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การศึกษาการซึมผ่านผิวหนังนอกกายของเอทโธโซมของตัวยาไมโคฟีโนลิกแอซิด

ธัญญลักษณ์ ลิ้มสุวรรณ, เฉลิมเกียรติ สงคราม, ธนภร อำนวยกิจ*

บทคัดย่อ

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บทนำ: เอทโธโซม คือ ระบบนำส่งยาเข้าสู่ผิวหนังแบบอนุภาคหรือถุงทรงกลมที่มีความอ่อนตัว ยืดหยุ่น สามารถเปลี่ยนแปลงรูปทรงไปตามเส้นทางที่เคลื่อนที่ผ่าน ประกอบขึ้นจากสารไขมันประเภทฟอสโฟไลปิด เอธานอลที่มีความเข้มข้นสูง และน้ำ มีลักษณะพิเศษที่น่าสนใจหลายประการในการเพิ่มการซึมผ่านผิวหนัง โดยในการศึกษานี้ ได้เตรียมตำรับเอทโธโซมของตัวยาไมโคฟีโนลิกแอซิด หรือ MPA และประเมินลักษณะต่างๆเพื่อหาสูตรตำรับเอทโธโซมที่เหมาะสมสำหรับศึกษาการซึมผ่านและการสะสมของตัวยาในผิวหนังนอกกาย **วัสดุและวิธีการทดลอง:** เตรียมสูตรตำรับเอทโธโซมของตัวยา MPA โดยวิธีธินฟิล์มไฮเดรชัน (Thin-film hydration) และประเมินลักษณะของเอทโธโซมที่เตรียมได้ในเรื่องของ ลักษณะทางกายภาพ ขนาดอนุภาค การกระจายของขนาดอนุภาค ศักย์ซีต้า สัณฐานวิทยา และประสิทธิภาพการกักเก็บตัวยา ศึกษาการซึมผ่านและการสะสมตัวยาในผิวหนังนอกกายของตำรับเอทโธโซมที่เหมาะสมเพื่อประเมินถึงศักยภาพในการเป็นระบบนำส่งยาเฉพาะที่โดยใช้ Modified Franz diffusion cell และทดสอบผ่านผิวหนังหมูแรกเกิด **ผลการทดลอง:** สูตรตำรับเอทโธโซมที่เหมาะสม (Etho-25) มีองค์ประกอบของไขมันเท่ากับ 4 เปอร์เซ็นโดยมวลต่อปริมาตรของ L-α-phosphatidylcholine from soybean หรือ SPC: Cholesterol from lanolin หรือ CHOL Polyoxyethylene sorbitan monooleate หรือ Tween 80: Deoxycholic acid หรือ DA ในอัตราส่วน 6:2:1:1 โดยโมล และมีเอทธานอล 30 เปอร์เซ็นต์โดยปริมาตรในฟอสเฟตบัฟเฟอร์ pH 7.4 เป็นตัวกลาง ซึ่งให้เอทโธโซมที่มีขนาดอนุภาค 370.90±7.91 นาโนเมตร (Polydispersity index หรือ *PI* = 0.270±0.02 นาโนเมตร), ศักย์ซีต้า -45.58±4.50 มิลลิโวลต์และมีประสิทธิภาพการกักเก็บตัวยา 56.01±1.10 เปอร์เซ็นต์ เมื่อศึกษาการซึมผ่านและการสะสมตัวยาในผิวหนังนอกกาย พบว่า ตำรับเอทโธโซมที่เตรียมได้มีปริมาณยาที่ซึมผ่านผิวหนังที่ 24 ชั่วโมงหรือ Q_{24} เท่ากับ 307.29±24.93 ไมโครกรัมต่อพื้นที่ผิว 1 ตารางเซนติเมตร มีค่า Steady-state fluxหรือ J_{ss} เท่ากับ 13.20±0.91 ไมโครกรัมต่อพื้นที่ผิว 1 ตารางเซนติเมตรต่อชั่วโมง มีค่าสัมประสิทธิ์การซึมผ่านของตัวยาหรือ K_p เท่ากับ1.32±0.09 $\times 10^{-3}$ เซนติเมตรต่อชั่วโมง ซึ่งมีค่าสูงกว่าเมื่อเทียบกับตำรับอื่นๆอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 และมีค่าระยะเวลาในการเริ่มซึมผ่านหรือ T_{lag} ของตำรับเอทโธโซมเท่ากับ 1.16±4.67 ชั่วโมง นอกจากนี้ตำรับเอทโธโซมยังให้ปริมาณยาที่สะสมในผิวหนังที่ 24 ชั่วโมง (45.46±6.80 ไมโครกรัมต่อพื้นที่ผิว 1 ตารางเซนติเมตร) มากกว่าตำรับตัวยา MPA ที่แขวนตะกอนในน้ำ, ตำรับลิโปโซม และตำรับลิโปโซมที่เตรียมโดย Rattanat, 2008 อย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 **สรุปผลการทดลอง:** จากผลการทดลองข้างต้นแสดงให้เห็นว่าระบบนำส่งเอทโธโซมสามารถเพิ่มการซึมผ่านและการสะสมตัวยา MPA ในผิวหนังนอกกาย ซึ่งนำไปสู่การมีประสิทธิภาพในการใช้เป็นระบบนำส่งยาทาเฉพาะที่

