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Stability and Release Kinetics of Natural Oil Microemulsions Containing Nicotinamide

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Synopsis

This research aimed to evaluate stability and release characteristics of nicotinamide-loaded microemulsions (MEs). Four MEs were prepared with Tween 80 as surfactant, Span 80 as cosurfactant, either virgin coconut oil or olive oil as oil phase, water as aqueous phase, and nicotinamide as an active ingredient. They were composed of 3% w/w nicotinamide and designated as MEC1-N, MEC2-N, MEO1-N, and MEO2-N. All samples were kept in clear glass containers at 4°C, room temperature (RT, 28° ± 2°C), and 45°C for 3 months. Afterward, they were observed for physical changes and analyzed for remaining nicotinamide by a validated high-performance liquid chromatography technique. MEC1-N and MEO1-N were compared for nicotinamide released through dialysis membrane using modified Franz diffusion cells. It was found that all samples were clear liquid and water-in-oil type. Phase separation was found in MEO2-N at all storage conditions. Discoloration was observed in all samples after being kept at 45°C for 3 months. MEC1-N, MEC2-N, and MEO1-N were both physically and chemically stable after being kept at 4°C and RT for 3 months. Release kinetics of MEC1-N and MEO1-N were the best fitted with the Higuchi model.

INTRODUCTION

Nicotinamide, also known as vitamin B3, is an attractive active ingredient in skin-care products because of its many benefits. It is well known as a skin-lightening agent because it can inhibit melanosome transfer from melanocytes to keratinocytes (1). Moreover, it can provide other advantages for skin conditions such as anti-acne, anti-aging, and anti-inflammatory (2,3). However, it is generally known that stratum corneum, the outermost layer of human skin, obstructs the penetration of all chemicals into deeper layers of the skin (4). Therefore, formulations are necessary for development of effective skin-care products. Microemulsions (MEs) are thermodynamically stable nanosystems composed of two normally immiscible phases, i.e., oil and water, which can simultaneously form as a

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single-phase liquid with the help of interfacial film of a surfactant (S) or a mixture of S and cosurfactant (CoS). MEs have been used in formulation development of many cosmetic products (5–7). MEs have been proposed to increase skin penetration of active ingredients by many possible mechanisms such as enhancer function of surfactant, hydration effect of aqueous phase, and carrier property of nano-sized droplets (5,8). Besides, the clear appearance and low viscous texture of MEs align with consumers' expectations on sensory properties of skin-care products. It has been reported that topical water-in-oil (w/o) MEs are able to make their cosmetic active ingredients be highly accumulated in the skin membrane rather than permeated to the receptor fluid, representative of blood circulation (9–11). Although w/o ME–containing nicotinamide was previously reported for its benefits (11), it contained synthetic oil and alcohol. Nowadays, consumers pay high concern to safety and global environment. Therefore, w/o MEs that were formulated from components with generally recognized as safe and natural status were focused in this study. Two nonionic surfactants, i.e., Tween 80 and Span 80, were used as an S and a CoS in the developed MEs, respectively. Both are generally recognized for low skin irritation potential and usually used as skin penetration enhancers (12). Natural oils, i.e., virgin coconut oil or olive oil, were studied as oil phase in the MEs. Virgin coconut oil is extracted from mature kernel of coconut (*Cocos nucifera*) and has long been used for skin application as a moisturizer (13). Olive oil is extracted from the flesh of olive fruit (*Olea europaea*) and provides moisturizing, antioxidant, and photoprotective effects (14). Water was used as aqueous phase. This study aimed to examine stability and release kinetics of the developed nicotinamide-loaded MEs.

EXPERIMENTAL METHODS

MATERIALS

Nicotinamide, olive oil, Tween 80 (polyoxyethylene 20 sorbitan monooleate), and Span 80 (sorbitan monooleate) were purchased from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Virgin coconut oil extracted from coconut meat by the cold-pressed process was procured from Chemipan Corporation (Bangkok, Thailand). Buffer solutions were prepared in-house and composed of potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate, and sodium chloride which were acquired from Univar (New South Wales, Australia) in appropriate concentrations. Triethylamine was obtained from Fluka Chemika (Busch, Switzerland). Acetonitrile was purchased from RCI LabScan (Bangkok, Thailand). All chemicals were of pharmaceutical or analytical grade and used without modification. Deionized water was prepared in-house and used throughout the experiment.

