Original Article

ผลของน้ำมัน น้ำและสารลดแรงตึงผิวผสมต่อคุณสมบัติทางเคมีกายภาพและการนำส่งผ่านผิวหนังของไมโครอิมัลชันฟีแนสเทอร์ไรด์: การประเมินและทำนายผลด้วยวิธีพื้นผิวตอบสนอง

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บทคัดย่อ

บทนำ ฟีแนสเทอไรด์เป็นยาชนิดรับประทานที่มีประสิทธิภาพในการรักษาโรคศีรษะล้านจากพันธุกรรม โดยออกฤทธิ์ยับยั้งเอนไซม์ 5 α- reductase ชนิดที่ 2 ปัจจุบันมีความพยายามในการพัฒนายาฟีแนสเทอไรด์ในรูปแบบนำส่งทางผิวหนังเพื่อหลีกเลี่ยงอาการข้างเคียงจากยาฟีแนสเทอไรด์ ซึ่งไมโครอิมัลชัน จัดเป็นระบบนำส่งยาที่มีความน่าสนใจอีกระบบหนึ่ง การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของน้ำมัน น้ำ และสารลดแรงตึงผิวผสมต่อลักษณะทางเคมีกายภาพของไมโครอิมัลชันที่กักเก็บยาฟีแนสเทอไรด์ และการซึมผ่านผิวหนังในหลอดทดลอง **วิธีดำเนินการวิจัย** สร้างแผนภาพวัฏภาคไตรภาคเทียมเพื่อหาบริเวณที่เกิดไมโครอิมัลชัน จากนั้นเลือกสัดส่วนขององค์ประกอบทั้งสาม ที่อยู่ในบริเวณที่เกิดไมโครอิมัลชัน 7 สัดส่วนเพื่อนำมาทดสอบคุณสมบัติทางเคมีกายภาพ ได้แก่ ขนาดอนุภาค การกระจายขนาดอนุภาค การนำไฟฟ้า และค่าความเป็นกรด-ด่าง ของตำรับ โดยเปรียบเทียบระหว่างตำรับที่มีและไม่มียาฟีแนสเทอไรด์ จากนั้นนำข้อมูลที่ได้ไปทำนายผลด้วยวิธีพื้นผิวตอบสนองและศึกษาการซึมผ่านผิวหนังของยาฟีแนสเทอไรด์ในไมโครอิมัลชัน **ผลการวิจัย** การสร้างแผนภาพวัฏภาคไตรภาคเทียมพบว่า ในระบบที่ประกอบด้วย Tween 20 และ propylene glycol สัดส่วน 3:1 ให้พื้นที่ของไมโครอิมัลชันสูงสุด และการเติมยาฟีแนสเทอร์ไรด์ความเข้มข้น 0.1 %w/w ไม่มีผลต่อคุณสมบัติทางเคมีกายภาพของไมโครอิมัลชัน และตำรับที่มีน้ำในปริมาณสูงจะส่งผลให้ยาซึมผ่านเข้าสู่ผิวหนังได้ดีว่าตำรับที่มีน้ำในปริมาณต่ำ เมื่อตำรับมีปริมาณสารลดแรงตึงผิวผสมเท่ากัน **สรุปผลการวิจัย** การทำนายผลด้วยวิธีพื้นผิวตอบสนองสามารถทำให้ทราบความสัมพันธ์ระหว่างส่วนประกอบทั้งชนิดและปริมาณในตำรับไมโครอิมัลชัน ที่มีอิทธิพลต่อคุณสมบัติทางเคมีกายภาพและการซึมผ่านผิวหนังของยาได้ ซึ่งทำให้สามารถนำมาพัฒนาหาตำรับไมโครอิมัลชันที่เหมาะสมที่สุดที่สามารถนำไปใช้ต่อไป

