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Original Article

MICROEMULSION-BASED HYDROGEL FOR TOPICAL DELIVERY OF INDOMETHACIN

WANTIDA CHAIYANA[1,*], RUNGSINEE PHONGPRADIST[1], PIMPORN LEELAPORNPISID[1], SONGYOT ANUCHAPREEDA[2]

[1]Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand, [2]Division of Clinical Microscopy, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand.

Email: wantida.chaiyana@gmail.com

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ABSTRACT

Objective: The present study aims to develop and characterize microemulsion from herbal infused oil of *Zingiber cassumunar* (HO) and microemulsion-based hydrogel (MBH) containing indomethacin. The release patterns of indomethacin from MBH were also investigated.

Methods: HO was produced by hot extraction of *Z. cassumunar* rhizome in coconut oil, and characterized for acid value, iodine value, and saponification value. The cytotoxicity of HO on human peripheral blood mononucleared cells (PBMCs) was also investigated. Pseudoternary phase diagram was constructed to study suitable compositions of microemulsion containing HO, oleic acid, Triton X-114, propan-2-ol, and water. Indomethacin was then incorporated into the microemulsion and finally blended with gel base (2% Carbopol 940 or 3% sodium carboxymethylcellulose) to produce MBH. The indomethacin MBH was characterized for appearance, pH, viscosity, and *in vitro* release characteristics.

Results: HO exhibited an acid value of 0.203 ± 0.004 mg of KOH/g, iodine value of 7.39 ± 0.15 g of I_2/100 g, and saponification value of 265.4 ± 7.3 mg of KOH/g with no cytotoxic effect on human PBMCs. The microemulsion region in the pseudoternary phase diagram of HO, oleic acid, Triton X-114, propan-2-ol, and water was 45.25%. Six microemulsions (ME1– ME6) containing 10% of HO and oleic acid mixture (1:1) as the oil phase and Triton X-114 and propan-2-ol (3:2) as surfactant mixture were formulated and characterized. The droplet size was in the range of 26 to 32 nm with polydispersity index less than 0.3. They showed a Newtonian flow behavior with the viscosity ranging from 15.12 ± 0.15 to 16.78 ± 0.12 Pas. The microemulsion was incorporated into hydrogel using 3% sodium carboxymethylcellulose or 2% Carbopol 940. Only ME1 – ME3 gave clear MBH; therefore, they were studied in the *in vitro* release of indomethacin, and the results indicated the sustained-release characteristic fitted the Higuchi model.

Conclusion: The topical MBH, containing microemulsion of HO, oleic acid, Triton X-114, propan-2-ol and water, might be a promising approach for sustained transdermal delivery of poor water-soluble compounds, including indomethacin.

Keywords: Sustained release, Microemulsion, Hydrogel, Indomethacin, *Zingiber cassumunar*, Herbal infusion oil, Coconut oil, Oleic acid.

INTRODUCTION

Indomethacin is a non-steroidal anti-inflammatory drug and also used for analgesic, and antipyretic [1]. The therapeutic action of indomethacin is believed to inhibit cyclooxygenase (COX) activity and thereby block the production of prostaglandins [2]. There are many isozymes of COXs including COX-1 and COX-2, which are different in physiological functions because of the disparate in their tissue expression and regulation [3]. COX-1 is constitutively expressed in almost all tissues, whereas COX-2 expression is highly restricted. Therefore, inhibition of COX-1 leads to many adverse events, especially serious gastrointestinal side effects such as bleeding, ulceration, and perforation of the esophagus, stomach, small intestine, or large intestine, which can be fatal [4-5]. Since indomethacin is a nonselective inhibitor of COXs [6], oral administration of indomethacin might cause such serious adverse events. The incidence of these adverse events might depend on the degree of enzyme inhibition and the daily fluctuations in enzyme activity, especially at the gastrointestinal tract. Transdermal application might be used to improve overall tolerability and offer many advantages over conventional oral medications, including smooth and continuous drug delivery, reduced C_{max} and steadier systemic drug levels [7].