DETERMINATION OF FATTY ACIDS IN THE STUDIED OILS

Fatty acid compositions of virgin coconut oil and olive oil were analyzed by gas chromatography with flame ionization detector (GC-FID, Agilent, Palo Alto, CA) as described in a prior report with some modifications (15). The flow rate of helium (carrier gas) was 1 mL/min and the split ratio was 50:1. Hydrogen gas and air were supplied to FID with flow rates of 30 and 300 mL/min, respectively. Separation was carried out on capillary column, Select Biodiesel for FAME, with a length of 30 m and diameter of 320 μm (Agilent). The injector and detector temperatures were set at 290° and 300°C, respectively.

PREPARATION OF NICOTINAMIDE-LOADED MES

Pseudoternary phase diagrams of systems containing various ratios of Tween 80:Span 80, either virgin coconut oil or olive oil, and water were constructed by titration method. Tween 80 and Span 80 were studied at the S:CoS ratios of 1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.3:0.7, 0.2:0.8, and 0.1:0.9. Afterward, four formulations of blank MEs were selected from the middle points of four large ME regions in four pseudoternary phase diagrams. In this study, the chosen systems were composed of 0.7:0.3 or 0.6:0.4 Tween 80:Span 80 as the surfactant blend, virgin coconut oil or olive oil as the oil phase, and water as the aqueous phase. Blank MEs were prepared by simply mixing all components in the determined weight ratios. Afterward, 3% w/w nicotinamide-loaded MEs were prepared by mixing 3% w/w of nicotinamide powder and 97% w/w of each blank ME with a magnetic stirrer until nicotinamide powder completely dissolved.

CHARACTERIZATION OF NICOTINAMIDE-LOADED MES

The obtained nicotinamide-loaded MEs were left at room temperature (RT) overnight before being characterized. They were visually observed for appearance. They were investigated for isotropic property under polarized light microscope (Olympus BX61, Tokyo, Japan) to confirm for ME formation. ME type was determined by drop dilution test into water as well as oil and by conductivity measurement with a conductivity meter (FiveEasy, Mettler Toledo, Greifensee, Switzerland). In case of drop dilution test into water, if the sample was miscible with water, it was defined as oil-in-water (o/w) because water was the external phase. By contrast, if the sample was immiscible with water, it was defined as w/o. In case of drop dilution test into oil, the results should be vice versa. The conductivity has been usually low in w/o, whereas high in o/w MEs (16). Transmission electron microscope (TEM, JEM-2010, JEOL, Tokyo, Japan) was used to illustrate the sample microstructure. Briefly, a sample was dropped on a Formvar carbon film on 200-mesh copper grid and left at RT until dry. Subsequently, the obtained sample was observed under TEM at magnification of ×100,000. Viscosity values were measured in triplicate by a rheometer (DV III Ultra Programmable Rheometer, Brookfield Engineering Laboratories, Middleboro, MA) using a spindle number SC4-31 with five different shearing speeds at 32° ± 1°C.

STABILITY EVALUATION OF NICOTINAMIDE-LOADED MES

Nicotinamide-loaded MEs were kept in clear glass containers at 4°C, RT, and 45°C for 3 mo. Their physical changes such as phase separation, turbidity, precipitation, and discoloration were observed every month. Their chemical stability was evaluated by analysis of amounts of nicotinamide remaining in the samples. Briefly, an accurate weight (0.05 g) of each nicotinamide-loaded ME was vigorously mixed and diluted with isotonic phosphate buffer solution (PBS) pH 7.4 into an appropriate concentration. Afterward, each obtained sample was filtered by a syringe filter and analyzed for nicotinamide content by validated high-performance liquid chromatography (HPLC) technique. All experiments were performed in triplicate.