คำสำคัญ: ฟีแนสเทอไรด์, ไมโครอิมัลชัน, ระบบนำส่งยาทางผิวหนัง, วิธีพื้นผิวตอบสนอง

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Effect of oil, water and surfactant mixture on the physicochemical properties and transdermal delivery of finasteride-loaded microemulsions: evaluation and prediction using response surface methodology

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Abstract

Introduction: Finasteride is an effective drug for the treatment of androgenetic alopecia by inhibition of type II 5α- reductase. However, oral administration finasteride has the undesirable systemic side effect about sexual dysfunction. Therefore topical delivery system of finasteride is a method to avoid this problem. Microemulsion (ME) is an interest delivery system for delivery of finasteride through the skin. The objective of this study was to investigate the effect of oil phase, water phase and surfactant mixture (Smix: surfactant and co-surfactant) on the physicochemical properties of finasteride-loaded microemusion. **Methods:** The pseudoternary phase diagrams were constructed to obtain the microemulsion area. Then the seven different ratios of microemulsion were selected to evaluate the physicochemical properties (droplet size, size distribution, conductivity and pH) by comparing between blank and finasteride-loaded microemulsion. The response surface method was used to predict the results by using computer program. The three finasteride-loaded microemulsion formulations with the same percentage of surfactant mixture were selected to study the *in vitro* skin permeation. **Results:** The pseudoterary phase diagram of microemulsion system with Smix at ratio 3:1 showed the largest area of microemulsion**,** therefore this system was selected to incorporate finasteride into the formulation. Finasteride did not affect the change in physicochemical properties of microemulsion. The *in vitro* skin permeation results indicated that the formulation with high concentration of water showed higher permeation flux than the formulation with low concentration of water. **Conclusion:** The prediction of finasteride-loaded ME by using response surface methodology showed the relationship between the amount of the composition in ME formulation and the physicochemical properties. This suggested that the optimal formulation can be developed for transdermal delivery of finasteride in treatment of androgenetic alopecia. However, other properties of the formulation, such as entrapment efficiency, skin retention, toxicity and stability of the formulation should be investigated.

Keywords: Finasteride, Microemulsion, Transdermal delivery system, Response surface methodology

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Introduction

Androgenetic alopecia (AGA) is the common problem among men and can affect the self confident in daily life. Finasteride (FN) is the effective drug for treatment of AGA approved by US FDA. FN is the selective type II 5α- reductase enzyme inhibitor and can reduce the converting of testosterone to dihydrotestosterone which is the cause of AGA (Saraswat and Kumar, 2003). However, oral finasteride has the undesirable systemic side effect such as decreased libido, ejaculation disorder, erectile dysfunction and gynecomastia (Kumar et al., 2007), Moreover, 5α- reductase enzyme which is the target of finasteride had found in the dermal papilla cell of hair follicles (Liu and Yamauchi, 2008), therefore development of transdermal delivery of finasteride in treatment of AGA is in demand to decrease the systemic side effects and improve the skin penetration of the drug. Many drug delivery systems were developed to enhance the skin permeation of finasteride such as liposome (Kumar et al., 2007), niosome (Tabbakhian et al., 2006), ethosome (Rao et al., 2015), polymersome (Caon et al., 2014) and liquid crystalline nanoparticle (Madheswaran et al., 2013).

Microemulsion (ME) is the special emulsion system consisting of oil, water, surfactant and co-surfactant and has the small droplet size which typically less than 150 nm (Jaipakdee et al., 2014). The application of ME for cutaneous drug delivery is becoming increasingly popular due to its high solubilization potential for both lipophilic and hydrophilic drug. As ME contains high concentration of surfactant system, it provides high skin permeability but may cause skin irritation to patients. Therefore, it is important to optimize the ME formulation in the development processes for suitable ME (Duangjit et al., 2015).

The aims of this study were to investigate the effect of the ratio of oil, surfactant mixture and water in development of finasteride-loaded microemulsion based on computer design and to optimize the ME system for transdermal delivery.

