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Development and Evaluation of Biocompatible Topical Petrolatum-liquid Crystal Formulations with Enhanced Skin Permeation Properties

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Abstract: Transdermal administration represents a major advancement over traditional pharmaceutical dosing methods. However, a frequent issue is inadequate penetration of the active medicinal component through the skin. As a result, in the current research, we assessed the utility of newly developed petrolatum-liquid crystal (LC) ointment formulations and characterized their biocompatibility and function in the transdermal drug delivery system. To begin, we made petrolatum-LC formulations using *p*-aminobenzoic acid (PABA) as a hydrophilic model molecule. The viscosity, small-angle X-ray scattering (SAXS), particle diameters, and z-potential were measured to assess the physicochemical properties of the formulations. A dialysis release technique was used to evaluate medication release from petrolatum-LC formulations. *In vitro* testing was performed to determine the potential to enhance skin penetration. The biocompatibility of the produced formulations was further tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and single-cell gel electrophoresis. According to the results, the novel petrolatum-LC formulations are biocompatible and effective in forming hexosomes. PABA skin penetration was significantly enhanced by the new petrolatum-LC formulations. According to this study, petroleum-LC formulations are more efficient than commercial petrolatum in terms of skin permeability improvement and PABA skin concentration.

Key words: petrolatum, liquid crystals, formulations, skin penetration, *p*-aminobenzoic acid

1 Introduction

The first line of defence is the skin, preventing foreign things from entering the human body system and preventing water loss from the body via evaporation[1, 2]. The stratum corneum is a complex outermost layer of the skin that acts as the initial physical barrier to any external elements, including medicines, entering the body, which also makes an obstacle in the drug penetration into the body[2]. However, owing to its simple and convenient accessibility, the skin remains an appealing route of drug administration, non-invasive, and therapeutically effective way for treating systemic and localized diseases. Two important factors characterize successful topical formulations which are the need to be thermodynamically stable and high drug partition across skin layers[3]. The ointment is one of the semi-solid pharmaceutical dosage forms that exist as a single-phase whether a hydrophilic or hydrophobic system with sufficient consistency, it allows active pharmaceutical ingredients to be dissolved and dispersed homogeneously[4]. Ointment dosage forms are meant to be applied topically

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as it provides many advantages than current topical dosage forms such as patches, lotions, and creams that are associated with several limitations.

One of the most significant disadvantages of ointments is their inability to penetrate the stratum corneum, the skin's outermost layer. If suitable ointment formulations cannot be established, the development of topical medicine delivery devices will be hindered. As a result, the choice of ointment bases required extensive consideration, as the degree of drug release varied across different types of bases, affecting the medication's efficacy[5, 6].

The formulations of new petrolatum-liquid crystals ointments are being investigated for their potential application in transdermal medication delivery systems, as well as their biocompatibility and function.

Petrolatum, a long-chain aliphatic hydrocarbon component, is found in the majority of ointment bases. The majority of white petrolatum is made up of naphthene, *iso*-paraffin and *n*-paraffin[7]. Petroleum jelly is often utilized for transdermal medication delivery because to its occlusive layer on the stratum cornea's uppermost surface. Drug penetration through this layer of human skin, however, remains a challenge[8]. Thus, the purpose of this study was to mix lipid-forming liquid crystals (LC) with petrolatum to create a novel formulation that would allow for more medication penetration through the skin.

LC are crystalline forms composed of lipids that exhibit both crystal and liquid properties. In comparison to molecules in a liquid, which scatter randomly, those in a crystal are highly ordered. Glycerol monooleate (GMO) is a self-assembling amphiphilic molecule (Fig. 1) that forms a variety of crystalline structures, some of which have advantageous mechanical properties for drug administration. Even when a small amount of water is added, GMO generate oily reverse micelles[9–11]. As additional water is added to the mixture during the lamellar phase, a mucous-like structure forms. When 20% and 40% water is introduced across a broad temperature range, GMO transforms into cubic or hexagonal phases. When exposed to high water concentrations and surfactants, GMO and phytantriol (PHT) are examples of amphiphilic lipids that spontaneously form LC systems. In lyotropic LC, hexagonal, cubic, and lamellar phases are often seen[12–14]. Due to the highly structured inner structures of cubic and hexagonal phase systems, a slow-release matrix for active medicinal components with variable molecular sizes and polarity has been created[14–16].

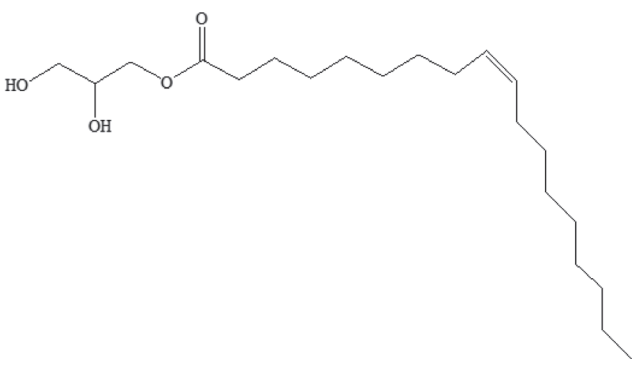

Fig. 1 Glyceryl monooleate (GMO) structure.

