Characterization and Stability Evaluation of Film-Forming Polymeric Solution Containing Clove Oil Microemulsion for Treatment of Cutaneous Candidiasis

Chaowalit Monton and Sukanya Settharaksa
Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University, Pathum Thani, Thailand
Email: {chaowalit, sukanya.s}@rsu.ac.th

Jirapornchai Suksaeree
Department of Pharmaceutical Chemistry, College of Pharmacy, Rangsit University, Pathum Thani, Thailand
Email: jirapornchai.s@rsu.ac.th

***Abstract*—This work sought to prepare, characterize, and evaluate the stability of Film-Forming Polymeric Solution (FFPS) containing clove oil microemulsion. The ratio of film-forming polymers—Polyvinyl Alcohol (PVA) and hydroxypropyl methylcellulose (HPMC F4M)—were varied from 100:0 to 75:25. The developed FFPS containing clove oil microemulsion, which contained clove oil 1%, dried within 10-15 minutes and formed the complete film after drying. The pH of the formulations was approximately 4.8-4.9. The pH and spread ability values were increased, while the viscosity decreased when the ratio of HPMC F4M increased. The standard marker, eugenol, in the formulation was approximately 0.5%. The formulation exhibited a good anti-*Candida albicans* property; the inhibition zone was approximately 1.2 cm. Eugenol release from FFPS containing clove oil microemulsion showed the Korsmeyer-Peppas release model. Moreover, the release mechanism was anomalous transport. The anti-*Candida albicans* activity was stable for 60 days, but it was decreased at 90 days of the experiment when stored at 25 and 40ºC, except when stored at 4ºC, the anti-*Candida albicans* activity was not significantly different compared with at the initial time. In summary, the developed FFPS containing clove oil microemulsion was an alternative product that could be used for the treatment of cutaneous candidiasis.**

***Index Terms*—film-forming polymeric solution, clove oil, microemulsion, cutaneous candidiasis**

I. Introduction

Candidiasis is a fungal infection caused by yeasts belongs to the genus *Candida*. There are more than 20 *Candida* species that can cause infection in humans—*Candida albicans* is the most common pathogen. It can be found in the intestinal tract, mucous membranes, and skin without causing infection. However, it can cause significant infections in some patients under immune compromised conditions [1]. Besides good general health and hygiene, the antifungal agents may be used to treat cutaneous candidiasis. However, many antifungal agents may cause adverse drug reactions [2].

Clove oil is a volatile oil that is steam distilled from the flower buds of *Syzygium aromaticum* (L.) Merr. & L. M. Perry. The major chemical compound of clove oil is eugenol [3]. The clove oil has potent activity against *C. albicans*, which can be developed as an ingredient in an anti-*C. albicans* products. However, clove oil is a poorly water-soluble compound, making it difficult to prepare an aqueous-based product. The application of the microemulsion technique could increase the solubility and stability of the formulations [4].

Film-Forming Polymeric Solutions (FFPS) are novel drug delivery systems that can deliver drugs via the skin. The FFPS formulation is composed of the active ingredient and non-active ingredients, i.e., film-forming agent, plasticizer, and other excipients such as penetration enhancers or solvent. After the application of the formulation on the skin in liquid form, the solvent evaporated, and then it will form a film. The obtained film can control drug release to the skin [5].

The aim of this work was to prepare, characterize, and evaluate the stability of FFPS containing clove oil microemulsion. The authors expected that the developed FFPS containing clove oil microemulsion was an alternative product that could be used for the treatment of cutaneous candidiasis.

II. Methodology

A. Preparation of FFPS Containing Clove Oil Microemulsion

The blank FFPS was composed of polyvinyl alcohol (PVA) and/or hydroxypropyl methylcellulose (HPMC F4M) 5%, glycerin 5%, ethanol 20%, and water 70%.