Transdermal drug delivery offers many advantages over other traditional routes; however, the barrier nature of the skin made it difficult for most drugs to be delivered through it [8]. Therefore, effective formulations which are able to deliver the therapeutic agents through this barrier would be essential. Practical water insolubility of indomethacin due to its low polarity [2], is a problem in the formulation development. There are many studies on solubility enhancing techniques of indomethacin such as using nanospheres, complexation, and microemulsion [9-11].

Microemulsion, belonging to a group of colloidal drug delivery systems, is an optically transparent, low viscosity, and thermodynamically stable dispersions of oil, water, and surfactant, frequently in combination with co-surfactants [12-14]. The systems can be differentiated from a coarse emulsion by visual inspection, when microemulsion is clear, whereas, coarse emulsion is opaque [14]. Depending on their microstructure, microemulsions can be categorized into the droplet and bicontinuous type. In a droplet type, the dispersed oil or water is surrounded by surfactant molecules forming micelles or reverse micelles in the continuous component, whereas, bicontinuous microemulsions are characterized by a sponge-like microstructure, with comparatively large oil and water domains intertwining, separated by a surfactant layer [15]. Microemulsion provides a promising alternative for transdermal delivery of both hydrophilic and lipophilic drugs [16]. Co-solubilization of components with different solubility would attain a synergistic effect for a specific therapeutic goal [13]. The use of microemulsions in pharmaceuticals is advantageous not only due to the low-cost and ease of preparation (zero interfacial tension and almost spontaneous formation), but also because of the improved bioavailability, stability (long shelf-life), high surface area (high solubilization capacity), very small droplet size (5-200 nm), and good appearance [13,16]. Small droplets of microemulsion have better chance to transport bioactive molecules in a more controlled fashion [13]. Microemulsions were also used as a protecting medium for entrapment of drugs from degradation, hydrolysis and oxidation [13]. Moreover, microemulsion can be used as a template for developing many formulations, including hydrogel and organogel.

Zingiber cassumunar Roxb. is an aromatic plant which is widely distributed in various parts of Thailand. It was used in folklore remedies, especially rheumatism and muscular pain [17-18]. The rhizome of *Z. cassumunar* has been used as a component in herbal compress balls and massage oil for muscular pain relief since the ancient time [19]. Recently, there were many studies of *Z. cassumunar* that related to the anti-inflammatory property, such as muscular pain and rheumatism [20]. *Z. cassumunar* was extracted using solvent extraction or hydrodistillation to obtain essential oil [18,21-22]. However, the herbal oil infusion from *Z. cassumunar* in folklore remedies has not been investigated.

Therefore, the aims of this study were to develop microemulsion from herbal infused oil of *Z. cassumunar* (HO) and microemulsion-based hydrogel (MBH) containing indomethacin as a topical delivery system.

MATERIALS AND METHODS

Materials

Indomethacin, oleic acid, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), phenolphthalein TS, triethanolamine, Carbopol 940, sodium carboxymethylcellulose (SCMC), and Triton X-114 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Biochrom AG (Berlin, Germany). Ficoll-paque plus was purchased from Lymphoprep™ Axis-Shield PoC AS, (Oslo, Norway). Disodium hydrogen phosphate, dipotassium hydrogen phosphate, sodium hydroxide, potassium hydroxide, potassium iodide, iodobromide, hydrochloric acid, and sodium thiosulfate were purchased from Fisher Chemicals (Loughborough, UK). RPMI 1640, Penicillin, Streptomycin, and Trypan blue were purchased from GIBCO™ Invitrogen (Grand Island, NY, USA). Hydrochloric acid was AR grade and purchased from Merck (Darmstadt, Germany). Ethanol, methanol, propan-2-ol, dimethyl sulfoxide (DMSO), hexane, ethyl acetate, and ether were AR grade and purchased from Labscan (Dublin, Ireland). Herbal infused oil was prepared from rhizome parts of *Zingiber cassumunar* and coconut oil were purchased from a local market in Chiang Mai province, Thailand.

Herbal infused oil preparation

The rhizomes of *Z. cassumunar* were sliced into small pieces and extracted by heated coconut oil for 3-4 h, then filtered to obtain HO and stored in a well-closed container protecting from light until further use.