PABA also known as vitamin Bx was selected as a hydrophilic model compound, which is widely found in foods as a cofactor of the vitamin B complex[17]. PABA is often used as an antioxidant because of its ability to scavenge reactive oxygen species. It is also available as a health supplement (vitamin B10)[18]. The potassium salt of PABA is used as a prescription drug for the treatment of skin disorders such as scleroderma, dermatomyositis and Peyronie's disease[19–21]. Based on these findings, PABA is considered as an active element in cosmeceuticals, dietary supplements and skin disorder medicines. However, as one of the most difficult difficulties in dermatologic therapy, overcoming the stratum corneum barrier to cutaneously deliver hydrophilic medicines, PABA was chosen as a well-known hydrophilic and mal-absorbed model agent in this study.

Petrolatum-LC formulations containing PABA were first prepared. Viscosities, particle diameters, and z-potential were measured to determine the physicochemical properties of these formulations. Small-angle X-ray scattering (SAXS) was used to confirm the LC phase structures in the prepared mixtures. A dialysis release technique was used to evaluate the PABA release from petrolatum-LC formulations. *In vitro* studies have also shown that LC formulations improved skin penetration. The biocompatibility of the developed formulations was further evaluated using single-cell gel electrophoresis (comet assay) and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

2 Materials and Methods

2.1 Materials

Petrolatum, GMO with a normal purity of >97%, PABA, stearyl alcohol, propylene glycol and sodium lauryl glycol carboxylate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Human keratinocytes cells (HaCaT) were obtained from American Type Culture Collection (ATCC). Other reagents and solvents were of special grade or HPLC grade and used without further purification.

2.2 Preparation of petrolatum-LC formulations

Table 1 shows the petrolatum-LC formulations used in this study PABA was utilized to encapsulate GMO, which was melted at a temperature of 70℃ before use. These formulations were produced by changing the amount of genetically modified organisms in them. A homogenizer was employed to ensure that the mixture was distributed uni-

Table 1 Composition of petrolatum-LC formulations.

Ingredients (%)	WP	WP-GMO5*	WP-GMO10	WP-GMO20
White petrolatum	20	20	20	20
Stearyl alcohol	25	25	25	25
Propylene glycol	12	12	12	12
Sodium lauryl glycol	1	1	1	1
GMO	0	5	10	20
Purified water containing PABA	41	37	32	22
Total %	100	100	100	100

The concentration of PABA was 10 mM in all prepared formulations.
Formulation code: WP = white petrolatum; GMO = glyceryl monooleate; Number = Percentage of GMO.
*: Rejected topical formulations.

formly.

2.3 Measurement of zeta potential and particle size

Petrolatum-LC formulation zeta potential and particle size were measured using a dynamic light scattering Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). Before analyzing the Petrolatum-LC samples, a vortex mixer was used to shake and dilute them. After determining the particle size of the formulations using particle size analysis, the zeta potential of the petrolatum-LC formulations was examined. The measurements were repeated 3 times.

2.4 Viscosity measurement

The stearyl alcohol. white petrolatum and GMO are melted together at about 75℃. The other agents, dissolved in the purified water, are added with stirring until the mixture congeals. Sodium lauryl sulfate is the emulsifying agent, with the stearyl alcohol and white petrolatum constituting the oleaginous phase of the water in oil ointment. The viscosity of petrolatum-LC mixtures was measured using a viscometer (Alpha Analytical, MCR 301) that allowed a sensitive determination of viscosity within a range of 0.3–15,000 mPa・s with an accuracy of 1% relative error.

2.5 SAXS measurement

A Nano-Viewer equipped with a Pilatus 100K/RL 2D detector (Anton Paar, Austria) was used to conduct SAXS measurements on petrolatum-LC mixtures. Cu K radiation with a wavelength of 1.54 and a voltage and current of 110 mA and 45 kV served as the X-ray source. It was decided that a 375 mm sample-to-detector distance would be used. Each sample was in a vacuum-resistant glass capillary cell and subjected to 25℃ for 10 minutes. SAXS pattern was plotted against the scattering vector length was plotted, with the scattering angle as the y-axis.

2.6 Release experiment

A dialysis membrane from Thermo Fisher Scientific with a molecular weight cutoff of 10 kDa was employed in a vertical diffusion cell with an effective diffusion area of 0.95 cm^2, and the receiver chamber was kept at 32℃. Phosphate-buffered saline was injected into the receiver chamber (PBS; pH 7.4). To initiate the release experiment, the donor cell was given 1.0 mL of the Petrolatum-LC formulation. PBS was added to the receiver chamber to maintain the same volume as the withdrawn aliquot (500 L). The amount of PABA emitted was determined using an HPLC (Shimadzu Ltd., Japan). The cumulative % PABA release was plotted against the square root of time (Higuchi model)[22].