Manuscript received June 18, 2020; revised August 31, 2020.

doi: 10.18178/ijpmbs.9.4.164-168

The PVA was heated until it almost dissolved using a heating magnetic stirrer and stands to cool. The HPMC F4M, glycerin, and ethanol were further added and mixed. It was kept in the refrigerator overnight to allow the polymer completely to swell. The clove oil microemulsion was prepared by simple mixing of clove oil 2%, Tween® 80 9%, polyethylene glycol 400 9%, and water 80%. A clear microemulsion was obtained. The equal ratio of blank FFPS and clove oil microemulsion was mixed to produce the FFPS containing clove oil microemulsion, so the formulation contained 1% of clove oil. The blank FFPS and FFPS containing clove oil microemulsion were coded as B and F, respectively. The B1 and F1 to B6 and F6 used different ratios of PVA to HPMC F4M—100:0, 95:5, 90:10, 85:15, 80:20, and 75:25, respectively.

B. Determination of Drying Time and Integrity of Film on the Skin

The 0.1 mL of each formulation was applied to the forearm of the researcher in the area of 1×1 inch^2 (n = 3). The drying time and integrity of film on the skin after drying were observed. The integrity of film on the skin was graded into three groups—A, B, and C—A complete film with no cracks and no flaking, a complete film with cracks or sporadic flaking, and a film partly or completely missing, respectively [6].

C. Determination of pH

The pH of the formulation was measured using pH meter (SevenCompact S220, Mettler Toledo, Switzerland). Each formulation was evaluated in triplicate; then, the mean and Standard Deviation (SD) were reported.

D. Determination of Viscosity

The viscosity of the formulation was measured using a viscometer (Brookfield DV-II+, USA) with the S00 spindle. It was measured every 10 seconds for one minute, so the six values were obtained. It was done three times. Then, the mean and SD were reported.

E. Determination of Spreadability

The one gram of FFPS (W) was dropped on the center of the glass plate and compressed with another glass plate. The spreading area (A) of the sample and the time taken to spread on the glass plate (T) were collected. The spreadability value was calculated by W×A/T. A unit of this value was g·cm^2/s [7]. Each formulation was performed in triplicate, and the mean and SD were reported.

F. Determination of Drug Content

Eugenol was selected as a standard marker. The eugenol in a concentration range of 10–200 µg/mL was used to construct a calibration curve. The 100 mg of the formulation was diluted using methanol in a 10-mL volumetric flask. Then, 100 µL of the obtained solution was added to a 10-mL volumetric flask and adjusted with methanol to the volume. It was filtered and injected into a high-performance liquid chromatography (HPLC) instrument. The eugenol content was calculated from the calibration curve.

G. HPLC Condition

Eugenol content analysis was performed using the HPLC instrument (Agilent 1260 Infinity, Agilent Technologies, USA). The analysis was done on the ACE Generix C18 column (150×4.6 mm i.d., 5 µm). The column temperature was controlled at 25°C. Isocratic elution of water and methanol in a volume ratio of 35:65 was used. The flow rate of mobile phase was set at 1 mL/min. The detection wavelength was 280 nm. The injection volume was 10 µL.

H. Determination of Anti-C. Albicans Activity

The anti-*C. albicans* activity was determined using the agar well diffusion technique. The Sabouraud dextrose agar contained in the Petri dish was drilled to produce a 0.7 mm well. A suspension of *C. albicans* ATCC 10231—Turbidity matched a 0.5 McFarland standard, was swabbed onto the surface of Sabouraud dextrose agar. The 50 µL of FFPS containing clove oil microemulsion and blank FFPS was filled into a well and was incubated at 37°C for 24 h. Each sample was performed in triplicate. The marketed product: topical cream of clotrimazole 1% was used as a positive control. The mean and SD of the inhibition zone were also reported.

I. Determination of Eugenol Release

Eugenol release from FFPS containing clove oil microemulsion was investigated using a modified Franz-type diffusion cell (Teledyne Hanson Research, Inc., USA). The effective diffusion area was 1.77 cm^2. The release medium was composed of a phosphate buffer solution (pH 7.4) and absolute ethanol at a ratio of 8:2 (12 mL). The temperature was controlled at 37±0.5°C and the stirring speed was set at 600 rpm. Either 500 µL of the formulation or 250 µL of microemulsion containing 2% clove oil was added to the donor compartment. The Spectra/Por® dialysis membrane (Spectrum Laboratories Inc., USA) with molecular weight cut-off 3500 was used. The one mL of the receptor medium was sampled to analyze the eugenol content after 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. The fresh release medium was replenished after the release medium was sampled. The content of the released eugenol was determined using HPLC (n = 3); then, the release profiles of eugenol were constructed. The release kinetics based on zero-order, first-order, Higuchi's, and Korsmeyer-Peppas models were analyzed by DDSolver—An add-in program in Microsoft Excel [8].