คำสำคัญ : เอทโธโซม, ไมโคฟีโนลิกแอซิด, การซึมผ่านผิวหนังนอกกาย, ระบบนำส่งยาทาเฉพาะที่

Abstract

***In vitro* skin permeation study of ethosome containing mycophenolic acid**

Limsuwan T, Songkram C, Amnuaikit T*

Introduction: Ethosome are soft, malleable vesicles carrier which mainly composes of phospholipids, ethanol in relatively high concentration and water. This carrier presents interesting features correlated with its ability to permeate through the skin. In this study, the ethosome containing Mycophenolic acid (MPA) was prepared and characterized to obtain the optimized

***ติดต่อผู้นิพนธ์**: โทร (66 74) 288849, โทรสาร (66 74) 428148, E-mail: chomchan.a@psu.ac.th

***Corresponding author:** Tel. (66 74) 288849, Fax. (66 74) 428148, E-mail: chomchan.a@psu.ac.th

formulation. The *in vitro* skin permeation of the optimized formulation was tested for evaluation suit of applying as topical delivery system for MPA. **Materials and method:** The ethosome containing MPA were prepared according to the thin-film hydration method. The characterization of ethosome was evaluated in terms of physical appearances, particle size, size distribution, zeta potential, surface morphology and entrapment efficiency. In addition, *In vitro* skin permeation and skin retention studies of the appropriate ethosome were evaluated using modified Franz diffusion cell were carried out with the newborn pig skin. **Results:** The optimized ethosomal formulation (Etho-25) was composed of 4% w/v L-α-phosphatidylcholine from soybean (SPC): Cholesterol from lanolin (CHOL): Polyoxyethylene sorbitan monooleate (Tween 80): deoxycholic acid (DA) with a molar ratio was 6:2:1:1 as lipid component and 30% v/v ethanol in phosphate buffer pH 7.4 as dispersion medium. This formulation gave ethosome with 370.90±7.91 nm vesicular size (Polydispersity index *PI*= 0.270±0.02 nm), the zeta potential of -45.58±4.50 mV and the entrapment efficiency of 56.01±1.10%. Further, the appropriate ethosome provided significantly higher skin permeation parameter such as the cumulative amount at 24 hours Q_{24} (307.29±24.93 µg/cm^2), the steady state flux J_{ss} (13.20±0.91 µg/cm^2/h) and the skin permeation coefficient K_p (1.32±0.09 ×10^{-3}cm/h) compared to other formulations. The lag time T_{lag} of ethosome was 1.16±4.67 hours. Furthermore, the ethosome system gave the MPA accumulated in the skin higher (45.46±6.80 µg/cm^2) than aqueous suspension, liposome and liposome that was developed by Rattanat, 2008 (p<0.05). Conclusions: These results indicated that the ethosome could enhance the skin permeation and retention of MPA that leading to the efficiency in topical delivery system.