2.7 Animals

Male hairless rats (8 weeks old) were housed in temperature-controlled rooms (25 ± 2℃) with a 12 h light-dark cycle (07:00–19:00 h). The rats were allowed free access to food and tap water. The animal experiment protocol was approved by the Animal Care and Use Committee of Wasit University (Kut, Wasit, Iraq).

2.8 HPLC conditions

When an *in vivo* or *in vitro* study sample (5 μL) containing methylparaben (10 μg/mL) was used as an internal standard, there were no significant differences between the experimental and control groups. The generated supernatant (20 μL) was analyzed using an HPLC system (Shimadzu, Japan). The HPLC system was comprised of a system controller (CBM-20A), a pump (LC-20AD), an autosampler (SIL-20AC), a column oven (CTO-20A), and a UV detector (SPD-M20A) (LC Solution). At a temperature of 40℃, the column utilized in the experiment was an ODS-3 from GL Sciences Inc. (Nihon Waters, K.K., Japan). The mobile phase was acetonitrile, and the reaction durations were as follows: 8: 52 (0–4 min), 35: 65 (4–14 min), and lastly, 8: 92

(14–20 min) with 0.1% phosphoric acid. Reduced the flow rate to 1.0 mL/min. PABA was found in the ultraviolet at a wavelength of 280 nm.

2.9 *In vitro* skin permeation study

Hairless rat skin was removed from the abdomen while the patient was sedated with a 50 mg/kg i.p. dose of pentobarbital as a sedative. A little amount of fat was removed from the skin samples, and the epidermis-facing side was placed in vertical diffusion cells facing the donor compartment. The receiver chamber was treated with the same PBS solution as the release experiment and kept at a temperature of 32℃. The tests on skin permeability were performed 60 minutes after hydration with PBS. To begin the *in vitro* skin penetration experiment, 1.0 mL of Petrolatum-LC formulation was administered to the donor cell. PBS was supplied to the receiver chamber to maintain the same volume as an aliquot (500 L) that had been removed from it.

2.10 MTT assay

Petrolatum-LC compositions were put to the test using HaCaT to determine their safety. A cell density of 8×10^3 was used in 96-well plates, and cells were incubated overnight at 37℃ in a humid environment composed of 95% air and 5% CO_2. Dilutions of the Petrolatum-LC formulations to 0, 5, and 10 mg/mL in Dulbecco's Modified Eagle Medium (DMEM) were introduced to the well. For 12 and 24 hours, the cells were cultured with different formulations of Petrolatum-LC. The wells were then filled with DMEM without fetal bovine serum (FBS) and incubated at 37℃ for four hours with 0.5 mg/mL MTT Solubilizing formazan was accomplished using dimethyl sulfoxide (DMSO) after the MTT medium had been withdrawn from the wells, and the 96-well plates were vibrated for 15 minutes while being shielded from light. The absorbance at the visible light wavelength of 490 nm was measured using a microplate reader (Palkin Elmer, USA). To calculate the relative cell viability, the absorbance of the test wells was divided by the absorbance of the control wells.

2.11 Analysis of DNA damage (Comet assay)

At a density of 4×10^5 cells/mL culture media, cells were plated in the multiwell system. A positive control, 50 μM H_2O_2, was added to the cells after they had grown for 24 hours before they were treated to various doses of petrolatum-LC formulations (0.1-1 mg/mL). Incubation for 3 minutes followed by addition of 1 mL of DMEM + 10% FBS medium followed by washing with PBS and addition of 300 μL of trypsin. Pipetting was used to separate the cells. The cell suspension was put in Eppendorf tubes and spun at 1000 rpm for three minutes to get rid of any remaining debris. The cells were resuspended in 100 μL of PBS after the supernatant had been removed and kept on ice. Single-

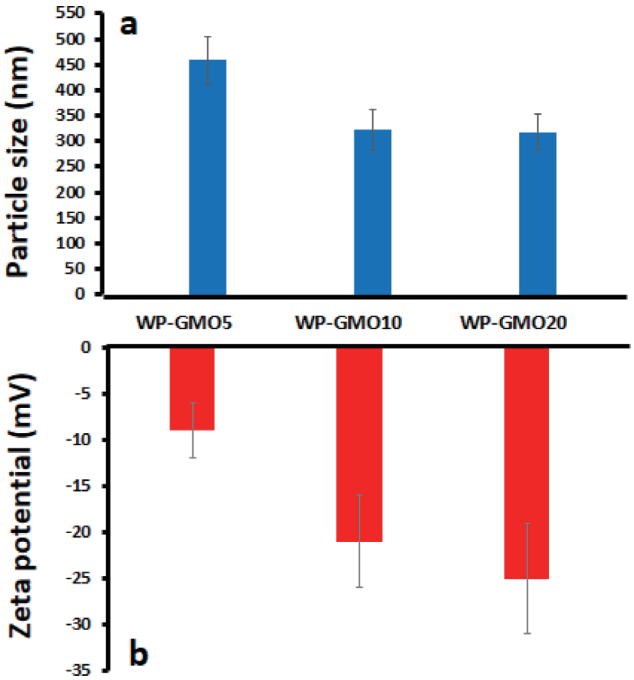

Fig. 2 Particle size (a) and zeta potential (b) of WP-LC formulations. Each point represents the mean ± SE of three experiments.