Materials and Methods

Materials

Finasteride was supported by Bangkok Lab & Cosmetic Co.,Ltd (Ratchaburi, Thailand). Cinnamon oil was purchased from New Sang Thong Trading.,L.P. (Bangkok, Thailand). Tween® 20 and propylene glycol were purchased from Namsiang International Co., LTD (Bangkok, Thailand). All other chemical reagents and solvents were analytical and HPLC grade.

Construction of pseudoternary phase diagrams

The pseudoternary phase diagrams of ME were constructed by the water titration method. Three components of ME including oil phase, water phase and mixture of surfactant and co-surfactant (Smix). In this study cinnamon oil was used as oil phase due to the capability to dissolve the drug. Tween® 20 was the

surfactant in this ME system and propylene glycol was used as co-surfactant. The surfactant mixtures were prepared in different weight ratio, 1:1, 2:1 and 3:1, of surfactant and co-surfactant. Then the Smix was dissolved in oil phase in the vial at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 (oil: Smix). Each vial of mixture was titrated drop-wisely with purified water from burette, and stirred with magnetic until the mixture became turbid (Duangjit et al., 2015).

The quantity of water required was recorded then the percentage of each component was calculated. The pseudoternary phase diagram was established to delineate the area of ME and plotted by using Prosim® ternary diagram software.

Preparation of finasteride-loaded microemulsion

From the ME area of pseudoternary phase diagram, 0.1% w/w finasteride was dissolved together with selected seven different ratios (Duangjit et al., 2015) as presented in Table 1. Finasteride was dissolved in cinnamon oil, then Smix and water were added, and stirred with a magnetic stirrer. Finasteride-loaded ME was stored in airtight containers.

Characterization of microemulsion and finasteride-loaded microemulsion

Droplet size, size distribution, electrical conductivity and pH of free ME and FN-loaded ME were characterized. The dynamic light scattering technique (Zetasizer Nano ZS, Malvern Instruments Worcestershire, UK) was used to evaluate droplet size and size distribution. Mean droplet size and PDI (Polydispersity Index) were recorded. The electrical conductivity was measured using a conductivity meter (S230 SevenCompact™, Mettler Toledo, Switzerland). The pH of microemulsion was determined using pH meter (S220 SevenCompact™, Mettler Toledo, Switzerland). Each measurement was determined in triplicate at 25°C, and then the average and standard deviation were calculated.

Computer design for prediction of response surface and optimal ME

Design Expert® software with a simplex lattice design was utilized to optimize the ME system. The casual factor consists of oil phase (X_1), surfactant system (X_2) and water phase(X_3), based on the area under the pseudo-ternary phase diagram. The upper and lower limits of each component were assign as follows:

$$5 \leq X_1 \leq 25\ (\%)$$
$$55 \leq X_2 \leq 75\ (\%)$$
$$10 \leq X_3 \leq 30\ (\%)$$
$$X_1 + X_2 + X_3 = 100\ (\%)$$

The physicochemical characteristics of ME such as droplet size (Y_1), size distribution (Y_2), zeta potential (Y_3), pH (Y_4) and electrical conductivity (Y_5) were define as response variables. The seven model formulations of FN-loaded ME were prepared from ME that had the biggest area of pseudo-ternary phase diagram.

***In vitro* skin permeation study**

The evaluation of finasteride from the

selected three ME formulation penetrated into rat skin. The protocols of experiment were approved by the ethics committee for the use of laboratory animal, Faculty of Pharmacy, Silpakorn university (Protocol Number:001/2560). Full thickness skin from Sprague Dawley (SD) rat (5-6 weeks old) was used as membrane. The whole subcutaneous layer was removed from rat skin carefully and then cleaned with phosphate buffer pH 7.4. The rat skin was stored at 20°C and thawed before used. The rat skins were mounted between donor chamber and receptor chamber of Franz diffusion cells. The stratum corneum side was faced upward into donor chamber. The receptor chamber was filled with 6.0 mL of 50% ethanol in phosphate buffer pH 7.4, and then the temperature at 32°C (the temperature of the skin) (Jaipakdee et al., 2014) was controlled using water jacket. The donor chamber was filled with 1 g of 0.3% w/w FN-loaded ME formulations after that 1.0 mL of the receptor medium was withdrawn from the receptor chamber at 0.5, 1, 2, 4, 6, 8 and 24 h , and the same volume of fresh medium was replaced. HPLC was used to analyze the amount of finasteride penetrating through the skin. The results were shown in permeation profile and the skin permeation flux was the slope of the linear portion of the profile.