IN VITRO RELEASE STUDY OF NICOTINAMIDE-LOADED MES

Two selected nicotinamide-loaded MEs prepared with the same S:CoS but different oils were evaluated for *in vitro* release profiles and kinetics. The release study was performed by modified Franz diffusion cells (Model 57-6 M, Hanson Research Corporation, Chatsworth, CA). A membrane model was dialysis membrane with the molar weight cutoff 3500 Dalton (Spectra/Por®3, Spectrum laboratories, New Brunswick, NJ). The membrane was cut into appropriate sizes and soaked in the receptor fluid for 30 min before placed between donor and receptor chambers of the diffusion cell. Twelve milliliters of degassed PBS was added into each receptor chamber, stirred at a speed 200 rpm by a magnetic stirrer, and thermostatically maintained at 37° ± 0.5°C. An accurate weight (1 g) of the sample was applied on the membrane with the diffusion area of 1.77 cm^2 in the donor chamber. At specified time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h), 500 µL of receptor fluid was withdrawn from the receptor chamber and immediately replaced with equal volume of PBS. The withdrawal samples were examined for nicotinamide concentrations by a validated HPLC technique. Experiments were carried out in triplicate for each formulation. The cumulative amount of released nicotinamide through the dialysis membrane into the receptor fluid (Q, µg/cm^2) was calculated by equation (1). Subsequently, the release data were further analyzed using three different kinetics models, i.e., zero order, first order, and Higuchi model as exhibited in equations (2)–(4), respectively.

$$Q = V_r C_t + \sum_{i=0}^{t-1} V_s C_i, \tag{1}$$

where C_t is the concentration of nicotinamide in the receptor fluid at each sampling time (t), C_i is the concentration of nicotinamide of the ith sample, and V_r and V_s are the volumes of the receptor fluid and the sample, respectively.

$$\text{Zero order}: Q_t = Q_0 - k_0 t \tag{2}$$

$$\text{First order}: \ln Q_t = \ln Q_0 - k_f t \tag{3}$$

$$\text{Higuchi model}: Q_t = k_H t^{1/2} \tag{4}$$

where Q_t is cumulative amount of nicotinamide released in time (t), Q_0 is initial amounts of nicotinamide in the formulations. The k_0, k_f, and k_H are release rate constants of zero order, first order, and Higuchi model, respectively.

ANALYSIS OF NICOTINAMIDE

The HPLC technique was slightly modified from previous studies (11,17). HPLC system (Agilent 1100 series, Palo Alto, CA) was used. Stationary phase was a reversed phase column (Luna® C18, 150 × 4.6 mm, 5 µm particle size, Phenomenex Inc., Torrance, CA). Mobile phase was a mixture of 0.1% triethylamine in 0.067 M monobasic potassium phosphate buffer pH 6.7 and acetonitrile (96.15:3.85 v/v). Mobile phase was filtered through the 0.45-µm nylon filter and degassed before use. Its flow rate was controlled at 1.0 mL/min. The injection

volume was 20 μL and the detecting wavelength was 260 nm. The analytical method was validated according to the International Conference on Harmonisation (ICH), now named the International Council for Harmonisation (ICH), guidelines (18).

RESULTS AND DISCUSSION

FATTY ACIDS IN THE STUDIED OILS

The types and amounts of fatty acids in virgin coconut oil and olive oil are presented in Table I. In addition, double bonds in all unsaturated fatty acids found in both oils were in *cis* configuration. The results showed that virgin coconut oil could be classified as saturated oil because it predominantly contained saturated fatty acids such as lauric acid (48.102%) and myristic acid (19.140%), whereas olive oil could be regarded as unsaturated oil because it mainly contained oleic acid, an unsaturated fatty acid (71.530%). The main components of both investigated oils were in agreement with a previous report (19); however, slight differences were found because of dissimilar geographic sources and extraction techniques.

CHARACTERISTICS OF NICOTINAMIDE-LOADED MES

Four blank MEs (designated as MEC1, MEC2, MEO1, and MEO2) were selected from four ME regions as exhibited in Figure 1 for further incorporating with nicotinamide.

Table I
Fatty Acid Composition in the Studied Virgin Coconut Oil and Olive Oil

Fatty acid (%)	Lipid numbers C:D[a]	Virgin coconut oil	Olive oil
Saturated type			
Caprylric	8:0	5.897	0.009
Nonanoic	9:0	0.014	0.013
Capric	10:0	5.814	0.000
Undecanoic	11:0	0.024	0.000
Lauric	12:0	48.102	0.000
Tridecanoic	13:0	0.032	0.000
Myristic	14:0	19.140	0.025
Pentadecanoic	15:0	0.010	0.007
Palmitic	16:0	8.978	11.320
Heptadecanoic	17:0	0.000	0.069
Stearic	18:0	3.082	2.786
Behenic	22:0	0.000	0.122
Lignoceric	24:0	0.000	0.030
Unsaturated type			
Palmitoleic	16:1	0.000	1.070
Oleic	18:1	5.775	71.530
Linoleic	18:2	1.026	10.968
Linolenic	18:3	0.000	0.633
Gondoic	20:1	0.000	0.275
Erucic	22:1	0.000	0.022

[a]C and D represent numbers of carbon atoms and double bonds in the fatty acid, respectively.