cell gel electrophoresis (SCGE or comet assay) was used to assess the amount of DNA damage. Completely frozen slides were coated in three layers: normal-melting-point agarose (NMA) as the first layer, then cell suspension, and low-melting-point agarose LMA as the second layer, followed by LMA without cell as the third layer. The last layer was 0.6% LMA without cell. Each slide was put in a horizontal electrophoresis tank after solidification at 4℃ and then submerged for 1 hour in lysing buffer comprising 2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris, and 1% Triton-X having pH 10 at that temperature. Slides were kept in the solution for 20 minutes to enable DNA unwinding and expression of alkali labile damage before electrophoresis, and then the tank was filled with newly produced electrophoresis solution of 300 mM NaOH and 1 mM Na2EDTA having pH 13. A 20-minute electrophoresis procedure at 4℃ with a voltage and current of 25 V and 0.3 A was then carried out. For fluorescence microscopy, the electrophoresis-stained slides were placed in humidified airtight containers, neutralized in neutralization buffer, stained with ethidium bromide, and viewed under a humidified, fluorescent microscope. A visual scale was used to classify the extent of DNA damage into five categories based on the quantity of DNA in the tail[24]. Harm levels range from 0 to 4, with 0 inflicting no damage and 4 inflicting heavy damage. Damage levels range from 5-95%, with 95% being the most damaging.

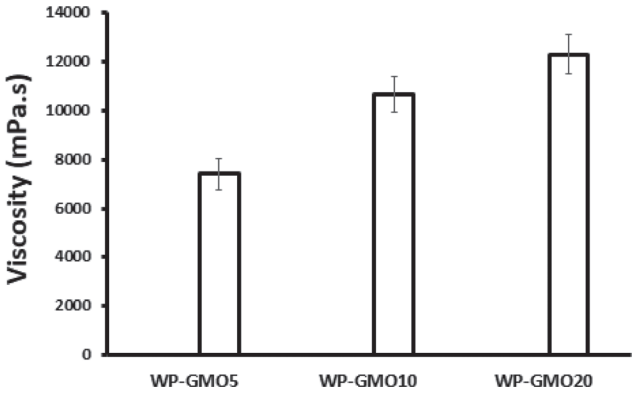

Fig. 3 WP-LC formulations' apparent viscosity. Each point represents the mean ± SE of three experiments.

3 Results

3.1 Determination of particle size and zeta potential

Figure 2 illustrates the z-potential and particle size of topical WP-LC formulations. A non-uniform mixture, phase separation and low z-potential values were observed with WP-GMO5 formulation. Moreover, this formulation was inconvenient for topical application. Because the low surface charge (z-potential) indicated low stability of the WP-GMO5, no further work was carried out with this formulation. These results could be related to the low percentage of GMO used in this formulation. When the percentage of GMO increased to 10 and 20% (WP-GMO10, WP-GMO20) the ointment formulations were tend to be uniform opaque white mixture without visible signs of aggregates. The z-potential is an important parameter for the evaluation of stability and bio-distribution[24, 25]. By increasing the GMO concentration, the z-potential became more negative, suggesting that the number of LC particles was increased with an increasing of GMO concentration. The negative z-potential of LC formulations could be related to the present of free oleic acid in the lipid phase and also can be explained by preferential adsorption of hydroxyl ions at the lipid-water interface.