J. Stability Evaluation of Anti-C. Albicans Activity of FFPS Containing Clove Oil Microemulsion

The FFPS containing the clove oil microemulsion, F1, was kept at 4, 25, and 40°C for 90 days. They were sampled to evaluate anti-*C. albicans* activity every 30 days.

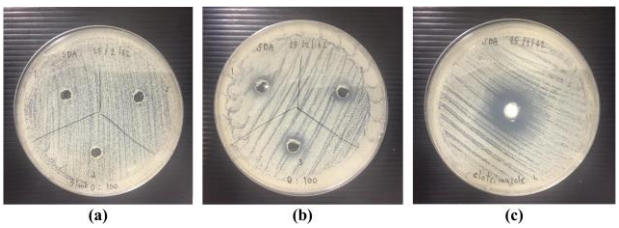
III. Results and Discussion

The blank FFPS could dry within 15-20 minutes, except B5 and B6, which contained PVA: HPMC F4M of 80:20 and 75:25, respectively, could dry within 10-15 minutes. The addition of clove oil microemulsion into blank FFPS to make FFPS containing clove oil microemulsion could decrease the drying time from 15-20 min to 10-15 min. All formulation gave a complete film after applying to the skin. The formulation was slight acidic pH. Increasing HPMC F4M ratio increased the pH value of the formulation, it could observe in both blank FFPS and FFPS containing clove oil microemulsion. The viscosity was decreased when the HPMC F4M ratio increased, while the spreadability was increased. This occurrence perhaps described by the practically insoluble in ethanol of HPMC, so it was precipitated when mixed with ethanol-contained formulation [9]. During the experiment of viscosity and spreadability, precipitation of HPMC provide less viscous mass of the FFPS formulations, so they exhibited less viscosity but easily spread. The eugenol content determined by HPLC was approximately 0.5%. According to the anti-*C. albicans* activity, all FFPS containing clove oil microemulsion formulations showed a good anti-*C. albicans* activity with the inhibition zone of approximately 1.2 cm, while the blank FFPS had no anti-*C. albicans* activity (Table I). The inhibition zones of blank formulation (B1), FFPS containing clove oil microemulsion (F1), and topical cream of clotrimazole 1%, the positive control, are shown in Fig. 1. The positive control showed the inhibition zone of 2.0 cm.

TABLE I. Physicochemical and Anti-*C. albicans* Properties of Blank FFPS and FFPS Containing Clove Oil Microemulsion

Formula	Drying time (min)	Integrity of film on the skin*	pH	Viscosity (cP)	Spreadability ($g \cdot cm^2/s$)	Eugenol content (%)	Inhibition zone (cm)**
B1	15-20	A	4.62±0.01	46.17±0.47	9.89±0.06	-	ND
B2	15-20	A	4.65±0.01	36.90±0.48	13.05±0.15	-	ND
B3	15-20	A	4.63±0.01	34.07±0.11	13.18±0.20	-	ND
B4	15-20	A	4.70±0.01	19.57±0.25	15.77±0.24	-	ND
B5	10-15	A	4.61±0.01	20.45±0.05	19.84±0.00	-	ND
B6	10-15	A	4.69±0.01	15.83±0.04	26.10±0.30	-	ND
F1	10-15	A	4.78±0.00	43.55±0.47	11.19±0.13	0.465±0.001	1.23±0.13
F2	10-15	A	4.80±0.01	44.06±0.31	13.09±0.00	0.493±0.002	1.20±0.09
F3	10-15	A	4.80±0.01	36.30±0.85	15.77±0.18	0.458±0.002	1.22±0.12
F4	10-15	A	4.82±0.01	31.83±0.56	15.66±0.09	0.522±0.002	1.23±0.10
F5	10-15	A	4.86±0.01	26.57±0.25	15.61±0.09	0.531±0.002	1.19±0.08
F6	10-15	A	4.86±0.01	19.36±0.03	26.28±0.15	0.487±0.002	1.20±0.10

* A: Complete film with no cracks and no flaking, **ND = not detected



(a) (b) (c)

Figure 1. Anti-*C. albicans* activity of (a) blank FFPS (B1), (b) FFPS containing clove oil microemulsion (F1), and (c) the positive control (topical cream of clotrimazole 1%).