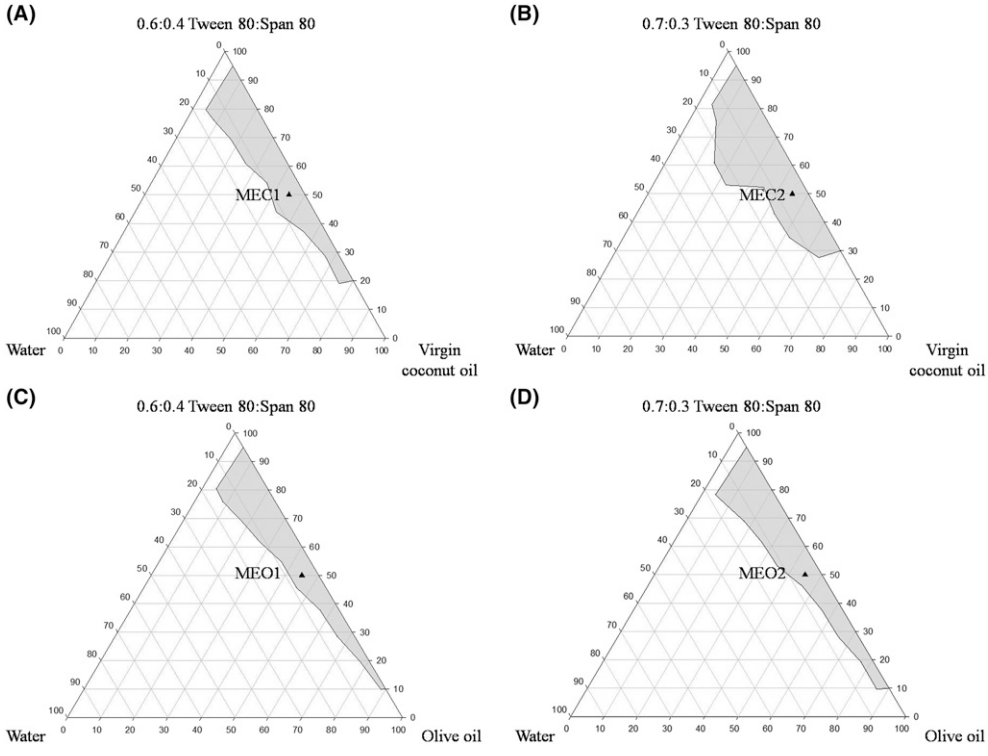

Figure 1. Pseudoternary phase diagrams and points of blank MEs in ME regions (shaded areas) of the systems consisted of (A) 0.6:0.4 Tween 80:Span 80/virgin coconut oil/water, (B) 0.7:0.3 Tween 80:Span 80/virgin coconut oil/water, (C) 0.6:0.4 Tween 80:Span 80/olive oil/water, and (D) 0.7:0.3 Tween 80:Span 80/olive oil/water.

The studied blank MEs are summarized in Table II. Virgin coconut oil could form MEs because its medium-chain fatty acids could easily penetrate into the interfacial film (20). Although olive oil contained high amount of oleic acid, a long-chain fatty acid, it could form MEs because of the structural similarity between its fatty acid chain and oleate hydrophobic tails of Tween 80 as well as Span 80 (21).

All obtained 3% w/w nicotinamide-loaded MEs (designated as MEC1-N, MEC2-N, MEO1-N, and MEO2-N) were clear yellowish liquids. Under polarized light microscope, all samples showed no birefringence (data not shown), indicating the isotropic property

Table II
Composition of Blank MEs

			Composition (% w/w)		
Formulation	S:CoS ratio (Tween 80:Span 80)	Oil	S:CoS	Oil	Water
MEC1	0.6:0.4	virgin coconut oil	50	45	5
MEC2	0.7:0.3	virgin coconut oil	50	45	5
MEO1	0.6:0.4	olive oil	50	45	5
MEO2	0.7:0.3	olive oil	50	45	5

Table III
Characteristics of Nicotinamide-Loaded MEs

Formulation	Dilution test		Conductivity (μS/cm)
	Into water	Into oil	
MEC1-N	immiscible	miscible	0.93 ± 0.01
MEC2-N	immiscible	miscible	1.92 ± 0.01
MEO1-N	immiscible	miscible	1.00 ± 0.08
MEO2-N	immiscible	miscible	0.94 ± 0.20

of MEs. As seen in Table III, the samples were immiscible with water but miscible with oil. Their conductivity values were very low. Data from drop dilution test and conductivity measurement indicated that all samples were w/o type because they revealed that the oil phase was the continuous or external phase (16). Figure 2 illustrates visual appearance and TEM photograph of MEC2-N demonstrated nano-sized internal droplets.