Statistical analysis

Each experiment was repeated at least three time and the results were expressed as mean ± S.D. Statistical analysis of all determination were performed using one-way analysis of variance (ANOVA) and Duncan's multiple range test. The *P* Values less than 0.05 would be considered as statistically significant.

Results

Construction of pseudoternary phase diagrams of ME and preparation of finasteride-loaded ME

The constructed pseudoternary phase diagrams of ME are presented in Figure 1. The ME system composed of cinnamon oil as oil phase and Smix (Tween®20 + propylene glycol) at 3:1 ratio showed the largest area (gray color) of ME. Therefore the ME system with Smix at 3:1 ratio was selected to incorporate with 0.1% w/w of finasteride. The seven formulations of ME are presented in Table 1. At the same formulation, blank ME without FN was also prepared and FN-loaded ME.

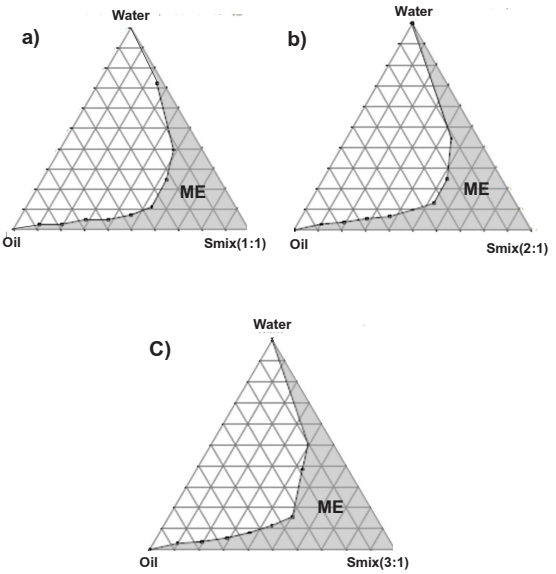

Figure 1. Pseudoternary phase diagram of ME a) Smix at 1:1 ratio, b) Smix at 2:1 ratio and c) Smix at 3:1 ratio.

Table 1 The amount of each component of seven ME formulation (%w/w) with Smix at 3:1 ratio

Formulation	Oil phase	Smix	Water phase
1	15	55	30
2	5	65	30
3	5	75	20
4	15	75	10
5	25	65	10
6	25	55	20
7	15	65	20

Characterization of microemulsion and finasteride-loaded microemulsion

The appearance of both finish blank ME and FN-loaded ME was clear solution. The average size and polydispersity index (PDI) of free ME and FN-loaded ME (0.1% FN) evaluated by using dynamic light scattering technique are presented in Table 2. The seven model blank ME had droplets size ranging from 232 to 366 nm, and FN-loaded ME had inner droplets size ranging from 207 to 391 nm. There was no significant difference in droplet size between blank ME and FN-loaded ME. However both group of ME showed the droplet size larger than 150 nm which might be caused from the composition of propylene glycol, long chain alcohol, in the formulation (Maghraby GM, 2008). In 2010, Shah RR et al. reported the droplet size of aceclofenac topical microemulsion was larger than 200 nm, however the evaluation of other properties such as optical birefringence by polarized light microscopy indicated isotropic system of microemulsion.

