C.

Properties of LC Nanoparticle Formulations for Drug Release

A vertical diffusion cell was used for the release experiment. Figure 4 illustrates the PABA release profiles from their TP20, TP30, and TP40 LC nanoparticle formulations. Higuchi's rule[14] was used to create all of the profiles. PABA levels released from the TP20, TP30, and TP40 formulations were 20.5 ± 1.9, 12.5 ± 1.7, and 7.5 ± 1.6, respectively, in comparison to the original dosing. The obtained study revealed unequivocally that the volume of released PABA decreased as the GMO concentration increased.

Penetrability of PABA into the skin through LC Nanoparticle Formulations

Figure 5 indicates the impact of TP20, TP30, and TP40 LC nanoparticle formulations on the time course of cumulative PABA penetration through hairless rat intact skin. PABA skin penetration was greatly increased after the application of the TP20, TP30, and TP40 formulations relative to drug solution permeation in H_2O. The enhancement skin penetration rate for

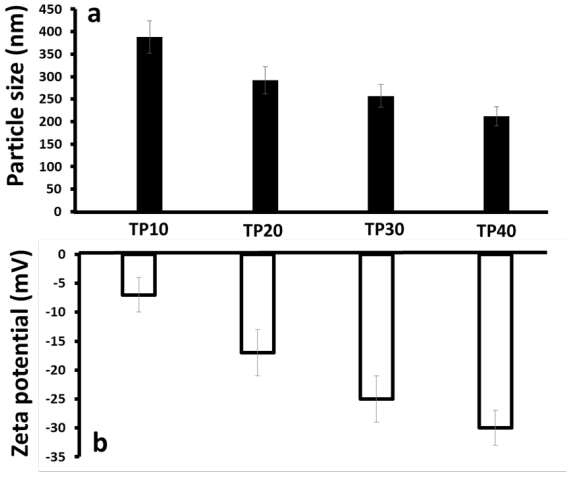

Figure 2: LC nanoparticle formulation particle scale (a) and zeta potential (b). Each point represents the mean ± SE of three experiments.

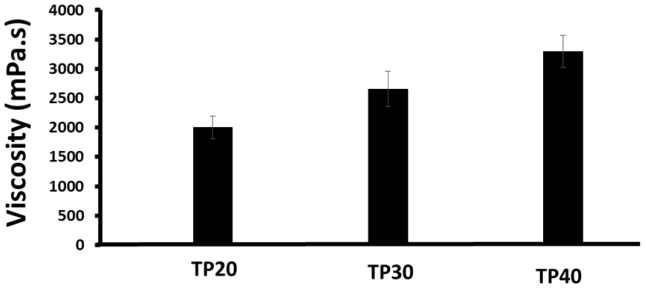

Figure 3: LC formulations' apparent viscosity. Each point represents the mean ± SE of three experiments.

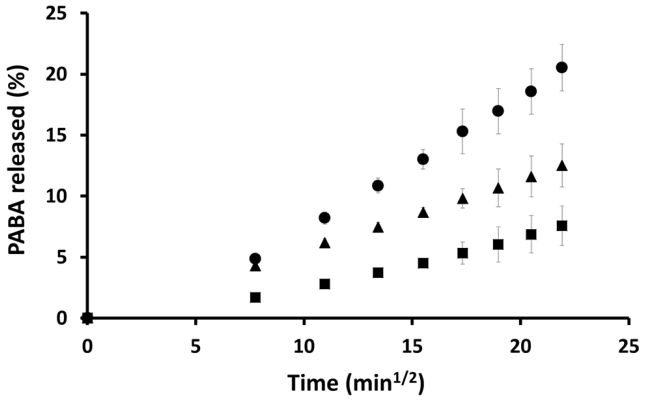

Figure 4: PABA release profiles are as follows: (●), TP20 formulation; (▲), TP30 formulation; and (■), TP40 formulation. Each point is the mean ±S.E. of three experiments.

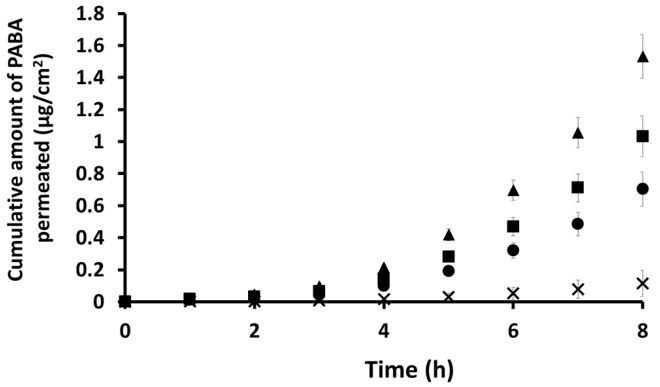

Figure 5: The effect of LC nanoparticle formulations on the cumulative amount of PABA over time. (●), TP20 formulation; (▲), TP30 formulation; (■), TP40 formulation. Each point represents the mean± SE of three experiments.