Thin layer chromatography of HO

Samples of HO were spotted on the TLC plate. The plate was subsequently developed in two developing solvent systems, including System 1 (methanol, hexane, and ethyl acetate in the ratio of 1.5:2.5:1) and System 2 (methanol, hexane, and ethyl acetate in the ratio of 1.5:2.5:1). After the solvents evaporated, the plate was observed in daylight, UV light at 254 nm and UV light at 366 nm.

Characterization of HO

Acid value determination

Acid value was determined by indirect titration method [23]. Briefly, 10 g of HO was mixed with 50 mL of ethanol/ether mixture (1:1). The mixture was subsequently shaken until homogeneous. Phenolphthalein TS was added as an indicator in the titration with 0.1 N NaOH. End point of the titration was indicated at the first permanent pink color which was persisted for at least 10 s. The acid value which was expressed as the amount of NaOH (in milligrams) necessary to neutralize free fatty acid contained in 1 g of oil was then calculated. The experiments were done in triplicate.

Iodine value determination

Determination of iodine value was carried out according to the AOCS official method with slight modification [24]. Briefly, 0.2 g of oil was dissolved in 10 mL of chloroform. The mixture was shaken until homogenous. Then 25 mL of iodobromide was added, and the reaction was carried out in the dark for 30 min. Potassium iodide solution (30 mL of KI in 100 mL of water) was then added to stop the reaction. The remaining iodine was titrated using 0.1 N sodium thiosulfate ($Na_2S_2O_3$) solution. The iodine value which is expressed as grams of halogen (calculated as iodine) absorbed by 100 g of substance was then calculated. The experiments were done in triplicate.

Saponification value determination

Saponification value was determined according to the AOCS official method with slight modification [24]. Briefly, 2 g of oil was dissolved in 25 mL of alcoholic KOH. After 30 min of reflux with the assistance of heating from water bath, 1 mL of phenolphthalein TS was added as an indicator for the titration. The sample was then titrated with 0.5 N HCl and the end point was indicated at the appearance of amber yellow color. The saponification value which was expressed as the milligrams of KOH necessary to neutralize the free acids and to saponify the esters present in 1 g of substance was then calculated. The experiments were done in triplicate.

Cell cytotoxicity of HO

The effect of HO on cell viability of peripheral blood mononuclear cells (PBMCs) was determined by a colorimetric technique using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [25].

PBMCs isolation

Whole blood (20-25 mL) was obtained from the same donor throughout the research. The whole blood sample was diluted with equal volume of phosphate buffer saline (PBS). After that, the diluted blood sample was carefully layered on Ficoll-Paque Plus and then centrifuged at 5000×g for 30 min at 18-20°C. The mononuclear cell layer was carefully collected. The cells were washed three times by PBS and resuspended in RPMI-1640 media with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 10% v/v fetal bovine serum (FBS). The viable PBMCs number was counted with equal volume of trypan blue solution.

Cell viability assay

The effect of HO on cell viability of PBMCs was determined by MTT assay. Briefly, 100 µL of PBMCs with the cell concentration of 1×10^5 cells/mL was added to the 96-well plate and incubated at 37°C, 5% CO_2 and 90% humidity incubator for 24 h. Then 100 µL of various concentrations of HO was added to the cells compared to untreated cells and incubated again in the same condition for 48 h. After that, 100 µL of media was removed from each well and 15 µL of MTT dye solution (5 mg/mL) was added into each well and further incubation for 4 h. Then all media were removed from the 96-well plate. Dimethylsulfoxide (DMSO) (200 µL) was added to each well to solubilize the formazan crystal. Finally, the plate was read at 540 nm using a microplate-reader. All the experiment was done in three times independent experiment.

Optimization of microemulsion form HO

Pseudoternary phase diagram construction

Pseudoternary phase diagrams of the oil mixture of HO and oleic acid with a weight ratio of 1:1 (Omix) was constructed using a slightly modified water titration method [26]. Triton X-114 was combined with propan-2-ol with a weight ratio 3:2 to obtain surfactant mixture (Smix). Omix and Smix were then mixed with various weight ratios (0:1, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 1:0) and the resulting mixtures were subsequently titrated with water under moderate agitation at room temperature. The samples were classified as microemulsion when they appeared visually as clear liquids. The different formulations were made in triplicate. The pseudoternary phase diagrams were drawn by OriginPro 8 program. The ME regions were measured by ImageJ 1.47v program.

