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Microemulsion of Erythromycine for Transdermal Drug Delivery

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ABSTRACT: The aim of this work was to prepare an erythromycin (EM) microemulsion (EM- microemulsion) for transdermal EM delivery using isotropic mixtures of oil and aqueous phases. The prepared EM-microemulsion is a white dispersion, with a suitable viscosity for transdermal delivery. In stability experiments, the EM-microemulsion showed no marked change in appearance for up to 3 weeks at 25°C. In accelerated stability experiments at 37 and 60°C, however, precipitated crystalline EM particles were observed in the EM-microemulsion. Diffusion of EM into the skin exhibited a first order release profile. Fluorescein (FL)-microemulsion penetrated to the dermis layer of skin. In conclusion, we confirmed that EM-microemulsion could serve as an excellent transdermal carrier of EM. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 4277–4282, 2013

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INTRODUCTION

A microemulsion is formed from isotropic mixtures of oil, surfactant, cosurfactant, and aqueous phase at appropriate ratios.[1–3] It is a colloidal, transparent, optically isotropic dispersion. It has several advantages compared to conventional formulations, such as enhanced drug solubility, good thermodynamic stability, and enhanced transdermal-delivery efficiency.[4–10] In particular, it does not tend to aggregate at room temperature. Thus, a microemulsion might be highly suitable for the incorporation of a hydrophobic drug.[11]

Many kinds of drugs can be incorporated in a microemulsion formulation for transdermal delivery.[12–16] The microemulsified drug shows increased thermodynamic activity, resulting in increased partitioning into the skin. Several ingredients in the microemulsion formulation that function as permeation enhancers might act by reducing the diffusional barrier of the stratum corneum.[17,18]

Erythromycin (EM), an antibiotic, is frequently applied as a cream. Several transdermal administration forms such as liposomes, emulsions, micelles, and microspheres have been explored to enhance the permeation of EM into the skin.[19] Among possible formulation choices, microemulsion is expected to provide significant stabilization of EM and to enhance its permeation into the skin.[20] Thus, preparation of a suitable emulsified EM formulation is likely to improve the drug's performance.

The purpose of this work is to prepare a suitable EM-emulsified formulation and evaluate its performance (Figure 1). In addition, fluorescence models using fluorescein (FL)-microemulsion were employed to analyze the permeation of the emulsified formulation.

MATERIALS AND METHODS

Materials

Erythromycin was purchased from Halcyon Labs (Gujarat, India). Caprylic/Capric triglyceride oil (CCT oil), polyethylene glycol (PEG)-100 stearate, tetraglycol, 1,3-propanediol and fluorescein (FL) were purchased from Sigma Chemical (St. Louis, MO). Steareth-21, composed of polyethylene glycol ethers of stearic acid (PEG-100 stearate), was purchased from Clariant (Houston, TX). Divinyl-dimethicone and silica silylate was obtained from Dow Corning (Seneffe, Belgium). All the other chemicals were of reagent grade. Deionized water (DW) was prepared using the Ultra Pure System from Romax (Hanam, Korea).

Preparation of the EM-Microemulsion

EM-microemulsion was prepared in the formulations shown in Table I. To prepare the oil phase, EM was first dissolved in ethanol. CCT oil and PEG-100 stearate were added to the EM solution, and the mixture was heated at 65°C. For preparation of the

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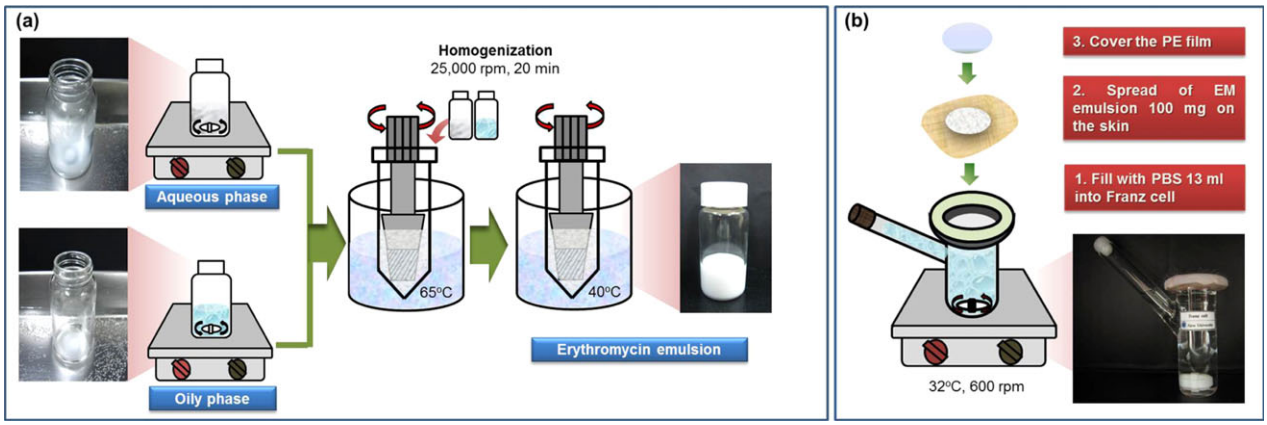