Keywords: Ethosome, Mycophenolic acid, *in vitro* skin permeation, Topical drug delivery systems

Introduction

Mycophenolic acid (MPA) was first discovered by Gosio in 1896, which was isolated from the fungus *Penicillium stoloniferum* (Orvis *et al.*, 2009). It has been used widely as an immunosuppressant in organs transplantations and in a variety of autoimmune diseases such as scleroderma, systemic lupus erythematosus and psoriasis. In 1995, MPA and its derivatives such as mycophenolate mofetil (MMF) and mycophenolate sodium were approved by The US Food and Drug Administration (FDA) as immunosuppressive drugs to prevent graft rejection after organ transplantation. The antiproliferative effect of MPA results from its potent, selective, uncompetitive, and reversible inhibition of inosine 5"-monophosphate dehydrogenase (IMPDH), the key enzyme that controls the rate of synthesis of guanine nucleotides in the de novo pathway of purine synthesis used in the proliferation of T and B lymphocytes, which leading to an inhibition of cell proliferation (Chaigne-Delalande *et al.*, 2008). In 1970s, Jones *et al.* demonstrated oral administration of MPA has effective and safe in the treatment of psoriasis. However, its oral administration has disadvantages that this drug has underwent extensive first-pass metabolism by the liver and its tolerability was limited by gastrointestinal (GI) upset; it caused nausea, soft stools, abdominal cramps, anorexia, diarrhea and frequent stools leading to the most patients requiring dose changes or discontinuation of treatment (Kitchin *et al.*, 1997; Arns, 2007; Orvis *et al.*, 2009). Using the topical route eliminates these side effects, increases patient compliance, targets of the active ingredient for a local effect and avoids first-pass metabolism. However, the insufficiency of skin permeation of MPA formulations leads to low clinical efficiency of topical administration (Geilen and Mrowietz, 2000). Therefore, an improved MPA formulation with a high degree of permeation could be useful in the treatment of psoriasis.

Ethosome are soft vesicles carrier, described by Touitou *et al.,* (2000). This carrier was modified form of liposome which mainly composes of phospholipids, ethanol in relatively high concentration and water. It was indicated that the physicochemical characteristics of ethosome allow this vesicular carrier to delivery of various active agents through the stratum corneum into the deeper layers of the skin or to the systemic circulation. One of the important characteristics of ethosome is that it has elasticity and deformability property tailored for enhanced delivery of active agents (Jain *et al.*, 2007). The better permeability of ethosome carriers may be due to the synergistic effect of combination of phospholipids vesicles, ethanol at high concentration and skin lipids. Ethanol interacts with skin lipid molecules in the polar head group region that the result in increasing fluidity and may finally lead to increase membrane permeability. In addition, ethanol may provide the lipid vesicles with soft, flexible and malleable characteristics which are easy to penetrate into deeper layers of the skin. The release of drug could be the result of fusion of ethosome with skin lipids and drug release at various points along the penetration pathway (Touitou *et al.,* 2000). Many studies have used ethosome as a carrier system of drugs delivery for the transdermal or topical administration. It has been reported to improve various drug deliveries both *in vivo* and *in vitro* skin, such as Acyclovir, Testosterone, Cannabidiol, Buspirone hydrochloride, Erythromycin, Ammonium glycyrrhizinate, Lamivudine, Minoxidil and 5-aminolevulinic acid (Elsayed *et al.*, 2007; Jain *et al.*, 2007; Fang *et al.*, 2008; Shumilov *et al.*, 2010).

In this study, the ethosome containing MPA was prepared and characterized to obtain the optimized formulation. The *in vitro* skin permeation of the optimized formulation was tested for evaluation suit of applying as topical delivery system for MPA.

Materials and methods

Materials

Mycophenolic acid (MPA) was kindly supplied by the Molecular Pharmaceutical Research Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand. It was synthesized by hydrolysis of mycophenolate mofetil (MMF), which was obtained from the extraction of Cellcept® capsule. L-α-phosphatidylcholine from soybean (≥30%, SPC) was purchased from Sigma-Aldrich. Cholesterol from lanolin (CHOL) and deoxycholic acid (DA) were also purchased from Fluka. Triton X-100 was purchased from Baker analyzed (New Jersey, USA). Polyoxyethylene sorbitan monooleate (Tween 80) was obtained from Srichand United Dispensary Co., Ltd (Bangkok, Thailand). All other reagents used in the study were of analytical grade.