3.2 Measurement of viscosity

A viscometer was used to determine the viscosity of the

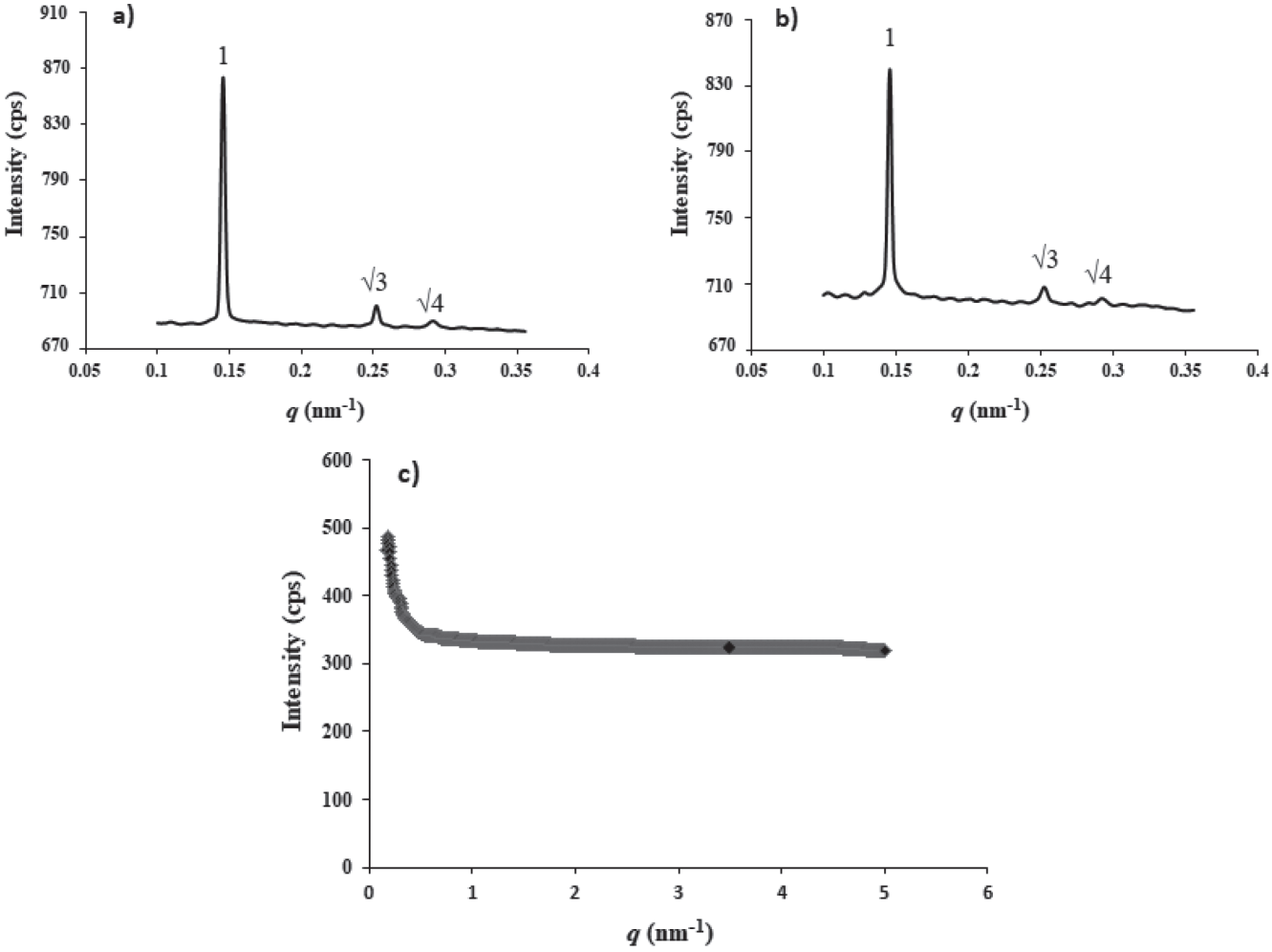

Fig. 4 SAXS charts of WP-GMO10 (a); WP-GMO20 (b) and WP (c) formulations.

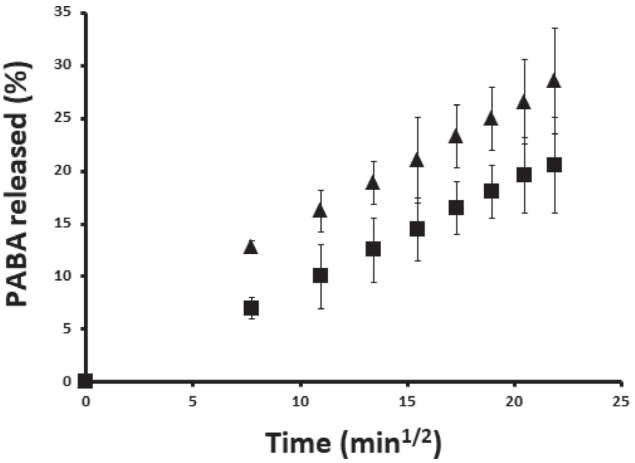

Fig. 5 PABA release profiles: (▲), WT-GMO10 formulation; and (■), WT-GMO20 formulation. Each point is the mean ± S.E. of three experiments.

WP-LC formulations. The resultant viscosity values are shown in Fig. 3. These values are mostly influenced by the quantity of GMO included in the formulation. The viscosity of the LC formulations increased as the GMO concentration increased. In view of the aforementioned results, the WP-GMO5 formulation has been discarded.

3.3 SAXS chart of WP- LC formulations

SAXS analyzed the WP-LC formulations' phase structure. (a) and (b) of the WP-GMO formulations, respectively, exhibit their X-ray diffraction patterns in Fig. 4. All produced formulations had H2 inverted hexagonal phase, as seen by the usual reflection patterns at around 1, $\sqrt{3}$, and $\sqrt{4}$. WP-LC formulations successfully formed hexosomes, as demonstrated by these findings.

3.4 Properties of WT-GMO nanoparticle formulations for drug release

The release experiment was carried out in a vertical diffusion cell. As shown in Fig. 5, the WT-GMO10 and WT-GMO20 LC nanoparticle compositions released PABA at different rates. All of the profiles were created with Higuchi's rule[22], which can be found here. There were 28.5 ± 5 and 20.4 ± 4.6 times more PABA released from the WT-GMO10 formulation than from the initial dosage of the WT-GMO. According to the findings, the concentration of GMOs led to a reduction in the amount of emitted PABA.