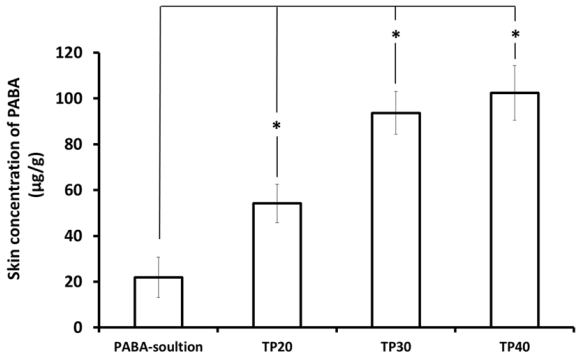

Figure 6: PABA accumulation in the skin following 8 hours of treatment of TP20, TP30, and TP40 formulations. Each column reflects the mean ± SE of three studies. *: Considerably different from the epidermis to the dermis (Student's t-test).

the TP20, TP30, and TP40 formulations were 7, 15, 4.2, and 10, respectively. These results suggest unequivocally that TP20, TP30, and TP40 are exciting modern topical formulations.

Skin Concentration of PABA

The concentration of PABA in the skin after application of the TP20, TP30, and TP40 LC nanoparticle formulations are visualized in Figure 6. PABA skin concentrations were significantly increased following the application of the TP20, TP30, and TP40 formulations relative to the medication solution. After the application of TP20, TP30, and TP40 formulations, the skin concentrations of PABA were 54 ± 8, 93 ± 9, and 102 ± 12 g/g, respectively.

DISCUSSION

Although LC nanoparticle formulations have earned considerable attention due to their excellent effectiveness as drug carriers,[15] there is still considerable disagreement among researchers regarding the concentration impact of LC-forming lipid on drug penetration through the skin. As a result, recent research has concentrated on strategies for optimizing the effectiveness of topical LC formulations.

The GMO is one of the most extensively researched LC-forming lipids for topical use and is generally regarded as the gold standard for the manufacture of LC formulations.[9] We first examined some topical LC formulations (Table 1). Our results demonstrate that it is not advisable to prepare LC oral dispersions with a GMO concentration of 50% (TP50) or greater, as these formulations are prone to being non-uniform aggregate mixtures. These results will aid future researchers in determining the optimal GMO concentration for topical LC formulations. PABA (MW = 137.14, ClogP = 0.98) was chosen as the hydrophilic model medication (MW = 137.14, ClogP = 0.98) since it is a cofactor for the vitamin B complex in a large variety of foods.[16] Due to its strong UVB absorbance and ability to defend against skin cancer, PABA is often used as an ingredient in sunscreen. The capacity of PABA to scavenge reactive oxygen species has been associated with protection against UV and free radical damage.[17] Due to its antioxidant function, it is also available as a dietary supplement (vitamin B10).[18] In the United States of America, the potassium salt of PABA is used to treat skin conditions such as scleroderma, dermatomyositis, and Peyronie's disease.[19-21] PABA has been designated as a significant component in cosmeceuticals, nutritional supplements, and skin condition medications as a product of these studies.

Our results indicate that as the GMO concentration increases, the particle size decreases and the zeta potential increases (Figure 2), assuming that the formulation's LC particle length, negative surface charges, and density improve as the GMO concentration increases. The zeta potential is a significant parameter for the stabilization and biodistribution of formulations.[22,23] Because of the low surface charge and low stability of TP10 formulations, no further work was done with these formulations. High surface charges, in general, induce electrical repulsion between particles, keeping them from aggregating. The existence of free oleic acid in the lipid phase will lead to the particles' negative charge, which explains the negative zeta potential values found in LC formulations. Furthermore, hydroxyl ion preferential adsorption at the lipid-water interface may explain the negative charge.[22]

Besides that, changes in the GMO dose used in the formulation directly impact the viscosity values (Figure 3).

These studies undeniably showed that growing the GMO content increased the viscosity of LC formulations. As a result of the drug release effects, differences in GMO concentrations can influence the diffusivity and rate of release of the drug entrapped in its formulations (Figure 4). Hydrophilic drugs are normally located at the lipid's polar head or in the water channels (water phase), while lipophilic drugs are found inside the lipid bilayer and amphiphilic drugs at the interface (lipid phase). The concentration of GMO, according to the collected release profiles, is the critical factor influencing drug diffusion in LC-formulations.

The GMO concentration has an important impact on the skin penetration results in vitro. PABA skin penetration was substantially increased in 20, 30, and 40% GMO (TP20, TP30, T40) with enhancement ratios of 7, 15, and 10, respectively (Figure 5). These findings imply that a low concentration of GMO, such as 20% (TP20), could decrease LC particles. As a result, it is important to overcome the barrier structure of the stratum corneum. On the other hand, a high GMO concentration, such as 40% (TP40), blocked the release of the drug from the formulation. As a result, significant rises in PABA skin concentrations were reported after applying the TP20, TP30, and TP40 formulations in comparison to the drug solution (Figure 6). Because of the high viscosity of TP40, it was challenging to wash out during the in vitro assay, which may clarify why TP40 had a higher skin concentration than TP30.

The exact mechanism by which LC systems increase transdermal drug penetration is unknown.[24,25] More study is required to understand how LC formulations improve skin penetration.

CONCLUSION

According to available data, LC formulations have the potential to enhance transdermal drug penetration. Our research suggests that the concentrations of LC-forming lipids are a critical factor that researchers in this area could acknowledge to optimize the effectiveness of LC formulations in various pharmaceutical applications. By understanding the influence of the LC-forming lipid concentration used in the LC preparation, researchers might establish LC methods that increase drug skin penetration.

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