Quantitative Analysis of MPA using High-Performance Liquid Chromatography (HPLC)

The HPLC apparatus (Massachusetts, United States) for quantitative determination of MPA was carried out with a reverse phase BDS HYPERSIL C18 column (250x4.6 mm, 5 µm) at room temperature. The mobile phase was a mixture of methanol/acetonitrile/phosphate buffer (10:30:60 v/v) with flow rate was maintained at 1.0 ml/min. the injection volume of 20 µl and the separation was monitored at 254 nm.

Formulation and preparation of ethosome containing MPA

The ethosome containing MPA were developed according to the thin-film hydration method, using MPA at the concentration of 10 mg/ml. The main composition of ethosome includes of 2-6% w/v SPC and hydroethanolic solution which is a mixture of phosphate buffer pH 7.4 with 10-30% v/v ethanol. The CHOL, Tween 80, PEG and DA were used as additives to improve the properties of ethosome. All the components of ethosome were varied in terms of type, concentration and the ratio. The process of preparing ethosome follows; firstly, MPA, SPC and other additives were dissolved in absolute ethanol and sonicate at 60°C for 30 minutes until all components were melted. Then, these components were added in a 500-ml round-bottomed flask followed by evaporation at 60°C using rotary evaporator to form the thin lipid film on the flask wall. The flask was continued on rotary evaporator for 20

minutes to ensure that the absolute ethanol were completely evaporated. Secondly, the thin lipid film was hydrated with hydroethanolic solution followed by shaking for 5 minutes which flask was tightly seal to prevent the ethanol in the formulation evaporates. Finally, these formulations were sonicate at 60°C for 30 minutes to achieve a complete ethosome formulations.

For comparison, the corresponding liposome containing MPA were also prepared using the same materials and procedure described for preparing ethosome but without ethanol. All formulations were prepared in triplicate.

Characterization of ethosome containing MPA

The characterization of ethosome was developed and evaluated in terms of physical appearances, particle size, size distribution, zeta potential and entrapment efficiency to as the optimized ethosomal formulation.

Visualization of ethosome using scanning electron microscopy (SEM)

Vesicles morphology were visualized by using Scanning Electron Microscope (SEM) (Quanta 400, FEI, Czech Republic) at an accelerating voltage of 20 kV. Prior to visualize, 100 μl of ethosome was diluted with 3 ml MiliQ water. The diluted ethosome was dropped on the cover slip with clean glass. The sample was coated with gold in a sputter coater under an argon atmosphere (50 Pa) at 50 mA for 70 seconds and was viewed under SEM at 30,000X magnification.

***In vitro* skin permeation studies**

The *in vitro* skin permeation of the optimized ethosome formulation and control formulations was measured through newborn pig skin using Franz diffusion cells. The control systems were used: (a) MPA liposomes; (b) 30% hydroethanolic solution of MPA; (c) aqueous suspension of MPA; (d) MPA liposome that was developed by Rattanat (2008). The effective diffusion area of the diffusion cell was 1.77 cm^2. The receptor compartment was filled with 11 ml of phosphate buffer saline solution pH 7.4 (PBS). The 1 ml of each sample was applied on the skin surface in the donor compartment. The newborn pig skin was maintained between the donor and the receptor compartment with the stratum corneum side facing upward into the donor compartment. The Franz diffusion cells were maintained at 37°C with stirring at 500 rpm throughout the experiment. The 1 ml sample of receiver medium was withdrawn through the sampling port of the diffusion cell at 0.5-, 1-, 2-, 4-, 6-, 8-, 12- and 24-hours time intervals. An equal volume of fresh PBS was replaced into the receptor compartment after each sampling. All withdrawn samples were analyzed by HPLC technique. For permeation data analysis, the cumulative amount of MPA permeated (Q_t, μg/cm^2) was plotted as a function of time. The steady state flux (J_{ss}) was calculated from the slope of linear portion of the plot. The lag time (T_{lag}) for MPA to permeate through pig skin before reaching the receptor fluid was calculated from the X-intercept of the plot. The permeability coefficient (K_p) of the MPA through pig skin was calculated from the equation; $K_p = J_{ss}/C_0$, where C_0 is the initial concentration of MPA in the donor compartment.