Particle size/size distribution of microemulsion

Particle size analysis was carried out using photon correlation spectroscopy (Zetasizer® version 5.00, Malvern Instruments Ltd., Malvern, UK). The sizing measurements were carried out at a fixed angle of 173°. The reported results are the mean and S.D. of at least ten measurements on the sample.

Formulation of indomethacin MBH

Indomethacin MBH preparation

Indomethacin was dissolved in the selected micro emulsions and then incorporated into gel base of 2% Carbopol 940 or 3% sodium carboxymethyl cellulose (SCMC) to form MBH. The final concentration of indomethacin in MBH was 1%.

Rheology study

Viscosity of the microemulsions and MBH were measured using a Brookfield DVIII rheometer (Brookfield Engineering Laboratories, Stroughton, MA) fitted with a bob spindle and plate spindle, respectively. Brookfield Rheocalc operating software was used to control the measurement. A sample volume of microemulsion was 70 mL, whereas that of MBH was only 1-3 g. The measurements were performed in triplicate at 25°C.

pH measurements

The pH of microemulsions and MBH were measured at 25±1.0°C by dipping the electrode into the test sample until reached the equilibrium, and reading became stable. The measurements were done in triplicate.

***In vitro* release studies**

The *in vitro* release studies of indomethacin were investigated using a slightly modified method of Shin, *et al*. [27]. Briefly, 1 mL of various MBH or gel base containing indomethacin was placed into 2.5×2.5 cm dialysis bag (Cellu Sep T2, Membrane Filtration Products, USA). Each dialysis bag was then introduced into a 50 mL of PBS pH 7.4 at 32°C. The medium was removed at definite time intervals. Once the medium was removed, the same amount of a fresh medium was immediately replaced. The investigation was carried out for 24 h. The amount of the released indomethacin was determined by UV spectrophotometry (Shimadzu, Japan) at 320 nm.

Statistical analysis

All data were demonstrated as a mean ± standard deviation (S.D.). Individual differences were evaluated by One-Way ANOVA: post-hoc test. In all cases, $p< 0.05$ indicates significance.

RESULTS AND DISCUSSION

TLC fingerprint of HO

HO was clear yellowish liquid with a characteristic odor. On the TLC plate that was developed with solvent **System 1**, there were two yellow spots detected under visible light with R_f value of 0.66 and 0.75. Absorbing compounds at the R_f value of 0.66, 0.75, and 0.83 diminished the uniform layer fluorescence and were detected as dark spots on a bright green background when excited with short-wave (254 nm) UV light and the native fluorescence compounds were visualized at the R_f value of 0.66, 0.75, and 0.83 when excited with long-wave (366 nm) UV light. Using the developing solvent **System 2**, there was only one yellow spot detected under visible light with R_f value of 0.64. However, two spots were detected at the R_f value of 0.64 and 0.77 when excited with short-wave and long-wave UV light.

Chemical characterization of HO

The characteristic of HO as the functions of acid value, iodine value, and saponification value are shown in table 1. The low acid value (lower than 2 mg KOH/g) indicated the tendency of oxidative stable of the oil [28]. Acid value is theoretically twice of free fatty acid value, which determines the free fatty acid content hydrolyzed from triglyceride. Since the rancidity is the result of the oxidation of free fatty acid in oils, this low acid value and free fatty acid value would be the factors that contribute to high oxidative stability [29]. The results were in good agreement with the previous study that the acid value of homemade coconut oil for HO production in this study was 0.40 ± 0.02 mg of KOH/g [29]. However, commercial coconut oil in the same study showed a higher acid value of 3.5± 0.9 mg of KOH/g because of the longer storage time [29]. Therefore, the life span of the oil might be a critical factor affecting the acid value.