Total hydrophilic-lipophilic balance (HLB) values of the systems with Tween 80:Span 80 ratios of 0.6:0.4 and 0.7:0.3 were 10.72 and 11.79, respectively. Although high total HLB values have usually indicated o/w type, all studied MEs were found to be w/o type. ME composition can modify the microenvironment of the surfactants and subsequently affect the geometric packing of surfactant molecules (22). Moreover, the preparation process of w/o ME is usually easier than that of o/w ME because it is easier for surfactant packing in interfacial film with the surfactant long tails arranged outward into the external oil phase (23). In this study, the amount of oil phase was 45% w/w, whereas that of the aqueous phase was only 5% w/w. Hence, the aqueous phase was too low to behave as the continuous phase. Some previous findings about the formation of w/o MEs with high total HLB values have been

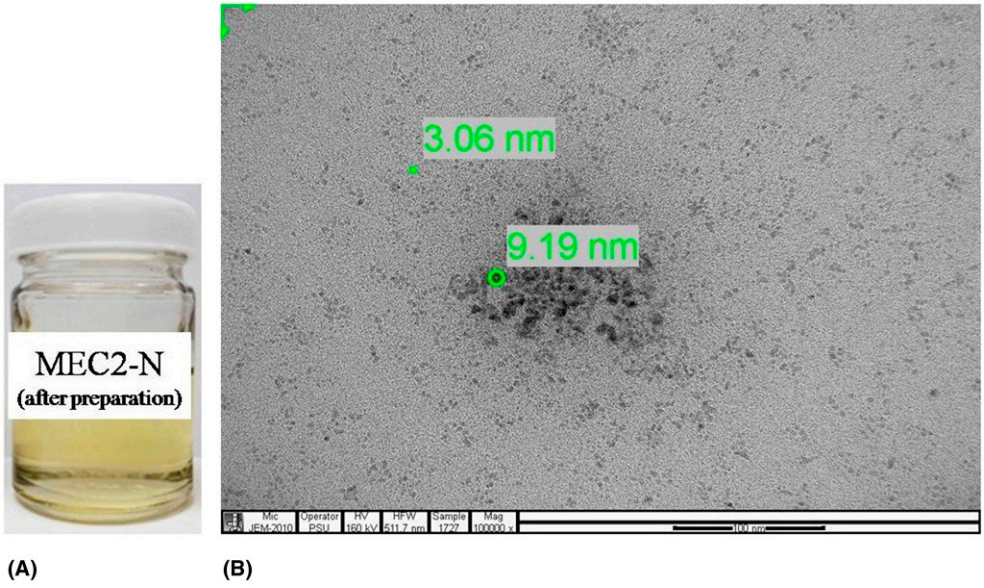

Figure 2. MEC2-N: (A) visual appearance and (B) TEM photograph with analyzed size.

reported. For example, w/o MEs of palm kernel oil esters originated when the HLB values of surfactant mixtures were 10.7–13.9 because of large volume of hydrophobic portion according to the structural similarity between palm kernel oil esters and the surfactant used (21). ME systems composed of 45% w/w Tween 80 (HLB 15), 45% w/w Eutanol G (2-octyldodecan-1-ol), and 10% w/w water were found to be of w/o type because of much lower fraction of the aqueous phase than that of oil phase (24).

VALIDATION OF HPLC ANALYSIS

No interfering peak was observed nearby the nicotinamide peak at the retention time of around 5.4–5.8 min as demonstrated in Figure 3. Other parameters, i.e., linearity of the calibration curve of nicotinamide concentrations between 1.25 and 40 μg/mL in terms of linear regression coefficient (r^2), accuracy in term of %recovery, precision in term of %relative standard deviation (%RSD), limit of detection (LOD), and limit of quantitation (LOQ) were summarized in Table IV. The results revealed that the HPLC technique was reliable according to the ICH guidelines (18). Hence, this analytical method was suitable for quantitative assay of nicotinamide.