***In vitro* skin retention studies**

At the end of the study (24 hours), the formulation was collected, the skin surface was cleaned by a swabs which was soaked ten times with 10 ml methanol was sonicated at room temperature for 30 minutes. After that, the samples was filtered through the filter paper and lysed with 30% v/v Triton X 100 and analyzed to estimate the amount of residual MPA in donor compartment after the end of the skin permeation studies by HPLC. The skin was removed from the diffusion cells and cut into small pieces by scissor. Then, the membrane was homogenized in 5 ml of methanol to extract the skin at 24,000 rpm, room temperature for 5 minutes and sonicated at room temperature for 30 minutes and centrifuged using a Hermle Z323K Centrifuge (Wehingen, Germany) at 6,000 rpm, 4°C for 30 minutes to separate the skin lipid. The clear supernatant was collected and determinate the amount of MPA retained in the skin by HPLC at 254 nm.

Statistical analysis

For statistical analysis, all the experimental data were presented as mean±standard deviation (SD).

All results were statistically evaluated using one-way analysis of variance followed by post hoc analysis for significant at $p<0.05$.

Results and discussion

Characterization of ethosome containing MPA

In this study, the ethosome formulations were prepared according to the thin-film hydration method, using the concentration of MPA 10 mg/ml. The characterization of the prepared ethosome was evaluated in terms of physical appearances, particle size, size distribution, zeta potential and entrapment efficiency. The optimized ethosome containing MPA was considered from the particle size less than 500 nm, a narrow size distribution, zeta potential more than 30 mV and entrapment efficiency more than 50%. The results of these studies were found that the formulation Etho-25 was selected as the optimized MPA ethosome. The Etho-25 including 4% w/v SPC: CHOL: Tween80: DA (6:2:1:1 molar ratio) as a lipid component and 30% v/v ethanol in phosphate buffer pH 7.4 as dispersion medium. It gave ethosome with 370.90±7.91 nm vesicular size (polydispersity index *PI* = 0.270±0.02), the zeta potential of -45.58±4.50 mV and the efficient entrapment of 56.01±1.10% as shown in Table 1. The physical appearance, optical micrograph and vesicles morphology of ethosome were visualized as show in Figure 1(A), (B) and (C), respectively. This formulation was improved the entrapment efficiency of the main compositions. The main composition including 4% w/v SPC as a lipid vesicle structure and 30% v/v ethanol mixed with phosphate buffer pH 7.4 as the dispersion media. It had low entrapment efficiency (16.52±0.08%). So, it was improved the entrapment efficiency by adding an additive such as CHOL, Tween 80 and DA. CHOL is a stabilizing agent by its molecule was inserted into lipid bilayer whereas the hydroxyl group was connected to the aqueous phase. The aliphatic chain paralleled to the acyl chains of SPC that result to the rigidity in the lipid vesicles increasing from intravesicle interaction leading to prevents leakage and reduces the membrane permeability of the ethosome vesicles. Moreover, the addition of CHOL in formulations could increase in both size and efficient entrapment (Lopez-Pinto *et al.*, 2005). Tween 80 could enhance the entrapment efficiency from its solubilizing effect (Yang *et al.*, 2007). Probably, it could increase stability property of ethosome by its hydrocarbon tail might be penetrate into the lipid bilayer of the vesicle (Xia S and Xu S, 2005; Yang *et al.*, 2007). The effects of DA as negative charge agent that on ethosome characteristic. It could increase the entrapment efficiency of ethosome due to it causes increased interbilayer from the electrostatic repulsion of it (Pinsuwan *et al.*, 2010). And the increase of negative surface charge can also help to prevent the aggregation of vesicle (Fang *et al.*, 2006). However, The appropriate formulation, not only effect of the additives but also depending on other factors such as influence of ethanol and phospholipids, the concentration ratio, type of drug incorporated, as well as the method and condition of preparation.