The blank ME and FN-loaded ME showed the narrow size distribution and the homogeneity of droplet size supported by PDI ranging from 0.39 to 0.76 for blank ME and ranging from 0.39 to 0.50 for FN-loaded ME.

Table 2 Droplet size, size distribution (PDI), conductivity, pH of blank and FN-loaded microemulsion formulations (mean ±SD), *n*=3)

ME	Size (nm)		PDI		Conductivity (mS/cm)		pH	
	Blank ME	0.1% FN	Blank ME	0.1% FN	Blank ME	0.1% FN	Blank ME	0.1% FN
1	232.67±16.34^{abc}	207.53±14.86^{a}	0.42±0.02^{a}	0.45±0.06^{ab}	0.052±0.003^{g}	0.047±0.000^{f}	6.61±0.02^{c}	6.52±0.03^{k}
2	259.65±22.88^{abc}	267.48±13.28^{bcd}	0.39±0.03^{a}	0.39±0.02^{a}	0.045±0.001^{f}	0.046±0.000^{f}	7.58±0.04^{j}	7.36±0.03^{l}
3	315.93±10.66^{de}	349.48±32.20^{ef}	0.40±0.07^{a}	0.45±0.07^{ab}	0.027±0.001^{cd}	0.027±0.003^{cd}	7.74±0.02^{l}	7.63±0.02^{k}
4	366.70±17.06^{f}	391.73±119.8^{f}	0.46±0.13^{ab}	0.43±0.03^{a}	0.014±0.000^{a}	0.016±0.002^{a}	7.32±0.01^{h}	7.26±0.02^{g}
5	310.62±47.92^{de}	231.07±37.43^{abc}	0.76±0.04^{c}	0.50±0.09^{b}	0.021±0.004^{b}	0.014±0.000^{a}	6.85±0.02^{d}	6.85±0.03^{d}
6	269.35±34.51^{cd}	214.60±14.88^{ab}	0.40±0.03^{a}	0.41±0.04^{a}	0.031±0.003^{e}	0.027±0.001^{c}	6.45±0.04^{a}	6.48±0.02^{a}
7	248.48±6.97^{abc}	230.48±19.38^{abc}	0.39±0.02^{a}	0.44±0.03^{ab}	0.029±0.001^{cd}	0.029±0.002^{de}	6.98±0.03^{f}	6.92±0.02^{e}

The conductivity and pH of blank ME and FN-loaded ME are presented in Table 2. Blank ME had the conductivity ranging from 0.0163 to 0.0530 mS/cm, and was close to FN-loaded ME that had the conductivity ranging from 0.0160 to 0.0471 mS/cm, indicating that these ME were classified as oil-in-water (>0.01 mS/cm) (Duangjit et al., 2015). Both two groups of ME formulation had pH ranging from 6.5 to 7.7. These results indicated that the addition of finasteride into ME did not change the physicochemical property of ME.

Computer design for prediction and optimal ME by using Design Expert®

The response surface comparing between blank ME and FN-loaded ME are presented in Figure 2. All of response variables were evaluated and sketched using the Design Expert® Software (Version 8), Approved No 009503 (Stat-Ease. Inc., Minneapolis, MN). The ratio of cinnamon oil (X_1), surfactant mixture (X_2) and water (X_3) were defined as causal factors, while the physicochemical characteristics such as droplet size (Y_1), size distribution (Y_2), zeta potential (Y_3), conductivity (Y_4) and pH (Y_5) were defined as response variables. The results of response surface exhibited uncomplicated relationships between the causal factors and the response variables.