Table 1: Acid value, iodine value, and saponification value of HO

	Acid value (mg of KOH/g)	Iodine value (g of I_2/100 g)	Saponification value (mg of KOH/g)
HO	0.20 ± 0.00	7.4 ± 0.2	265± 7
Coconut oil[23]	0.40 ± 0.02	6.6 ± 0.2	274 ± 2

Iodine value could be used to indicate a saturated or unsaturated fat component of the oil. Iodine value of oleic acid (an unsaturated fat containing 1 double bond), is 90g of I_2/100 g, whereas linoleic acid (unsaturated fat containing 2 double bonds) and linolenic acid (unsaturated fat containing 3 double bonds) are 282 and 274 g of I_2/100 g, respectively. The low iodine value of HO represented the low number of reactive double bonds in the molecule, place HO in the non-drying oil group which is unable to be solidified when exposed in a thin film to the air [30].

The results related to the previous study that the major components of coconut oil were saturated fats, including lauric acid (48%) and myristic acid (17%), whereas, the only small amount of unsaturated fats (7% of oleic acid and 2% of linoleic acid) were found [31]. Therefore, oxidative cleavage of unsaturated bond decreases and the oxidation likely slows down. The iodine value in this study was in a good agreement with the previous report [29].

High saponification value represents a high number of ester content or carboxylic functional groups per unit mass of HO. The results may suggest that HO is suitable for self-emulsification process and microemulsion formation [32]. The saponification value in this study was also in a good agreement with the previous report [29].

Cytotoxicity of HO

The cell viability of human PBMCs after exposure to HO for 48 h is shown in fig. 1. The HO was very safe since it had no toxic effect on human PBMCs with a 100% of cell viability were observed even at high concentration (50 %v/v) were used.

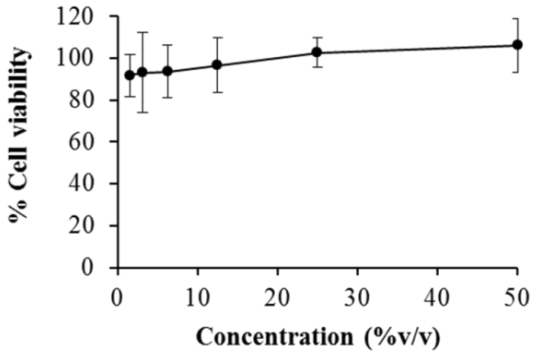

Fig. 1: Dose-response curve of viability of PBMCs versus concentrations of HO

Indomethacin MBH

Since one of the unique factors associated with microemulsions is the presence of different structures formed by altering the curvature of interface, construction of the phase diagrams enables determination of aqueous dilutability and range of compositions that form a microemulsion region [33]. Pseudoternary phase diagram of HO and oleic acid (1:1)/Triton X-114 and propan-2-ol (2:1)/water is shown in fig. 2. The microemulsion region in the phase diagram was 45.25%. Oleic acid was mixed with HO and used as an oil phase in the system since oleic acid has been reported to act as a penetration enhancer by lipid fluidization and phase separation [34-35]. Six formulations in the microemulsion region (ME1 - ME6) were formulated and characterized for their particle size and size distribution as the results shown in fig. 3. The particle size was in the range of 26 to 32 nm which were in the microemulsion range [36].

The particle size distributions were intermediate (PDI<0.3). All formulations were optically isotropic and transparent since their particle sizes were less than the wavelength of light [37].

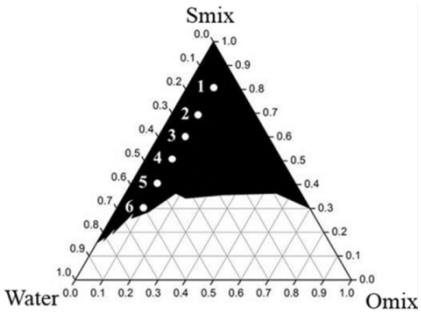

Fig. 2: Pseudoternary phase diagram of HO and oleic acid (1:1)/Triton X-114 and propan-2-ol (3:2)/water. The dark area represents the microemulsion region. Formulation ME1 - ME6 are composed of 10% HO and oleic acid with various ratios of Smix and water

The low viscosity of microemulsion is inappropriate for the topical use. Therefore, the viscosity was increased after adding thickening agents. Thickening agents not only changed the appearance of the system but also influenced the release of drugs [38]. Recently, various gelling agents, including Carbopols, xanthum gum, carrageenan, sodium alginate, ethyl cellulose, and hydroxypropyl methylcellulose have been used to prepare the MBH in order to improve the viscosity of microemulsions [39].