The only F1 was selected to evaluate the release profile and stability of anti-*C. albicans* activity, due to it exhibited the most stable characteristic under visual observation. The release profiles of FFPS containing clove oil microemulsion was compared with clove oil microemulsion. It was found that clove oil microemulsion showed higher eugenol release than FFPS containing clove oil microemulsion. The release profiles based on zero-order, first-order, Higuchi's, and Korsmeyer–Peppas models are shown in Fig. 2.

The release kinetic parameters of eugenol from FFPS containing clove oil microemulsion and clove oil microemulsion are shown in Table II. Between the main-release kinetic models, i.e., zero-order, first-order, Higuchi's, and Korsmeyer–Peppas. The release of eugenol from both the FFPS containing clove oil microemulsion and clove oil microemulsion approached to the Korsmeyer–Peppas and Higuchi's model, respectively. The release exponent (n) indicated that the drug transport mechanism of the eugenol was anomalous transport [10]. However, the drug release from the FFPS system could also fit with other kinetic models, such as the zero-order [11], [12] and Higuchi's model [7], [12]-[14].

TABLE II. Release Kinetic Parameters of Eugenol from FFPS Containing Clove Oil Microemulsion and Clove Oil Microemulsion

Release kinetic		FFPS containing clove	Clove oil
Zero-order	k_0	6.15±0.58	6.93±0.17
	T_{la}	0.14±0.08	0.02±0.13
	R^2	0.9638±0.0106	0.9542±0.0195
First-order	k_1	0.08±0.01	0.10±0.00
	T_{la}	0.38±0.03	0.33±0.07
	R^2	0.9789±0.0047	0.9764±0.0103
Higuchi's	k_H	17.79±1.75	20.16±0.47
	T_{la}	1.64±0.08	1.53±0.17
	R^2	0.9970±0.0021	0.9928±0.0059
Korsmeyer-Peppas	k_{KP}	20.20±3.77	18.97±3.56
	T_{la}	1.81±0.07	1.25±0.49
	R^2	0.9988±0.0004	0.9917±0.0068
	n	0.43±0.06	0.52±0.09

* The k_0, k_1, k_H, and k_{KP} were released at a constant rate according to the zero-order, first-order, Higuchi's, and Korsmeyer–Peppas models, respectively. The T_{lag} is lag time, and n is the release exponent.