Figure 1. Schematic representation of (a) the preparation of EM-microemulsion and (b) transdermal delivery of EM from EM-microemulsion. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

aqueous phase, glycerin, tetraglycol, and divinyl-dimethicone were dissolved in DW and heated at 65°C. The oil and aqueous phases were then mixed at 65°C and 25,000 rpm in a T-10 basic Ultra-Turrax Homogenizer (IKA Werke GmbH, KG, Germany). After 20 min, silica silylate was added and mixed at 40°C and 25,000 rpm. Finally, the EM-microemulsion was incubated at room temperature for 20 min. FL-microemulsion was prepared by a similar method.

Viscosity Measurements

The viscosity of the EM-microemulsion was measured using a circulating bath with a programmable controller (TC-502P, Brookfield Engineering Laboratories, Middleboro, MA) and a Brookfield DV-III ultra viscometer equipped with a programmable rheometer. The viscosity was investigated using a T-F spindle rotating at 1 rpm at 20°C.

Stability Measurements

The stability of the EM-microemulsion was investigated by observing its clarity, phase separation, and crystallinity at 25, 37, and 60°C for 1–4 weeks. The crystallinity of the EM-microemulsion and EM alone were measured by performing X-ray diffraction (High Resolution X-Ray Diffractometer, Ultima III, Rigaku,

Table I. The Preparation of EM-Microemulsion

	Content (g)									
	Aqueous phase				Oily phase			Drug	Thickening agent	
No.	Deionized water	Glycerin	Tetraglycol	Divinyl-dimethicone	Ethanol	CCT oil	PEG-100 stearate	Erythromycin	Silica silylate	Result
F-1	3.76	1.00	4.00	3.00	0.50	0.40	1.60	0.20	0.50	●
F-2	3.26	0.05	0.20	0.15	0.50	0.40	1.60	0.20	0.03	●
F-3	3.26	0.05	0.20	0.15	0.50	0.40	0.16	0.20	0.03	○
F-4	3.27	0.05	0.20	0.15	0.50	0.40	0.16	0.20	0.03	●
F-5	3.27	0.05	0.20	0.15	0.50	0.40	0.80	0.20	0.03	●
F-6	3.27	0.05	0.20	0.15	0.50	0.40	0.70	0.20	0.03	●
F-7	3.73	0.05	0.20	0.16	0.40	0.40	0.80	0.24	0.03	○
F-8	3.73	0.05	0.20	0.16	0.40	0.40	1.50	0.24	0.03	●
F-9	2.80	1.00	2.80	0.06	0.10	1.20	0.12	0.40	0.12	●
F-10	2.56	1.60	2.80	0.06	1.12	0.20	0.12	0.40	0.01	●
F-11	2.46	1.60	2.80	0.06	1.12	0.20	0.12	0.40	0.01	●
F-12	2.18	1.60	2.80	0.04	0.10	1.20	0.12	0.40	0.01	●
F-13	6.53	0.10	0.40	0.15	1.00	0.80	1.40	0.40	0.05	○
F-14	6.40	0.10	0.30	0.30	0.90	0.70	1.40	0.40	0.05	○
F-15	6.55	0.10	0.40	0.30	1.00	0.80	1.00	0.40	0.05	○
F-16	6.25	0.10	0.40	0.30	0.80	0.70	1.40	0.40	0.05	●
F-17	6.15	0.10	0.20	0.35	0.80	0.50	0.80	0.48	0.05	○
F-18	7.35	0.10	0.40	0.32	1.00	0.80	1.50	0.48	0.05	◎

◎: good ○: precipitation ●: phase separation.