Table 1 The physical characteristics of the optimized ethosome formulation

No.	Main compositions		Additives		Vesicle size (nm)	Polydispersity Index (nm)	Entrapment efficiency (%)	Zeta potential (mV)
	SPC (%w/v)	Ethanol (%v/v)	Compositions	Molar ratio				
Etho-25	4	30	SPC:CHOL:Tween80:DA	6:2:1:1	370.90±7.91	0.270±0.02	56.01±1.10	-45.58±4.50

Each data represents the mean±SD (n = 3). SPC, L-α-phosphatidylcholine from soybean; CHOL,Cholesterol from lanolin; DA, deoxycholic acid; Tween 80, polyoxyethylene sorbitan monooleate.

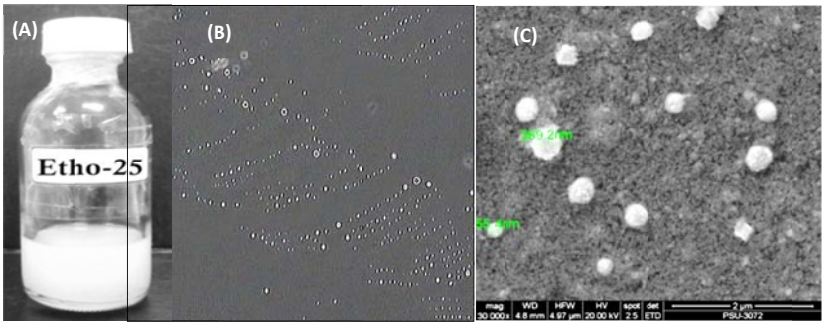

Figure 1 the physical appearance (A), optical micrograph image (×400 magnification) (B) and Scanning Electron microscope or SEM (×30,000 magnification) (C) of the optimized formulation (Etho-25)

***In vitro* skin permeation and skin retention studies**

In vitro skin permeation and skin retention of the optimized ethosome formulation were experimented using modified Franz-type diffusion cell were carried out. The newborn pig skin was used as a skin model in these studies. The ability of ethosomal carrier to deliver MPA through pig skin was evaluated for study the possibility of the ethosome as the topical delivery system through human skin. The skin permeation and retention of MPA from ethosome was tested and compared with MPA from aqueous suspension, hydroethanolic solution at the same percentage of ethanol (30%v/v), liposome at the same composition and concentration of lipid component but without ethanol in the formulation and liposome that was developed by Rattanat, 2008.

The skin permeation profiles and the permeation parameters e.g. steady state flux J_{ss}, permeation coefficient K_p and lag time T_{lag} of this study are shown in Figure 2 and Table 2, respectively. It could be seen clearly in the permeability profile that throughout the assay period of 24 hours. The cumulative amount at 24 hours (Q_{24}) of MPA ethosome formulation permeated through pig skin was 307.29±24.93 μg/cm^2 (Figure 2). The Q_{24} from ethosome was 3.58-fold higher than that from the hydroethanolic solution (85.91±14.73 μg/cm^2), 1.66-fold higher than that from the liposome (185.32±18.27 μg/cm^2) and 1.72-fold higher than that from the liposome that was developed by Rattanat, 2008 (178.88±13.31 μg/cm^2). For MPA from aqueous suspension, the drug could not be detected in receptor fluid throughout the experiment. This incident could be confirmed that the MPA as a lipophilic drug, which has slightly solubility in water (13 μg/ml at 250^{0}C), it might still remained on stratum corneum lipid and not penetrate into the skin. Further, the steady state flux J_{ss} (13.20±0.91 μg/cm^2/h) and the skin permeation coefficient K_p (1.32±0.09 ×10^{-3}cm/h) from ethosome significantly higher (p<0.05) than other formulations. Furthermore, the lag time of ethosome (1.16±4.67 hours) longer than other formulations. From the skin permeation study, these results were proved that the ethosome system was more effective in delivering MPA than other formulations.