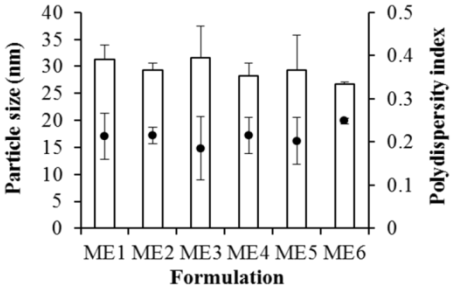

Fig. 3: Particle size and size distribution of microemulsions ME1 – ME6

In this study, each microemulsion formulation was incorporated into gel base of 3% SCMC or 2% Carbopol 940. ME1, ME2, and ME3 gave the clear appearance with both gel bases. Higher content of water in microemulsions resulting in the insufficient amount of Smix made the MBH translucent or turbid. Therefore, ME1, ME2, and ME3 were selected for incorporation of indomethacin since the clear appearance was still observed after the addition of gel bases. Indomethacin could be dissolved well in each microemulsion and produced clear gel after incorporated with gel bases of 3% SCMC producing S-ME1, S-ME2, S-ME3, and 2% Carbopol 940 producing C-ME1, C-ME2, and C-ME3, respectively.

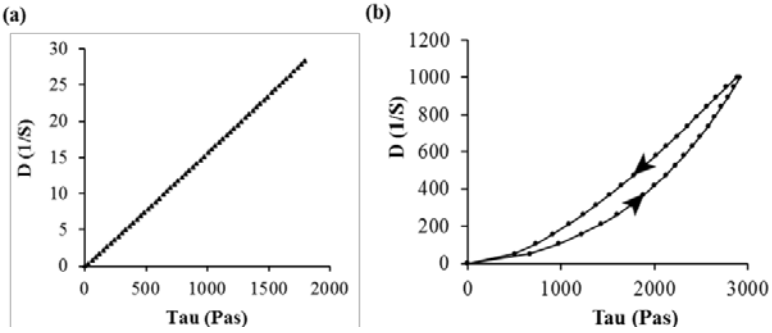

Fig. 4: Rheogram of microemulsion, ME1 (a) and MBH using gel base of 2% Carbopol 940 which contained 1% indomethacin, C-ME1 (b)

The rheograms of microemulsion as shown in fig. 4a revealed the Newtonian's flowed behavior, which stress versus strain rate curve is linear and passes through the origin. The viscosity was calculated from the well-known Newtonian equation; $\tau = \mu(du/dy)$, when τ is the shear stress (Pa), μ is the viscosity and du/dy is the velocity gradient perpendicular to the direction of shear or strain rate (s^{-1}). The viscosity of microemulsions as shown in table 2 was very low leading to the confirmation of microemulsion formation [40-41]. The rheogram of MBH containing 1% indomethacin as shown in fig. 4b exhibiting pseudoplastic flow with thixotropic property indicating a good characteristic of the gel for external use [42]. The viscosities of MBH were calculated using the following equation, $\eta'_a = \eta'_\infty + [(\eta'_0 - \eta'_\infty)/(1+(kF)^n)]$, where η'_a is the apparent dynamic viscosity, η'_∞ is the infinite dynamic viscosity, η'_0 is the zero frequency dynamic viscosity, k is the frequency at which formulation first displays frequency dependent viscosity, n is the slope of the frequency- dependent region, and F is the oscillatory frequency [43-44]. The viscosities of MBH, using 2% Carbopol 940 or 3% SCMC are shown in table 2. The results were in line with the previous study showing that Carbopol 940 gave clearer gel with higher viscosity comparing to SCMC [45-46]. There was no statistical difference in viscosities of MBH using the same gel base incorporated with various microemulsions since the viscosity of the gel base was much more prominent. However, increasing in water content from ME-1 (10% water) to ME-3 (30% water) led to lower trend in viscosity of S-ME3 compared to S-ME2 and S-ME1, respectively. The pH of MBH was in the range of 5.0 to 5.5, which is extremely matched with human cutaneous pH and appropriate for using as a topical formulation. Acidity of cutaneous has many important roles such as select and maintain the normal cutaneous microbiota, protect the skin against infection and activate pH-dependent enzymes involved in the process of keratinization [47].