3.5 Penetrability of PABA into the skin after application of WT-GMO formulations

The impact of LC nanoparticle formulations WT-GMO10 and WT-GMO20 on the time course of cumulative PABA penetration in hairless rats with undamaged skin is illustrated in Fig. 6. When compared to the WT formulation, the WT-GMO10 and WT-GMO20 formulations dramatically

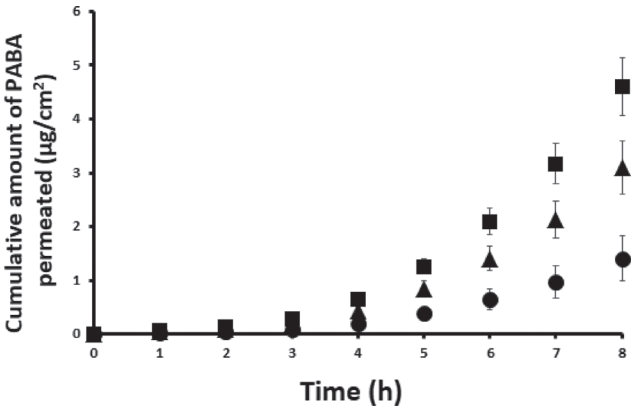

Fig. 6 The effect of LC nanoparticle formulations on the cumulative amount of PABA over time. (●), WT formulation; (▲), WT-GMO10 formulation; (■), WT-GMO20 formulation. Each point represents the mean ± SE of three experiments.

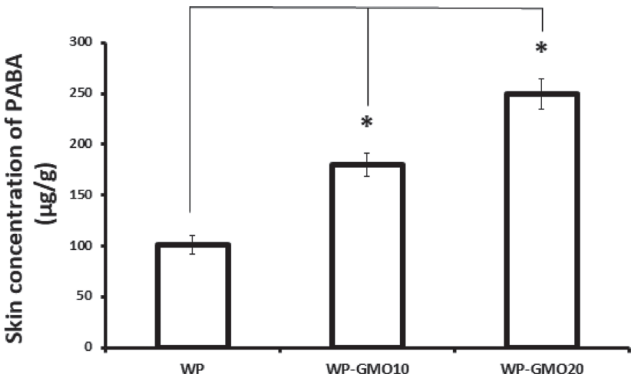

Fig. 7 PABA accumulation in the skin following 8 hours of treatment of WT, WT-GMO10, and WT-GMO20 formulations. Each column reflects the mean ± SE of three studies. *: $p < 0.05$ significantly different from WT (Student's t-test).

enhanced PABA skin penetration. Improved skin penetration required 2.2 and 3.3 days for the WT-GMO10 and WT-GMO20 formulations, respectively. According to our findings, WT-GMO10 and 20 are promising modern topical formulations.

3.6 Skin concentration of PABA

Figure 7 depicts the amount of PABA present in the skin following application of formulations WT, WT-GMO10, and WT-GMO20. The WT-GMO10 and WT-GMO20 formulations dramatically enhanced PABA skin concentrations compared to the WT. The skin concentrations of PABA were 101 ± 9, 180 ± 11, and 250 ± 15 μg/g after application of the WT, WT-GMO10, and WT-GMO20 formulations, respectively.

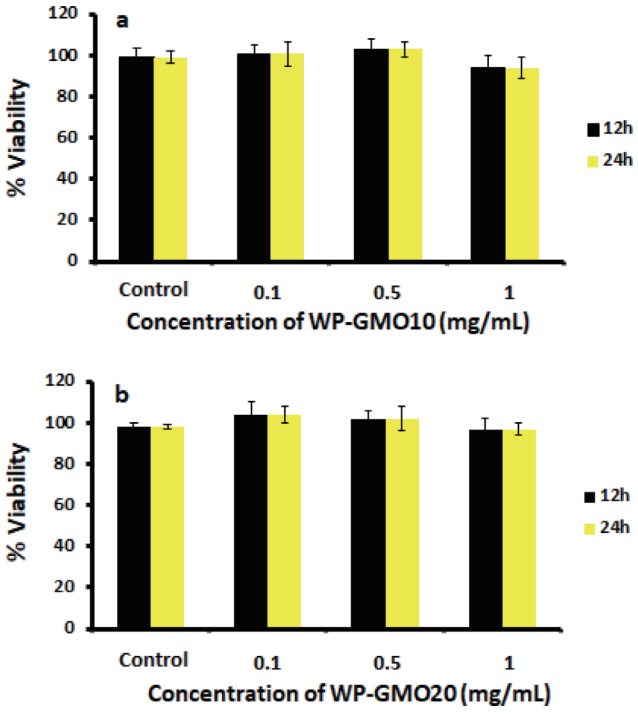

Fig. 8 Cell viability of HaCaT cells after incubation with WT-GMO10 (a) and WT-GMO20 (b) formulations of different concentrations for 12 h and 24 h, respectively.

3.7 Evaluating the biocompatibility with MTT assay

Figure 8 illustrates the proliferation of HaCaT cells when various doses of WT-GMO10 (a) and WT-GMO20 (b) formulations are used. At formulation concentrations of 0.1 and 0.5 mg/mL for 12 and 24 hours, the HaCaT cell viability was more than 98%, with no significant difference from the control group. Even at 1 mg/mL vehicle concentration, cell viability remained more than 90%, suggesting that the WT-GMO10 and WT-GMO20 formulations were safe for HaCaT cells.