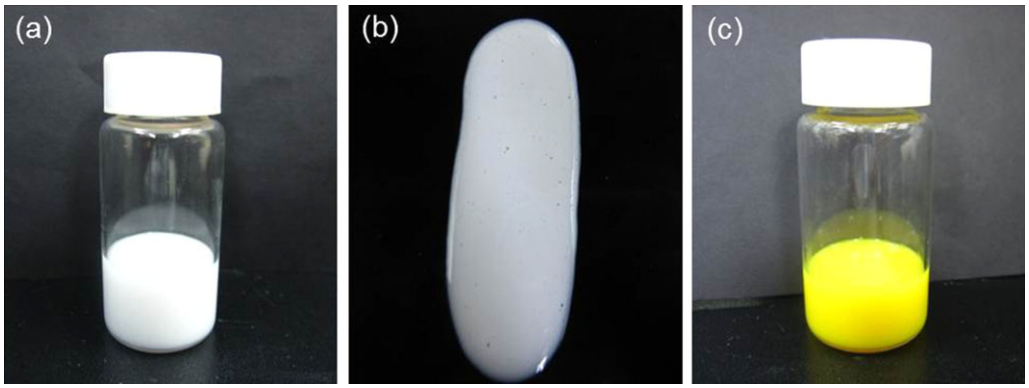

Figure 2. Pictures of (a,b) EM-microemulsion and (c) FL-microemulsion. The formulation for the selected emulsion is presented in Table I. Of all the emulsions prepared F-18 was the most suitable as it was spread evenly on mouse skin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Japan). A Ni filter at 35 kV and 15 mA were used to generate the radiation. The samples were placed in a quartz sample holder and scanned from 10 to 60°C at a scanning rate of 5°C min^{-1}. The degree of crystallinity was calculated as the ratio of the crystalline peak areas to the total area under the scattering curve.

EM Permeation Studies

For permeation studies, the skins of male hairless nude mice (6 weeks) were obtained from the epidermis region. The excised skins were washed with saline solution, examined for integrity, and then stored in a refrigerator at −83°C until needed for experimental use.