Table 2: Viscosity of microemulsion and MBH

Formulation	Viscosity		
	Microemulsion (Pas)	MBH (Pas) Carbopol 940	SCMC
1	15.12 ± 0.15	4300 ± 521	2192 ± 539
2	15.37 ± 0.10	4695 ± 1646	2132 ± 183
3	16.78 ± 0.12	5128 ± 243	1249 ± 47

***In vitro* release studies**

The *in vitro* of indomethacin MBH release profiles of indomethacin are shown in fig. 5. To determine the release patterns of indomethacin from each formulation, cumulative release was plotted versus time or square root of time to show the zero order or Higuchi released pattern, respectively. The equation responsible for zero

order is $Q = K_0t$, where Q is the cumulative release of indomethacin, K_0 is the zero-order rate constant expressed in units of concentration/time, and t is the time. On the other hands, the equation responsible for Higuchi's release is $Q = K_Ht^{1/2}$, where K_H is the Higuchi dissolution constant. K_H, K_0 and square of correlation coefficients (R^2) of each plot are shown in table 3. The release of indomethacin from all formulations was clearly described by the Higuchi model since the linear plots with resultant R^2 over 0.97 were found when accumulative release of indomethacin was plotted versus the square root of time, besides the formulation of 3% SCMC, which shows low R^2 value (0.726). Higuchi describes a drug release as a diffusion process based on the Fick's first law which the rate controlling step is the diffusion through the topical gel base [48-49]. According to the Fick's first law; diffusion coefficient of each formulation could be defined using the following equation, $J = -D(d\rho/dx)$, where J is the flux of a drug which is simply defined as the mass or number of molecules moving through a given crosssectional area (12.5 cm^2) during a given period of time, D is the diffusion coefficient, and $d\rho/dx$ is the concentration gradient. The velocity of diffusion is related to the diffusion coefficient, which is dependent on the size of the solute molecule and the viscosity of the formulations as described by the Stokes–Einstein's equation, $D = RT/(6\pi\eta N_0 r_A)$, where R is the gas constant, T is absolute temperature, r_A is the radius of spherical solute, N_0 is Avogadro's number, and η is the viscosity of the formulations. Therefore, viscosity is one of the major factors affecting the release of compound from the formulations. The results were in a good agreement since among 6 formulations of MBH, S-ME3 which was the formulation with the lowest viscosity had the highest release rate (K_H). In contrast, C-ME1 which exhibited the highest viscosity had the lowest release rate. Comparing to the traditional gel formulation, MBH showed the sustained-release pattern. The results were in a good agreement with many previous studies. Fouad *et al.* developed poloxamer MBH containing microemulsion of Capryol®, Labrasol®, Transcutol®, and water for sustained transdermal delivery of diclofenac epolamine [50]. Josef *et al.* developed the sustained delivery of hydrophobic drugs using composite hydrogels, prepared by embedding oil-in-water microemulsions in hydrophilic hydrogels of alginate solution crosslinking with calcium ions [51].

Baboota *et al.* developed MBH of betamethasone dipropionate and salicylic acid containing the microemulsion prepared by oleic acid: sefsol (1.5:1), Tween 20, isopropyl alcohol, and distilled water [38]. Moreover, many anti-inflammatory drugs have been formulated into MBH such as betamethasone dipropionate, diclofenac epolamine, triptolide, etc. to provide sustained transdermal delivery [38,50]. In this study, MBH containing HO, oleic acid, Triton X-114, propan-2-ol, water, and hydrogel base showed a sustained-release manner. Therefore, it may prolong the effective duration. The MBH would be another promising approach for transdermal delivery of indomethacin and maybe applied to other poor water-soluble compounds. The transdermal delivery of the optimized MBH and evaluation in animal skin or animal model are needed to be further investigated.