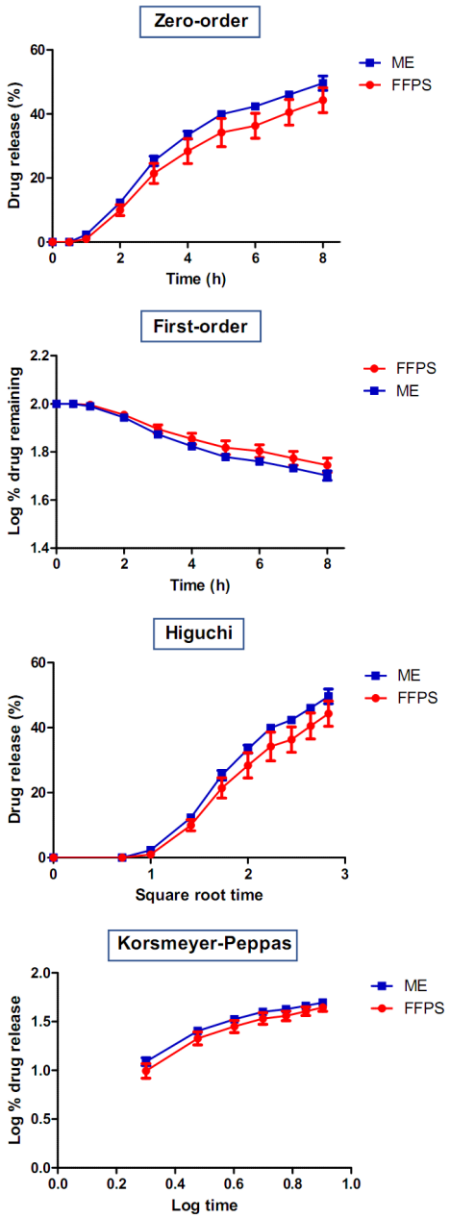


Figure 2. Release profiles of eugenol from FFPS containing clove oil microemulsion (FFPS) and clove oil microemulsion (ME) based on zero-order, first-order, Higuchi's, and Korsmeyer–Peppas models.

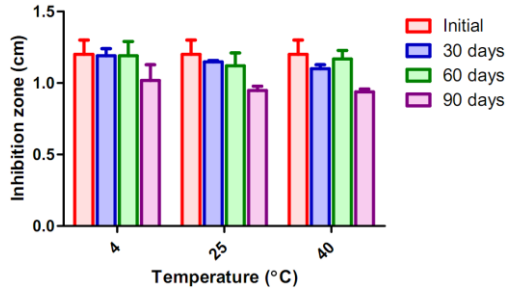


Figure 3. Anti-*C. albicans* activity of FFPS containing clove oil microemulsion after stored at 4, 25, and 40°C.

The inhibition zone was observed after stored the FFPS containing clove oil microemulsion (F1) for 90 days at different temperatures: 4, 25, and 40°C (Fig. 3). The anti-*C. albicans* activity was stable for 60 days, but the activity was significantly decreased after stored for 90 days, except when stored at 4°C. The decreasing anti-*C. albicans* activity when the formulation was stored at 25 and 40°C, might relate to the volatile property of clove oil. However, the formulation remains exhibited the good *C. albicans* activity, although some clove oil evaporated from the formulation. The author suggested that the formulation should be stored in refrigerator to preserve the shelf-life. Our further work will improve the stability of the formulation to prolong the shelf-life of the product.

IV. Conclusions

The ratio of PVA and HPMC F4M affected the physicochemical properties of FFPS containing clove oil microemulsion. The developed FFPS containing clove oil microemulsion dried within 10-15 min and formed the complete film after drying. Increasing of HPMC F4M ratio increased the pH and spreadability values, while decreased the viscosity. The developed formulation showed a good anti-*C. albicans* property. Eugenol release from FFPS containing clove oil microemulsion was approximately 40% within 8 h. The anti-*C. alicans* activity was stable at least 90 days when stored the formulation at 4ºC. The improvement of its stability will be performed in further work. In summary, the developed FFPS containing clove oil microemulsion was an alternative product that could be used for the treatment of cutaneous candidiasis.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

CM is a project leader, designed the experiments, contributed to the experimental parts, analyzed and interpreted the results, and drafted the manuscript. SS is contributed to the experimental parts, analyzed and interpreted the results. JS analyzed and interpreted the data. All authors have read and approved the final version of the manuscript.

Acknowledgment

The authors would like to thank Miss Kannika Kittiyarangsit and Miss Kittaporn Santichat for her research assistance.

References

[1] E. Palese, M. Nudo, G. Zino, V. Devirgiliis, M. Carbotti, E. Cinelli, *et al.*, "Cutaneous candidiasis caused by *Candida albicans* in a young non-immunosuppressed patient: an unusual presentation," *Int. J. Immunopathol. Pharmacol.*, vol. 32, pp. 1-4, 2018.

[2] N. J. Gogtay, U. P. Kulkarni, and T. S. Panchabhai, "Adverse reactions to antifungal agents," *Adverse Drug React Bull*, vol. 251, pp. 963-966, 2008.

[3] M. H. Alma, M. Ertaş, S. Nitz, and H. Kollmannsberger, "Chemical composition and content of essential oil from the bud of cultivated Turkish clove (*Syzygium aromaticum* L.)," *BioResources*, vol. 2, pp. 265-269, 2007.