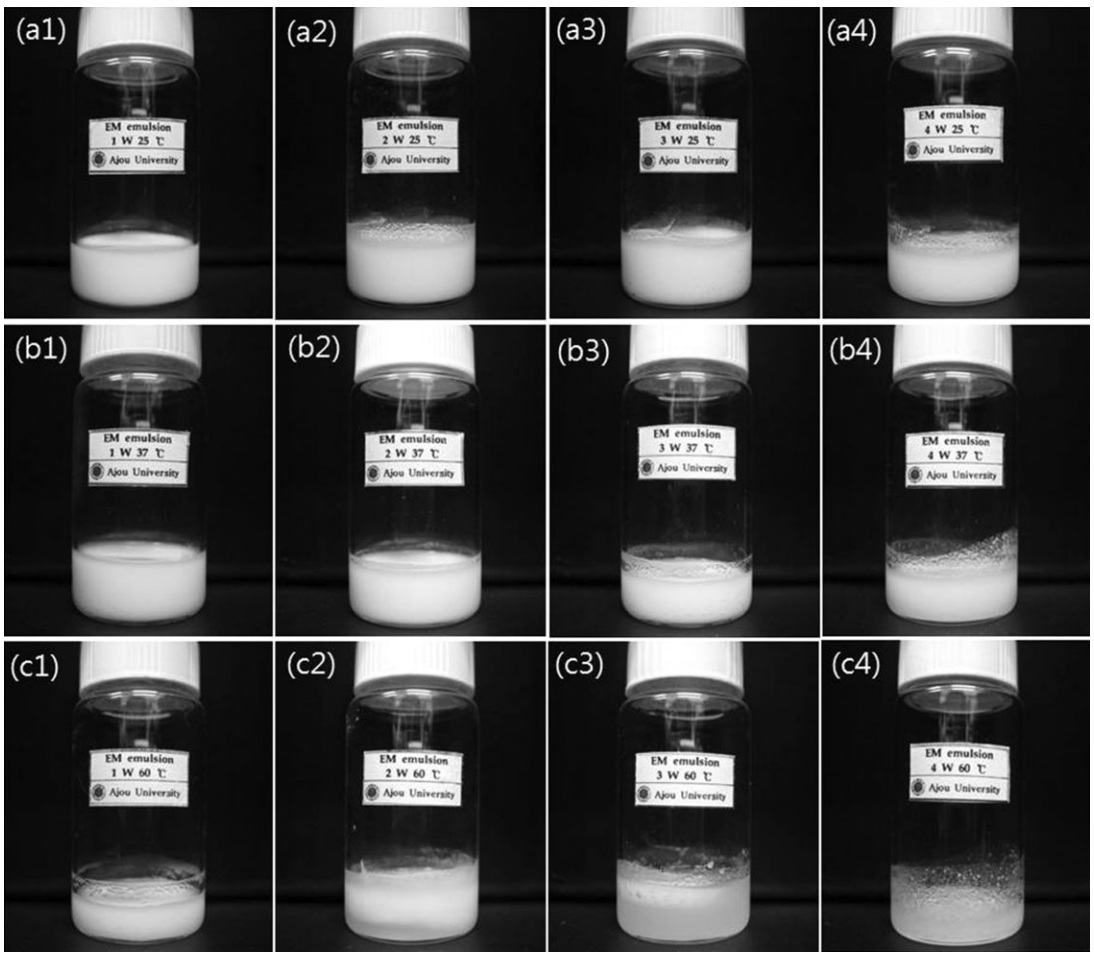

Figure 3. Pictures of EM-microemulsions after incubation for 1–4 weeks at (a) 25°C, (b) 37°C, and (c) 60°C. The numbers indicate incubation weeks.

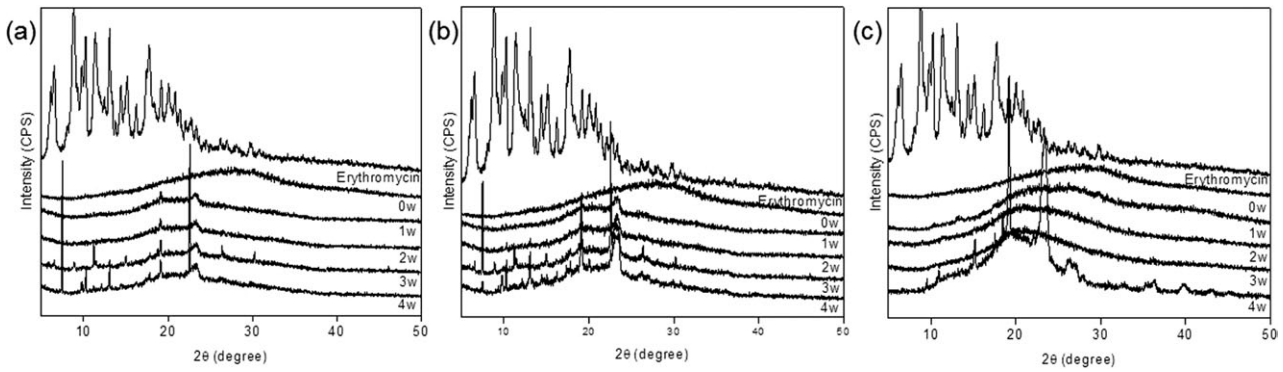

Figure 4. X-ray diffraction diagrams of EM-microemulsions after incubation for 1–4 weeks at (a) 25°C, (b) 37°C, and (c) 60°C.

Permeation experiments were performed using Franz diffusion cells with an effective area of 1.77 cm^2. The excised skin samples were clamped onto the cells. EM-microemulsion (100 mg) was applied to the excised skin samples, which were then covered with a polyethylene (PE) film. The reservoir chamber in the diffusion cells was filled with 13 mL of PBS. The cells were maintained at 32°C ± 0.5°C and stirred at 600 rpm throughout the experiment. For each experiment, 500 μL of medium was extracted at predetermined time intervals, and then the same volume of the pure medium was immediately added into the reservoir chamber. The extracted solutions were filtered through a 0.45-μm pore size cellulose membrane filter and analyzed by performing high-performance liquid chromatography (HPLC). The amount of EM was analyzed using a HPLC system (Agilent Technologies 1200 series (Waldbronn, Germany) with detection at 480 nm. A Sunfire C18 column (150 × 4.6 mm^2, 5 μm) was used. The mobile phase consisted of acetonitrile, methanol, 0.2*M* ammonium acetate, and DW (45/10/10/35 v/v). The column temperature was 30°C, and the detector wavelength was 215 nm. The column was eluted at a flow rate of 0.8 mL min^{-1}. Three independent release experiments were performed for each EM-microemulsion. The amount of released EM in the extracted solutions was determined by using a reference to a standard calibration curve prepared from solutions containing known concentrations of EM in PBS.