3.8 Evaluating the biocompatibility with the comet assay

Figures 9, 10, and 11 show the impact of the WT-GMO10 and WT-GMO20 formulations on DNA damage. HaCaT cells treated with 50 M hydrogen peroxide as a positive control suffered significant DNA damage, the study showed. Grades 3 and 4 damage made up the majority of the total damage. There was no apparent DNA damage in the HaCaT cells treated with WT-GMO10 or WT-GMO20 up to 1 mg/mL, the findings likewise showed.

4 Discussion

Studies on semisolid transdermal drug administration, particularly ointments, provide a significant challenge because of the unique physicochemical characteristics of

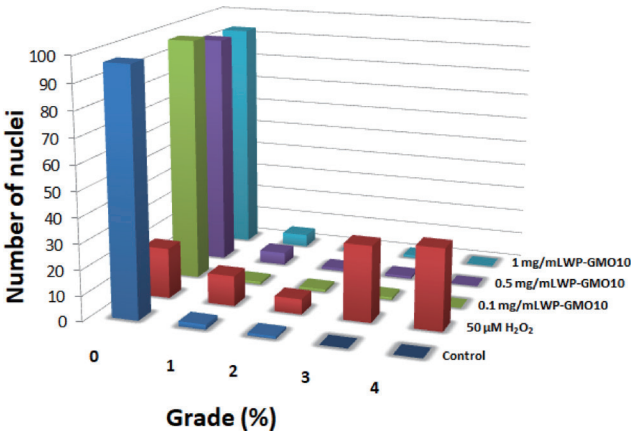

Fig. 9 Effect of WT-GMO10 formulation on DNA damage in HaCaT cells estimated with the comet assay. Data are presented as a percentage of grade (n = 3).

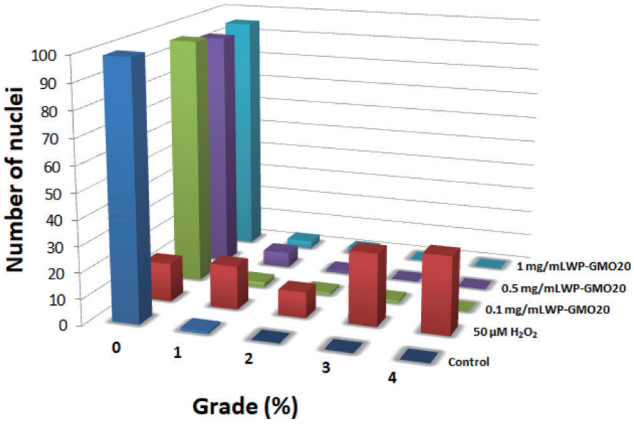

Fig. 10 Effect of WT-GMO20 formulation on DNA damage in HaCaT cells estimated with the comet assay. Data are presented as a percentage of grade (n = 3).

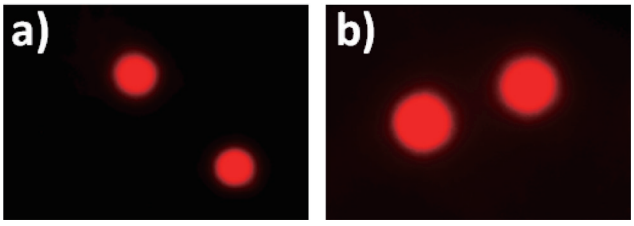

Fig. 11 Comet assay images of HaCaT cell lines treated with 1 mg/mL of WT-GMO10 formulation (a) and 1 mg/mL of WT-GMO20 formulation (b).

the skin layer and the complexity of the resulting formulations. For example, drug concentration and homogeneity are physicochemical features of ointment as well as droplet size and distribution as well as its rheological parameters, which play a vital role in figuring out the drug's stability and how that affects its release rate. Percutaneous perme-

ability and drug research physicochemical characteristics are therefore critical in evaluating transdermal administration, particularly in ointment[26].

We conducted this investigation to evaluate if petrolatum-LC formulations may serve as a new type of transdermal medication delivery method. As one of the most difficulties in dermatologic therapy, overcoming the stratum corneum barrier to cutaneously deliver hydrophilic medicines, PABA was chosen as a well-known hydrophilic and mal-absorbed model agent in this work.

We began by testing the physicochemical properties of the new petrolatum-LC compositions (Table 1). Particle size tends to decrease and zeta potential becomes more negative when GMO content increases (Fig. 2), indicating that the formulation's low-coordinate particle count, negative surface charges (NSC), and density all increased as a consequence. The stability and biodistribution of formulations are markedly influenced by zeta potential[24]. High surface charges inhibit particle agglomeration by causing electrical repulsion between them[25]. The presence of free oleic acid in the lipid phase may lead to the negative charge on the particles in LC formulations showing negative zeta potential values. It's also possible that the negative charge is due to the preferential adsorption at the water-fat contact of hydroxyl ions. Changes in the GMO concentration employed in the preparation had a substantial impact on the viscosity values of petrolatum-LC formulations. Figure 3 shows that the viscosity of LC formulations rose as GMO content increased. These findings were in line with those of earlier research[16] that looked at LC formulations.