[4] A. Simonazzi, A. G. Cid, M. Villegas, A. I. Romero, S. D. Palma, and J. M. Bermúdez, "Nanotechnology applications in drug controlled release," in *Drug Targeting and Stimuli Sensitive Drug Delivery Systems*, A. M. Grumezescu, Ed., William Andrew Publishing, 2018, ch. 3, pp. 81-116.
[5] I. Z. Schroeder, P. Franke, U. F. Schaefer, and C. M. Lehr, "Delivery of ethinylestradiol from film forming polymeric solutions across human epidermis *in vitro* and *in vivo* in pigs," *J. Control Release*, vol. 118, pp. 196-203, 2007.
[6] I. Z. Schroeder, P. Franke, U. F. Schaefer, and C. M. Lehr, "Development and characterization of film forming polymeric solutions for skin drug delivery," *Eur J Pharm Biopharm*, vol. 65, pp. 111-121, 2007.
[7] W. Pichayakorn, J. Suksaeree, P. Boonme, T. Amnuaikit, W. Taweepreda, and G. C. Ritthidej, "Deproteinized natural rubber film forming polymeric solutions for nicotine transdermal delivery," *Pharm Dev Technol*, vol. 18, pp. 1111-1121, 2013.
[8] Y. Zhang, M. Huo, J. Zhou, A. Zou, W. Li, C. Yao, and S. Xie, "DDSolver: An add-in program for modeling and comparison of drug dissolution profiles," *AAPS J*, vol. 12, pp. 263-271, 2010.
[9] R. C. Rowe, P. J. Sheskey, and M. E. Quinn, *Handbook of Pharmaceutical Excipients*, 6th ed., London: Pharmaceutical Press, 2009.
[10] M. L. Bruschi, "5-Mathematical models of drug release," *Strategies to Modify the Drug Release from Pharmaceutical Systems*, pp. 63-86, 2015.
[11] K. Frederiksen, R. H. Guy, and K. Petersson, "Formulation considerations in the design of topical, polymeric film-forming systems for sustained drug delivery to the skin," *Eur J Pharm Biopharm*, vol. 91, pp. 9-15, 2015.
[12] H. Garvie-Cook, K. Frederiksen, K. Petersson, R. H. Guy, and S. N. Gordeev, "Biophysical elucidation of the mechanism of enhanced drug release and topical delivery from polymeric film-forming systems," *J. Control Release*, vol. 212, pp. 103-112, 2015.
[13] W. Pichayakorn, J. Suksaeree, P. Boonme, W. Taweepreda, T. Amnuaikit, and G. C. Ritthidej, "Transdermal nicotine mixed natural rubber-hydroxypropylmethylcellulose film forming systems for smoking cessation: In vitro evaluations," *Pharm Dev Technol*, vol. 20, pp. 966-975, 2015.
[14] R. Asasutjarit, P. Larpmahawong, A. Fuongfuchat, V. Sareedenchai, and S. Veeranondha, "Physicochemical properties and anti-*Propionibacterium acnes* activity of film-forming solutions containing alpha-mangostin-rich extract," *AAPS PharmSciTech*, vol. 15, pp. 306-316, 2014.

Chaowalit Monton is a lecturer of College of Pharmacy, Rangsit University. He got the Bachelor of Pharmacy (first class honors) from Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand in 2008. Then, he got the Doctor of Philosophy in Pharmacy from Rangsit University, Thailand in 2019. His research interests are quality control of pharmaceutical and herbal products, optimization, and osmotic pump drug delivery system.

Sukanya Settharaksa is a researcher of Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University. She got the Bachelor of Science (Microbiology) from Faculty of Science, Prince of Songkla University, Thailand in 2005. Then, she got the Master of Pharmaceutical Sciences from Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand in 2008. Her research interests are microbiology, cell culture, and biological activity test.

Jirapornchai Suksaeree is an assistant professor of Department of Pharmaceutical Chemistry, College of Pharmacy, Rangsit University. He got the Bachelor of Science (Polymer Science) from Faculty of Science, Prince of Songkla University, Thailand in 2008. Then, he got the Doctor of Philosophy (Pharmaceutical Sciences) from Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand in 2012. His research interest is transdermal drug delivery system.