Histological Analysis

At predetermined time intervals after administration of FL-microemulsion on excised skin, the skin tissues were fixed with 10% formalin and embedded in paraffin. The embedded specimens were sectioned (4 μm) along the longitudinal axis of the skin and stained with hematoxylin and eosin (H&E). The fluorescent images were obtained using an Axio Imager A1 (Carl Zeiss Microimaging GmbH, Göttingen, Germany) and analyzed using the Axiovision Rel. 4.8 software (Carl Zeiss Microimaging GmbH).

Statistical Analysis

The data from the EM permeation studies were obtained from independent experiments with $n = 3$ for each data point. All the data are presented as mean ± standard deviation. The results were analyzed by one-way ANOVAs using the Prism 3.0 software package (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

Preparation of EM-Microemulsion

Various studies with several parameters were carried out to find an appropriate formulation for creating a stable microemulsion with acceptable solubilizing capacity for EM (Table I). The following three solubilizers were used: glycerin, tetraglycol, and divinyl-dimethicone. The PEG-100 stearate was tested as a surfactant in oil phase. Silica silylate contributed viscosity to the EM-microemulsion formulations and was included in all further studies. The microemulsion was prepared as a white dispersion of EM in isotropic mixtures of the oil and aqueous phases.

The uniformity of the microemulsion can be related to the composition of isotropic mixtures of the oil and aqueous phases. From the various outcomes, we sought a composition that provided an acceptable formulation (Table I). Certain formulations gave rise to precipitation from the microemulsion, others exhibited phase separation. Formulation F-18 produced a uniform microemulsion, indicating that EM was successfully incorporated into the emulsions. The F-18 formulation was selected for subsequent experiments.

The prepared EM-microemulsion was evaluated visually. As shown in Figure 2(a,b), a white emulsion was formed. It possessed suitable viscosity (~4 × 10^3 cP) for transdermal delivery. In the case of fluorescence permeation experiments, FL-emulsions showed yellow coloration [Figure 2(c)].

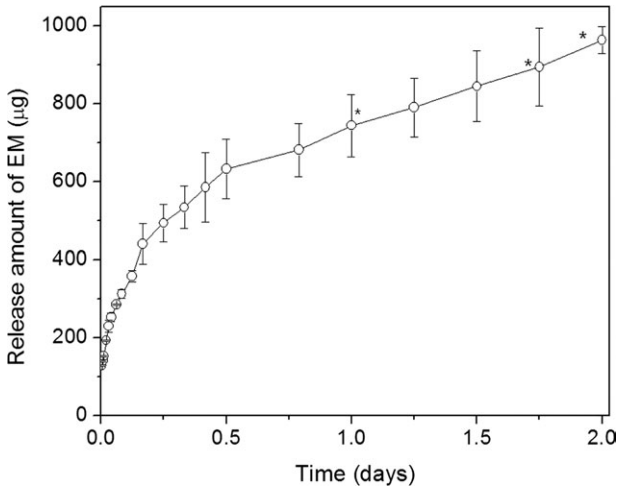

Figure 5. The release profiles of EM released from EM-microemulsion at 37°C. (*$P < 0.05$).