Table 3: Higuchi release rate constant (K_H), zero-order rate constant (K_0) and square of correlation coefficient (R^2) of indomethacin release from each gel formulations

Formulation	Higuchi's model		Zero order model	
	K_H	R^2	K_0	R^2
Carbopol	1.250	0.990	0.218	0.971
C-ME1	0.812	0.983	0.143	0.991
C-ME2	0.698	0.991	0.118	0.920
C-ME3	0.626	0.998	0.107	0.941
SCMC	1.553	0.726	0.231	0.519
S-ME1	0.720	0.974	0.122	0.906
S-ME2	0.821	0.993	0.140	0.928
S-ME3	1.057	0.991	0.179	0.920

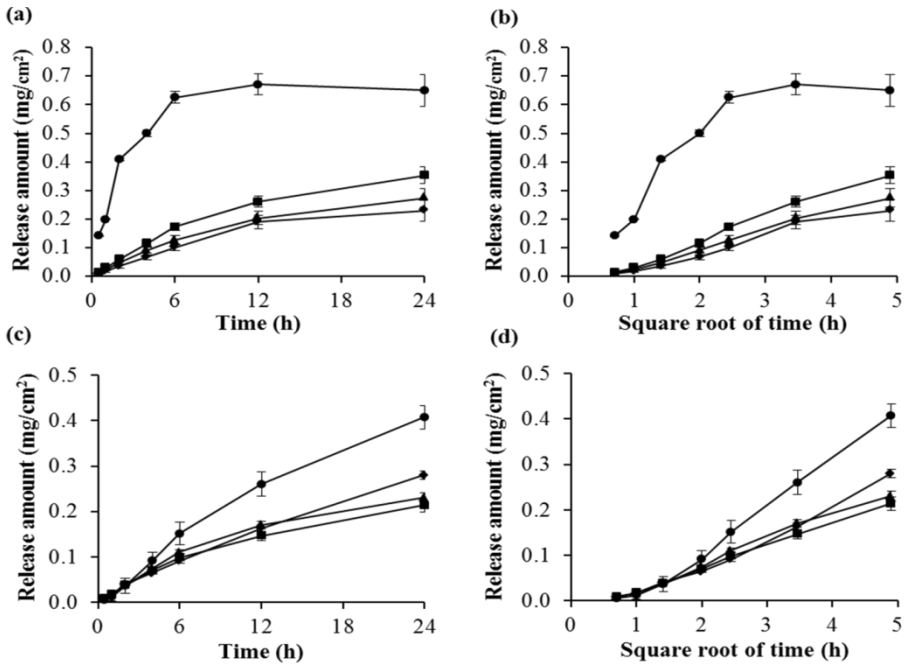

Fig. 5: Drug release profiles plotting the cumulative release of indomethacin from the formulations using 3% SCMC versus time (a) and square root of time (b), as well as, cumulative release of indomethacin from the formulations using 2% Carbopol 940 versus time (c) and square of time (d) which incorporated with indomethacin (●) or indomethacin in ME1 (◆), ME2 (▲), and ME3 (■)

CONCLUSION

HO, obtained by hot oil extraction of *Zingiber cassumunar* in coconut oil, showed safety profile on human PBMCs. HO was mixed with oleic acid (Omix) and used in the construction of pseudoternary phase diagrams. Triton X-114 and propan-2-ol were used as a surfactant and a co-surfactant, respectively. The microemulsion region in the phase diagram was 45.25%. Microemulsion (ME1 – ME6) containing 10% Omix exhibited the droplet size in the range of 26 to 32 nm with intermediate polydispersity index (PDI < 0.3). The viscosity ranged from 15.12 ± 0.15 to 16.78 ± 0.12 Pas and the flow behavior were in line with Newtonian's flow. ME1 – ME3 gave clear MBH using 3% SCMC or 2% Carbopol 940 as gelling agents. *In vitro* release study indicated the sustained-release characteristics with Higuchi's pattern of indomethacin from each MBHs. The topical MBH, containing microemulsion of HO, oleic acid, Triton X-114, propan-2-ol and water, might be a promising approach for sustained transdermal delivery of poor water-soluble compounds, especially indomethacin.

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CONFLICT OF INTERESTS

The authors report no conflicts of interest.

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