STABILITY OF NICOTINAMIDE-LOADED MES

Phase separation was found in MEO2-N after storage at all studied conditions for only 1 mo. The reason was unclear; however, it may be caused by the effect of entropy changing of water molecules by attraction with a hydrophilic active ingredient on the instability of the interfacial film (25). Darkened color was found in all nicotinamide-loaded MEs and their blank counterparts after being kept at 45°C for 3 mo. Discoloration of the samples was due to oxidation of either virgin coconut oil or olive oil in MEs. Similarities were found in previous reports of MEs and ME-based gels prepared from different oils and stored at high temperatures such as MEs prepared from Eutanol G at 45°C (10), MEs prepared from isopropyl palmitate at 45°C (11), and ME-based gels prepared from soybean oil stored at 60°C (17). Discoloration has adversely affected consumer confidence; therefore, nicotinamide-loaded MEs should not be stored at high temperatures. However, the color of virgin coconut oil and olive oil did not change after being stored under the same condition, i.e., at 45°C for 3 mo. Formation of w/o MEs could accelerate oxidation when compared with bulk oils because large surface area of MEs allowed interactions between water-soluble transition metals and oil (26).

Viscosity values of non-separated nicotinamide-loaded MEs, i.e., MEC1-N, MEC2-N, and MEO1-N were measured every month during stability study as exhibited in Table V. All samples possessed rheological property as Newtonian flow (data not shown). The percentage of nicotinamide remaining in nonseparated MEs was summarized in Table VI. It could be observed that high storage temperature seemed to adversely affect chemical stability of nicotinamide-loaded MEs. Stability of most vitamins, including nicotinamide, is influenced by many factors such as oxygen, light, and temperature (27). However, chemical instability of nicotinamide-loaded MEs after being kept at 45°C for 3 mo was not obviously noted. Entrapment of a hydrophilic cosmetic ingredient in the internal aqueous droplets of w/o MEs has been found to enhance chemical stability because of the protection effect (28). Average nicotinamide remaining in the samples after storage at 4°C and RT for 3 mo exhibited tendency of chemical stability.