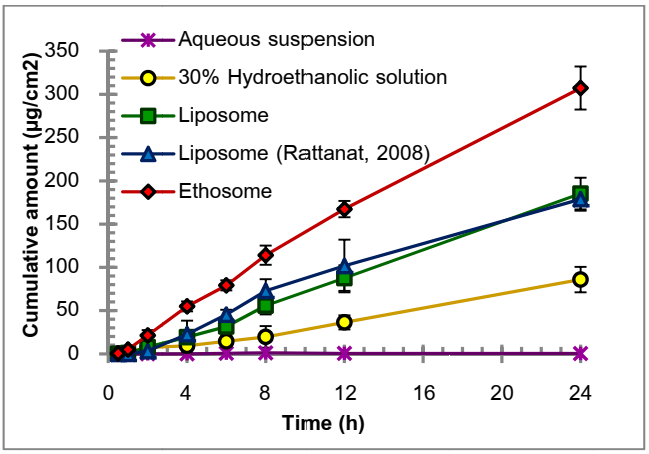

Figure 2 *In vitro* cumulative amount-time profiles of MPA permeated across pig skin from the aqueous suspension, 30% hydroethanolic solution, liposomes, liposomes that was developed by Rattanat (2008) and ethosome. Each point represents the mean±SD (n=5).

Table 2 *In vitro* skin permeation parameters of MPA from the aqueous suspension, 30% hydroethanolic solution, liposomes, liposomes that was developed by Rattanat (2008) and ethosome

Formulations	J_{ss} (µg/cm^2/h)	K_p (×10^{-3} cm/h)	T_{lag} (h)
Aqueous suspension	*UD	*UD	*UD
30% Hydroethanolic solution	3.55±0.58	0.36±0.06	3.26±4.57
Liposomes	8.02±0.60	0.80±0.06	8.97±3.25
Liposomes (Rattanat, 2008).	8.01±0.36	0.80±0.04	4.80±2.37
Ethosome	13.20±0.91	1.32±0.09	1.16±4.67

Each data represents the mean±SD (n = 5). *UD, undetected; J_{ss}, steady state flux; K_p, permeation coefficient. T_{lag}, Lag time

In the skin retention study, the amount of the MPA accumulated in the pig skin was analyzed after the end of the skin permeation at 24 hours (Figure 3). The ethosome system and hydroethanolic solution gave comparable amount of MPA accumulated in the pig skin (45.46±6.80 µg/cm^2 vs. 49.73±3.71 µg/cm^2; p<0.05). The MPA ethosome accumulated singnificantly more MPA in pig skin than that from the aqueous suspension, liposome and liposome that was developed by Rattanat (2008) which was 7.72±3.45, 29.50±8.27 and 24.54±5.90 µg/cm^2, respectively. These results indicated that ethosome has effective at delivering MPA to and through the skin to the systemic circulation. The better permeability of ethosome carriers may be the result of the addition of ethanol into the formulation. Ethanol has long been used as a skin permeation enhancer for topical and transdermal delivery. It could increase MPA solubility leading to enhance of the entrapment efficiency of MPA in the formulation, could alteration of stratum corneum's barrier property and could increasing in thermodynamic activity due to evaporation of ethanol. However, the skin permeation not only effect of the ethanol but also depending on phospholipids and skin lipids. Touitou *et al.* (2000) suggested a hypothetical model of ethosome for enhance the penetration of drug through the stratum corneum lipid. It was explained that the better skin permeation of ethosome may be due to the synergistic mechanism between high concentration of ethanol, phospholipids vesicles and skin lipids. The stratum corneum lipid at physiological temperature are densely packed and highly conformationally ordered. Ethanol interacts with lipid molecules in the polar head group region that the result in increasing fluidity and may finally lead to increase membrane permeability. In addition, it may provide the vesicles with soft flexible characteristics which are easy to penetrate into deeper layers of the skin. The malleable ethosome vesicle can forge paths in the disordered stratum corneum. The release of drug could be the result of fusion of ethosome with skin lipids and drug release at various points along the penetration pathway.