The droplet size of blank ME and o.1% FN-loaded ME increased when the concentration of surfactant mixture were increased and incorporation of 0.1% FN into ME did not significantly affect the droplet size of ME (Figure 2a.). The size distribution of FN-loaded ME was not significantly different from blank ME. However, both blank ME and FN-loaded ME showed the narrow size distribution when the formulation had high percentage of water (Figure 2b). In the response surface of conductivity prediction, both blank ME and FN-loaded ME had high electrical conductivity (>0.01 mS/cm), so they could be classified as water-in-oil ME (Duangjit et al., 2015). The conductivity of ME was influenced by the composition of the ME. When the water content was increased, the electrical conductivity increased (Figure 3c). The response surface of pH presented that the pH of ME depended on the composition of ME. The pH of both blank ME and

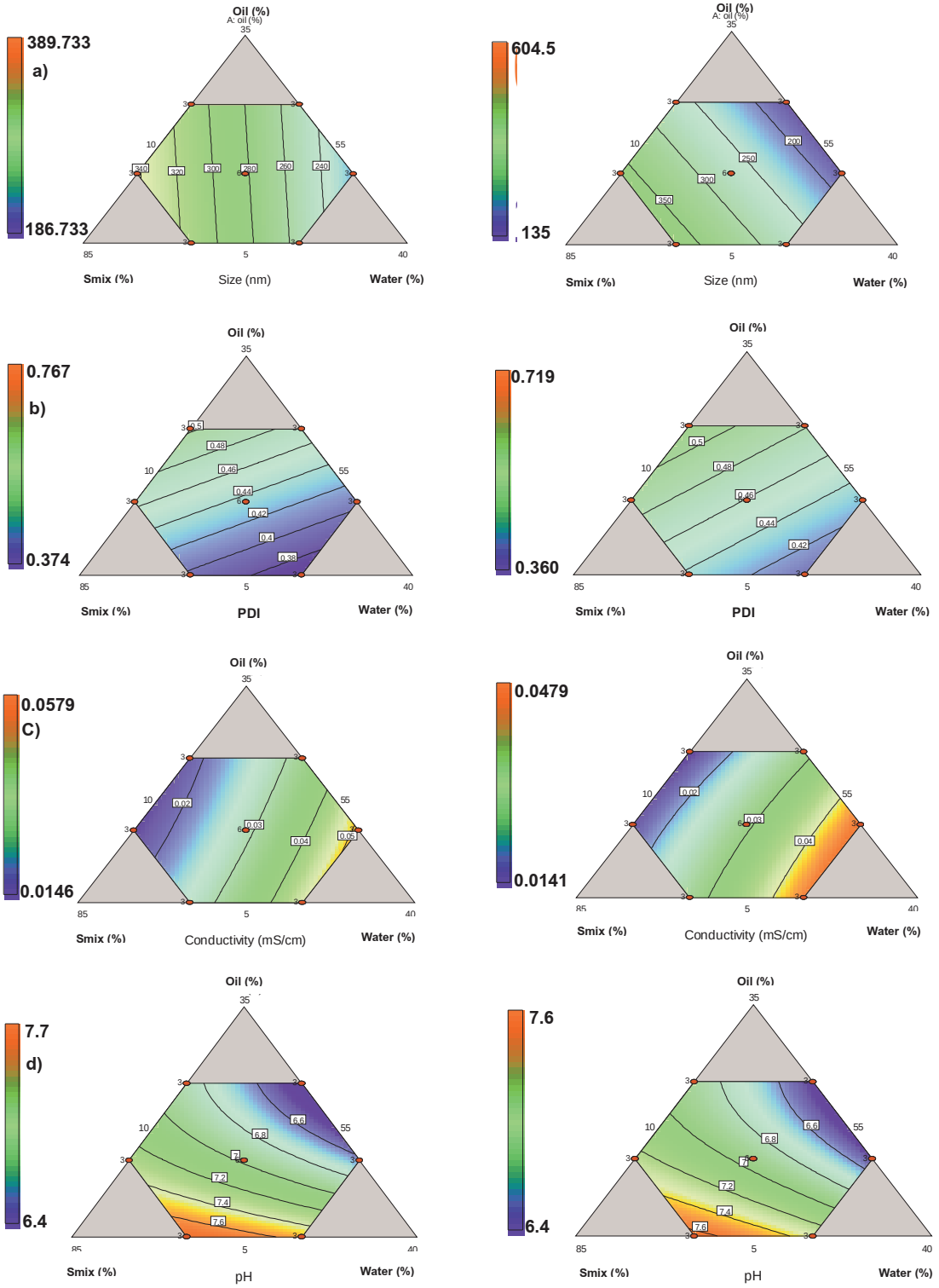

Figure 2 The response surface of a) droplet size, b) PDI, c) conductivity and d) pH for free ME (left column) and FN-loaded ME (right column).