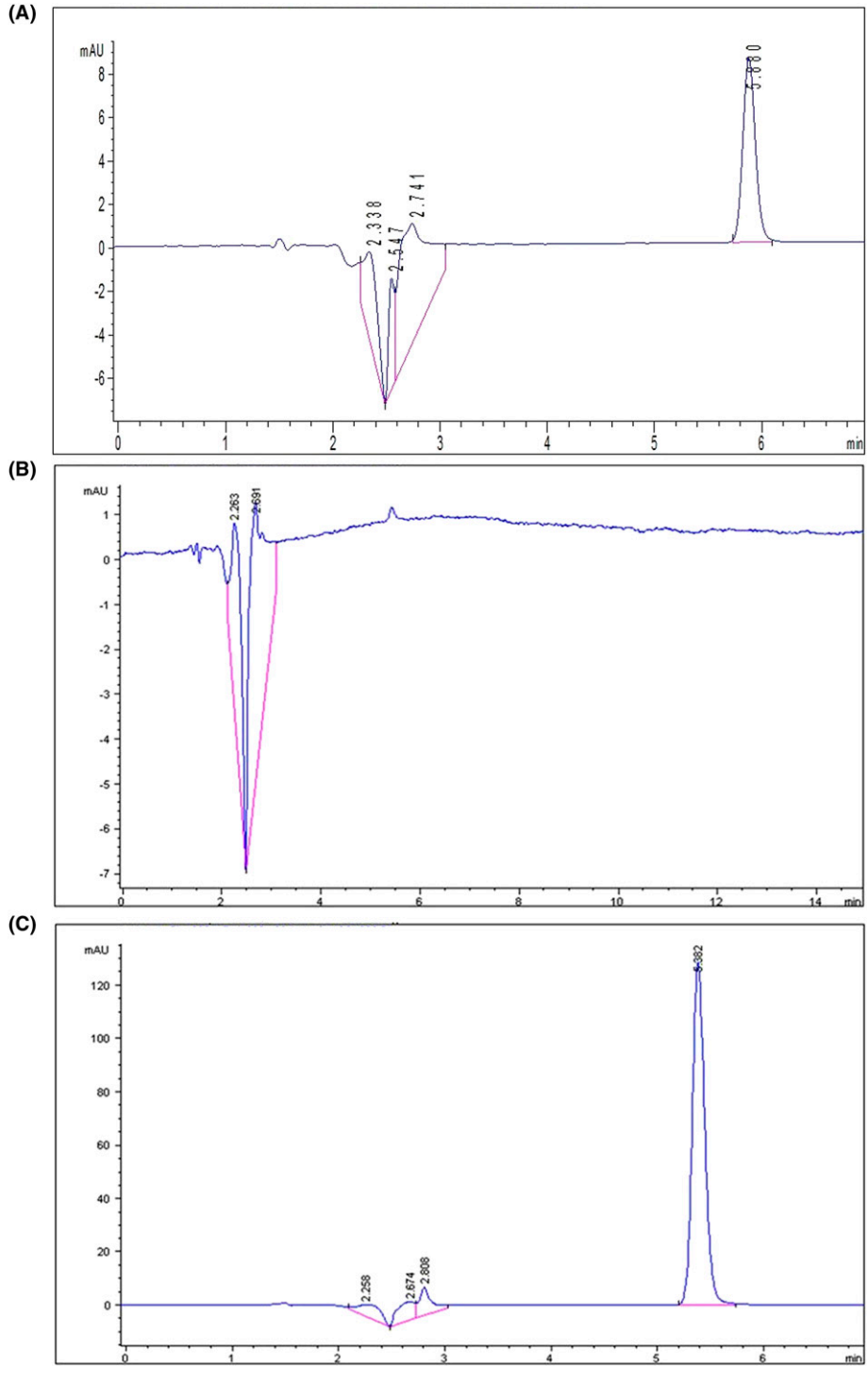

Figure 3. HPLC chromatograms of (A) 1.25 µg/mL nicotinamide standard solution, (B) a blank ME (MEO2), and (C) a nicotinamide-loaded ME (MEO2-N).

Table IV
Validation Results of HPLC Technique for Nicotinamide Analysis

Parameter	Value
Linearity (r^2)	1.0000
Accuracy (%recovery)	98.26 ± 0.06–100.95 ± 0.13
Intra-day precision (%RSD)	0.03–0.19
Inter-day precision (%RSD)	0.03–1.10
LOD (μg/mL)	0.02 ± 0.00
LOQ (μg/mL)	0.05 ± 0.00

Table V
Viscosity of MEC1-N, MEC2-N, and MEO1-N after Storage at Three Different Temperatures for 3 mo

		Viscosity (cps) at 100 rpm		
Time (month)	Temperature	MEC1-N	MEC2-N	MEO1-N
Initial[a]	–	222.65 ± 0.17	259.94 ± 0.00	230.55 ± 0.17
1	4°C	178.23 ± 4.55	225.58 ± 2.94	189.06 ± 2.79
	RT	169.31 ± 3.09	209.06 ± 3.09	207.31 ± 8.13
	45°C	169.31 ± 2.94	209.06 ± 6.44	207.31 ± 7.73
2	4°C	203.39 ± 1.53	246.51 ± 17.66	221.05 ± 13.05
	RT	211.65 ± 2.48	252.40 ± 2.05	225.75 ± 6.09
	45°C	226.20 ± 2.98	242.70 ± 10.88	256.55 ± 0.25
3	4°C	211.75 ± 6.60	239.05 ± 1.70	224.62 ± 15.15
	RT	218.09 ± 1.16	232.75 ± 8.61	238.08 ± 13.45
	45°C	202.36 ± 3.46	241.65 ± 1.24	190.19 ± 2.08

[a]Viscosity values were initially measured when the samples were left at RT overnight after preparation.

Table VI
Percentage of Nicotinamide Remaining in MEC1-N, MEC2-N, and MEO1-N after Storage at Three Different Temperatures for 3 mo

	Nicotinamide remaining (%)		
Formulation	4°C	RT	45°C
MEC1-N	90.10 ± 1.31	93.44 ± 2.40	88.22 ± 0.41
MEC2-N	91.53 ± 2.50	90.76 ± 1.89	90.46 ± 0.60
MEO1-N	94.42 ± 1.71	91.59 ± 1.36	89.49 ± 2.11