These formulations formed an H2 inverted hexagonal phase after being tested by SAXS on their phase structure. The results exhibited typical reflection patterns at approximately 1, $\sqrt{3}$, and $\sqrt{4}$ for topical LC formulas (Figs. 4a, 4b). However, no reflection patterns were observed with WP formulation (Fig. 4c). Although the percentage of stearyl alcohol was high with WP formulation, we couldn't obtain the hexagonal reflection patterns of hexosomes in the mixtures that do not contain GMO. Hexosomes peaks were obtained only in formulations that contain 10 and 20% of GMO. We believe that the presence of 10 and 20% of GMO could be the reason of forming Hexosomes. GMO is a very well-known self-assembling amphiphilic molecules that form a variety of crystalline structures with useful mechanical properties of special interest in drug delivery. In the presence of a small amount of water, GMO forms reversed micelles characterized by an oily texture[10]. As more water is added, a mucous-like system is formed that corresponds to the lamellar phase. GMO forms cubic or hexagonal phases when more water is added (20-40%) over a wide temperature range. These phases are highly viscous, in the presence of high amounts of water in temperatures ranging from 20-70℃; the cubic and hexagonal phases might exist in a stable condition[11, 13]. Previous research has demonstrated that the LC phases of GMO such as the cubic and hexagonal phases, increased transdermal drug delivery. As a transdermal absorption enhancer, GMO probably acts by causing a temporary and reversible disruption of the lamellar structure of the lipid bilayer in the stratum corneum and, in this way, increasing intercellular lipid fluidity[14]. The hexagonal phase has been found to offer various benefits in previous research[27, 28]. More medicines can be integrated despite their insolubility because of the bigger surface area available for interacting with biological membranes. We were convinced that several variables, including temperature, lipid type, entrapped drug physicochemical characteristics, lipid concentration, and surfactant type, might alter the phase structure of LC formulations. To fully understand the impact of these variables on the phase transition of LC formulations, more research studies are required. The most important thing is to create functional hexosomes or cubosomes using LC formulations. The potential of cubosomes above hexosomes, or vice versa, as a drug delivery method, has not been demonstrated in a way that can be quantified.

The concentration of GMO affected the drug release patterns from petrolatum-LC formulations, as seen in Fig. 5. These findings showed that when the GMO content was raised, the formulation's drug diffusivity was reduced. The GMO concentration had a significant impact on the findings of *in vitro* skin permeation testing (Fig. 6). PABA skin penetration improved significantly in WP-GMO10 and WP-GMO20 with enhancement ratios of 2.2 and 3.3, respectively. Although the release rate of PABA was higher in WP-GMO10 compared with WP-GMO20, the skin penetration of WP-GMO20 was markedly higher than WP-GMO10. These findings suggesting that a low concentration of GMO such as 10% (WP-GMO10) could lead to a reduction in hexosomes particles which are necessary to overcome the barrier function of stratum corneum. On the other hand, hexosomes particles are higher in WP-GMO20 due to the high content of GMO which lead to improve the skin penetration and reduce the release rate of PABA due to its entrapped with these particles. The detailed mechanism of the enhanced oral drug absorption and transdermal drug penetration by LC systems is not fully understood yet[9, 16]. Further studies must be conducted to clarify the absorption-enhancing mechanism of LC formulations.

As a result of these findings, petrolatum-LC formulations outperformed commercial WP for skin permeability augmentation. Petrolatum-LC formulations resulted in significantly higher skin concentrations of PABA as a result (Fig. 7). The method by which LC systems increase skin permeability is still a mystery[29]. Skin permeability may be improved by using a cubic structure that has a comparable nanostructure to the skins. This would boost the contact between skin and formulation. According to earlier research, the hexagonal phase may aid in the fusing of LC

with the stratum corneum and deeper layers of the skin, enhancing medication transport to the skin. Furthermore, the hexosome system may be integrated into substances regardless of their solubility due to its greater skin-interaction surface area and high fluidity[27, 28]. More research is needed to determine how LC phase shape affects medication penetration through the skin. In addition, HaCaT was used to examine the biocompatibility of petrolatum-LC formulations. When tested with HaCaT, the petrolatum-LC formulations had minimal cytotoxicity (Fig. 8). Our findings were following those of other research using HaCaT cell lines to assess the safety of several recently produced formulations[30, 31]. We found that petrolatum-LC formulations had no genotoxic reaction against HaCaT cells even when used at 1 mg/mL (Figs. 9-11), indicating that they may be used safely in pharmaceutical applications.

5 Conclusion

Petrolatum-LC formulations showed potential for increasing medicine transdermal penetration in this study. By gaining a better knowledge of the impacts of GMOs used in petrolatum-LC formulations, researchers may be able to develop LC formulation methods that seek to increase the skin penetration of medications.

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