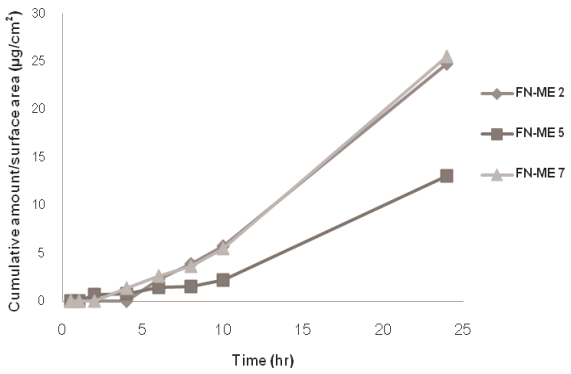

Figure 3 The skin permeation profile of FN-loaded ME 2, FN-loaded ME 5 and FN-loaded ME 7

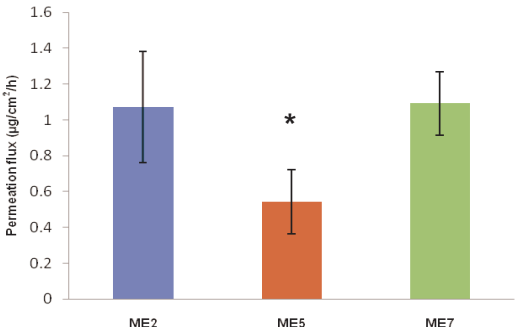

Figure 4 Permeation flux of FN-loaded ME2, FN-loaded ME5, FN-loaded ME7, presented as mean ± SD (n=3). *P<0.05

FN-loaded ME was ranging from 6.4-7.6 and theformulation became weak base when it had high percentage of oil and water (Figure 3d). The incorporation of 0.1% FN did not affect the pH. However, the zeta potential of finasteride-loaded ME could not be analyzed with this software, but all of formulation showed the neutral charge relating to the charge of each composition in ME formulations.

***In vitro* skin permeation study**

The three FN-loaded ME that had the same concentration of S_{mix} at 65 %w/w (ME2, ME5 and ME7 from Table 1) were selected to study the skin permeation. The skin permeation profile is shown in Figure 3. The skin permeation flux of the three FN-loaded ME2, ME5 and ME7 were 1.072, 0.543 and 1.092 µg/cm^2/h, respectively (Figure 4). The flux of FN-loaded ME5 was significantly lower than another two formulation at $p < 0.05$. The FN-loaded ME5 had water concentration at 10 %w/w while FN-loaded ME7 and ME5 had water concentration at 20% and 30% w/w. The results indicated that high concentration of water can enhance the skin permeation of FN-loaded ME due to the increasing of skin hydration. Many factors can affect the skin permeation profile of FN-loaded ME such as the type and concentration of oil, surfactant and co-surfactant. Therefore, other factors affecting skin permeation and skin permeation mechanism of ME should be investigated.

Discusstions and Conclusion

The type and amount of oil phase, water phase and surfactant mixture in ME formulation affected the physicochemical properties of ME supported by the prediction of finasteride-loaded ME by using response surface and optimization with Design Expert® software. High concentration of water in ME resulted in high skin permeation flux. This suggested that the optimal formulation can be developed for transdermal delivery of finasteride in treatment of androgenetic alopecia. However, other properties of the formulation, such as entrapment efficiency and drug retention in the skin should be performed to support the data of the final products before clinical study.

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