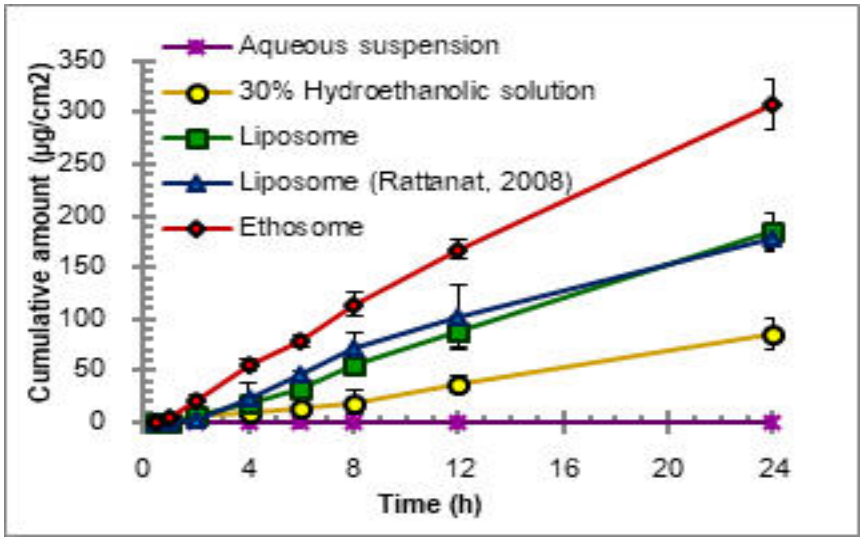

Figure 3 *In vitro* amount of MPA accumulated in pig skins after 24-hours *in vitro* skin permeation experiment. Each bar represents the mean±SD (n = 5). *UD = undetected

Table 3 The amount recovery of MPA from various formulations in the donor compartment, pig skin and receptor compartment after the end of the skin permeation studies at 24 hours.

Formulations	Amount recovery (% of the applied dose)			
	Donor compartment	Pig skin	Receptor compartment	Total
Aqueous suspension	85.40±9.38	0.14±0.07	*UD	85.56±9.39
30% Hydroethanolic solution	93.64±4.58	0.88±0.07	1.39±0.26	94.92±4.80
Liposomes	93.92±11.91	0.52±0.16	2.98±0.32	97.43±12.11
Liposomes (Rattanat, 2008).	88.67±5.61	0.43±0.12	2.81±0.24	91.91±5.51

Data are expressed as % of total MPA in the applied dose (mean±SD, n = 5). *UD = undetected.

Moreover, the distribution of MPA in three compartments (donor, pig skin and receptor) was calculated to evaluate the performance and reliability of the method. So, the amount of MPA remaining in the donor compartment was analyzed after the end of experiment at 24 hours (Table 3). The total MPA recovery from the three compartments for ethosome, aqueous suspension, hydroethanolic solution, liposome and liposome that was developed by Rattanat (2008) was close to 100% of applied dose. This indicates that this method has performance and reliability.

Conclusion

In this study, the ethosome containing MPA to apply for a topical delivery system was developed. The appropiate ethosomal formulation (Etho-25) was composed of 4% w/v SPC with CHOL, Tween80 and DA as additives with a molar ratio of SPC:CHOL:Tween80:DA was 6:2:1:1. The dispersion medium was 30% v/v ethanol in phosphate buffer pH 7.4. This formulation gave ethosome with 370.90±7.91 nm vesicular size (PI = 0.270±0.02 nm), the zeta potential of -45.58±4.50 mV and the entrapment efficiency of 56.01±1.10%. The appropriate ethosome provided significantly higher skin permeation parameter such as Q_{24} (307.29±24.93 μg/cm^2), J_{ss} (13.20±0.91 μg/cm^2/h) and K_p (1.32±0.09 ×10^{-3}cm/h), compared to aqueous suspension, hydroethanolic solution, liposome and liposome that was developed by Rattanat (2008). Further, the T_{lag} of ethosome was 1.16±4.67 hours. Furthermore, the ethosome system gave the MPA accumulated in the skin higher than (45.46±6.80 μg/cm^2) various formulations. These results indicated that the ethosome could enhance the skin permeation and retention of MPA that leading to the efficiency in topical and transdermal delivery system.

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