RELEASE PROFILES AND KINETICS OF NICOTINAMIDE-LOADED MES

MEC1-N and MEO1-N were selected for evaluation of *in vitro* release behavior because these formulations had the same ratios of components but different oil type. In addition, these MEs provided no appearance change and their active remaining was more than 90% after being stored at 4°C and RT for 3 mo. The release profiles of MEC1-N and MEO1-N in Figure 4 showed that nicotinamide was slowly released from both MEs. The amount of nicotinamide released from MEO1-N was significantly higher than that from MEC1-N ($p < 0.05$, t-test) because of different degrees of oil unsaturation. Olive oil contained 71.530% unsaturated oleic acid, whereas virgin coconut oil contained 48.102% saturated

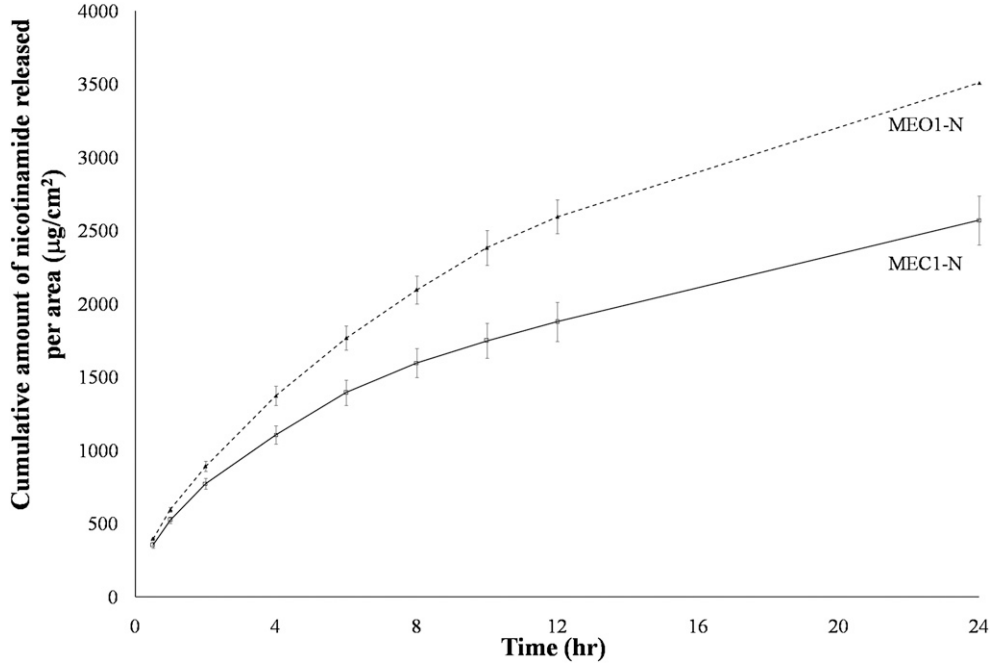

Figure 4. *In vitro* release profiles of MEC1-N and MEO1-N through dialysis membrane.

Table VII
Release Parameters of MEC1-N and MEO1-N

	Zero order		First order		Higuchi model	
Formulation	r^2	k_0[a] (µg/cm^2/h)	r^2	k_f[a] (1/h)	r^2	k_H[a] (µg/cm^2/h$^{1/2}$)
MEC1-N	0.9175	106.29 ± 6.94	0.7129	0.08 ± 0.00	0.9977	615.87 ± 41.04
MEO1-N	0.9104	132.36 ± 4.97	0.7044	0.08 ± 0.00	0.9956	769.09 ± 31.71

[a]The k_0, k_f, and k_H are release rate constants of zero order, first order, and Higuchi model, respectively.

lauric acid and 19.140% saturated myristic acid as exhibited in Table I. This finding was in good agreement with a previous report that higher degrees of unsaturation of triglyceride oils in ME systems resulted in weaker interfacial interactions (29). Release profiles of both MEC1-N and MEO1-N were the best fitted with the Higuchi model as illustrated in Table VII. Hence, the release of nicotinamide from both MEs was dependent on the diffusion mechanism (30).

CONCLUSION

Our findings indicated that among four developed nicotinamide-loaded MEs (MEC1-N, MEC2-N, MEO1-N, and MEO2-N), only MEO2-N was not stable. Other three formulations were physically and chemically stable when kept at 4°C and RT during the period of 3 mo. However, storage at 45°C should be avoided for all samples. MEC1-N and

MEO1-N provided slow release profiles and their release kinetics were the best fitted with the Higuchi model. The current study presented that natural oil MEs were possibly promising nano-carriers for topical delivery of nicotinamide.

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