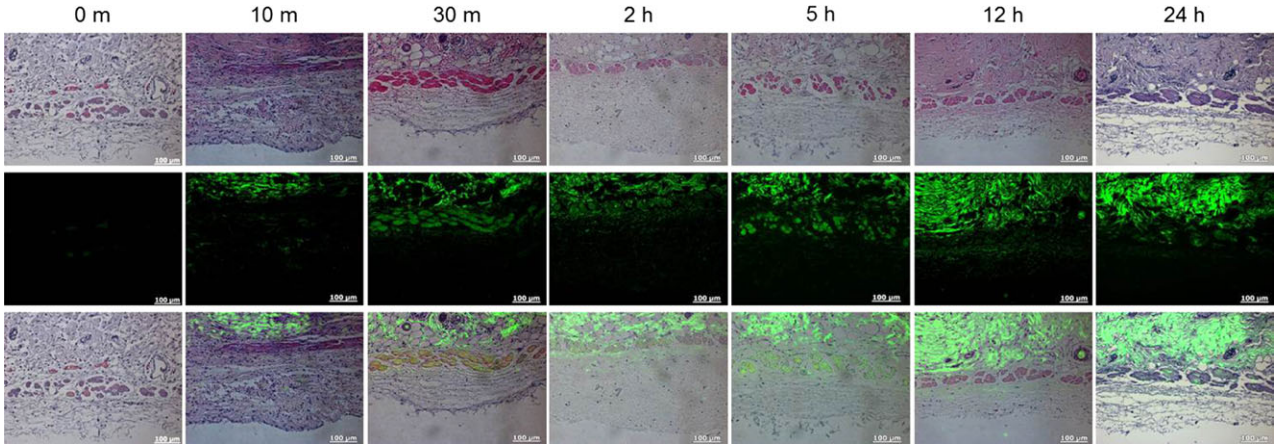

Figure 6. (Upper) Images of hematoxylin and eosin (H&E) images, (middle) fluorescence images, and (bottom) merged H&E and fluorescence images after transdermal delivery of fluorescein-microemulsion at each time point. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Stability of the EM-Microemulsion

The primary objective of this study was to develop stable formulations. It is well known that stability is a key characteristic of safe and effective transdermal drug-delivery formulations. Thus, both accelerated and long-term stability tests were carried out on the EM-microemulsion.

Stability was examined at 25, 37, and 60°C for 4 weeks (Figure 3). The EM-microemulsion at 25°C showed no marked change in appearance up to 3 weeks, but precipitated particles were just discernible after 4 weeks. At 37°C, precipitated EM particles in the EM-microemulsion were observed after 3 weeks. A large amount of precipitation was observed at 60°C even at 2 weeks. This indicates that EM precipitation is significantly high in the accelerated studies at elevated temperatures.

X-ray diffraction was used to study the crystalline character of the EM-microemulsion (Figure 4). Sharp, high intensity reflections are produced by highly crystalline EM. The crystallinity of EM is around 62%. The examination of the crystalline transition of EM in accelerated stability experiments at 37 and 60°C as well as room temperature is important to determine whether an optimized formulation stabilizes EM or not. The emulsified EM generated an almost amorphous diffractogram with very weak peaks, indicating less than 1% crystallinity as a result of the emulsification. The peaks assignable to crystalline EM steadily increased over time at 25, 37, and 60°C. The growth in peak intensities on storage indicates a dynamic crystalline transition of EM. However, the emulsified EM was stable for at least 1 month at room temperature and for 2–3 weeks even at 37°C.

Skin Permeation of EM

Next, the permeation of EM through mouse skin was evaluated (Figure 5). For permeation studies, 100 mg of EM-microemulsion was applied to the mouse skin and covered with commercial PE film. The EM released from the microemulsion permeated through the skin into the reservoir chamber. Diffusion profiles were obtained by plotting the percentage of permeated EM as a function of time. EM exhibited almost first order diffusion profile, and the released amount of EM reached 10 mg cm^{-2} after 2 days. This result indicates that EM from the microemulsion had a uniform permeation rate through the skin.

To better understand the penetration of EM into the reservoir chamber from the top surface of the skin, the penetration of FL-microemulsion into the skin was examined and stained. Figure 6 shows H&E, fluorescence, and the merged images of H&E images and fluorescence. Fluorescence was directly imaged across the skin layers and was observed to increase as a function of time. FL penetrated the skin to some depth, even to the dermis layer. This indicates that the microemulsion can serve as an excellent transdermal carrier of EM into skin.

CONCLUSION

In the present study, an EM-microemulsion for transdermal EM delivery was developed successfully. The prepared EM-microemulsion was stable for at least 1 month at room temperature. The skin permeation behavior of the EM-microemulsion exhibited a first order diffusion profile. Penetration into the dermis layer of the skin was inferred using FL-microemulsion. On the strength of these encouraging results, further investigation is now in progress on the animal model using the EM-microemulsion prepared in this work and needs the improving the self-life of the EM microemulsion.

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