Original Article

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Characterization and Stability of Vitamin A Palmitate Microemulsions Composed of Isopropyl Palmitate, Water and Polyoxyethylene-10-Oleyl Ether

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Abstract

The systems composed of isopropyl palmitate (IPP) as oil phase, water as aqueous phase and polyoxyethylene-10-oleyl ether (POE-10-OE) as nonionic surfactant were investigated. The results indicated that microemulsion region of IPP/water/POE-10-OE was 13.69% of total area of a ternary phase diagram. Two microemulsion formulations as representatives of low and high surfactant concentrations were selected to be incorporated with vitamin A palmitate (VA). The systems were clear yellowish liquids and no birefringence was observed under cross-polarized light microscopy. They were found to be micreomulsions of water-in-oil (w/o) type. Their rheological properties showed low viscosity with Newtonian flow behavior. After loaded, VA-loaded microemulsions did not change the characteristics of their blank counterparts. Regarding stability, the VA-loaded microemulsions were physically stable but chemically unstable after storage at room temperature for 28 days.

Key words: Microemulsions, Vitamin A palmitate, Characterization, Stability

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INTRODUCTION

Microemulsions are thermodynamically stable, transparent, low-viscosity dispersions of oil and water stabilized by an interfacial film of a surfactant. In pharmaceutical and related fields, microemulsions are widely used due to their good appearance, thermodynamic stability, high solubilization power and ease of manufacture. Microemulsions have clear appearance and give aesthetic looking. They are thermodynamically stable, resulting in long-term stability. Due to high solubilization power of microemulsions, both hydrophilic and hydrophobic active compounds can be incorporated in these systems. When using appropriate amount of compositions, microemulsions spontaneously form. Owning to many advantages, they are often used to deliver drugs via several routes including topical route and also used as vehicles for several cosmetic and cosmeceutical agents[1-4]. In addition to those benefits, microemulsions can provide high permeation of the actives through the skin because of several proposed mechanisms as previously reviewed[2].

The free radical theory proposed by Harman[5] has been accepted as the most important and well known theory about skin aging. Reactive oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical, cause oxidative damage to cellular macromolecules, i.e., proteins, carbohydrates, lipids and etc[6]. Hence, active compounds having antioxidant activity can be considered as antiaging agents. Topical application of vitamin A palmitate (VA) or retinyl palmitate, the most stable of available vitamin A esters, is a well-accepted treatment as an antiaging agent since the stimulatory effects of this vitamin tend to oppose changes that occur with aging[7-8]. VA has to be hydrolyzed by esterase to retinol, which is further oxidized to retinoic acid, the active form, by alcohol dehydrogenase. These enzymes are available in the skin[9]. In order to achieve the activity of VA, the skin permeation enhancement of the formulations is necessary since the epidermis or the outermost layer of the skin is mainly responsible for the barrier function and mechanical resistance[10]. From the above reasons, formulation as microemulsions may be suitable to efficiently deliver VA into the skin.

Different types of surfactants and oils can be used for preparation of microemulsions. In this study, polyoxyethylene-10-oleyl ether (POE-10-OE) and isopropyl palmitate (IPP) were used as surfactant and oil phase, respectively. POE-10-OE or $C_{18:1}E_{10}$ is widely used in topical pharmaceutical formulations and cosmetics primarily as an emulsifying agent for water-in-oil (w/o) and oil-in-water (o/w) emulsions due to its low irritation potential. It has been also used in several microemulsion formulations[11-17]. IPP is acceptable oil for using in topical pharmaceutics and cosmetics. It can be also used in preparation of microemulsions[16-20].

The aims of the present study were to develop and to investigate phase behavior of the nonionic system composed of IPP, purified water and POE-10-OE. The microemulsions containing VA were prepared and determined physicochemical characteristics and stability.

MATERIALS AND METHODS

Materials

Vitamin A palmitate (VA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyoxyethylene-10-oleyl ether (POE-10-OE, Sympatents-AO/100®) was purchased from Kolb Distribution Ltd (Hedingen, Switzerland). Isopropyl palmitate (IPP, Palmester 1517®) was purchased from East Asiatic (Bangkok, Thailand). Isopropanol was purchased from BDH Chemicals Ltd (Poole, UK). Purified water was used throughout the experiments. All chemicals were of pharmaceutical grade and used as received without further purification.

Construction of Phase Diagram

POE-10-OE was mixed with IPP at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Calculated amount of purified water was added at 10% increment to obtain water concentrations in the range of 0-90% w/w. The samples were prepared by adding an appropriate amount of each component in the individual bottle and then vigorously mixing with a vortex mixture. The samples were stored at room temperature for at least 24 hours in order to reach equilibrium before further investigation. The obtained samples were classified as microemulsions when they appeared as clear isotropic liquids, as emulsions when their appearance was milky or turbid liquids and as gels when no change in the meniscus after tilting to an angle of 90°C was found. The microemulsion region was finally constructed on a triangular graph. No attempts were made to identify the regions of other association structures[15]. A cut-and-weigh method was used to determine the percentage of the total area of the phase diagram covered by the microemulsions[19].

Preparation and Characterization of Samples

Two formulations were selected at concentrations of POE-10-OE of 20% w/w and 60% w/w which were representatives of low and high surfactant systems and designated as ME-L and ME-H, respectively. ME-L composed of IPP 77% w/w, water 3% w/w and POE-10-OE 20% w/w while ME-H composed of IPP 35% w/w, water 5% w/w and POE-10-OE 60% w/w. They were prepared by simple mixing the compositions. For preparation of VA-loaded microemulsions, VA was incorporated at a concentration of 1% w/w to each microemulsion formulation and designated as ME-L-VA and ME-H-VA, respectively.

All samples were then characterized for the following properties. They were observed for their visual appearance. Their microscopic appearance was investigated using cross-polarized light microscopy (Olympus, Japan) at a magnification of ×40 in order to distinguish microemulsions from liquid crystals. Microemulsion type was tested by dilution method. A drop of each microemulsion was placed on copper electron microscopy grids and the sample was quickly frozen at liquid nitrogen, followed by freeze-dried at -55°C. Subsequently, the shape of the sample was demonstrated by the transmission electron microscopy (TEM CM-12, Philips, Netherlands). Rheological behavior and viscosity values of the samples were investigated in triplicate using a Brookfield DV-III programmable cone and plate rheometer (Brookfield Engineering Laboratories, USA) fitted with a CP-42 cone spindle. Brookfield Rheocalc operating software controlled the rheometer[16,18,21,22].

Determination of Drug Content

To determine the total drug content of the microemulsions, accurate weight (≈ 10 mg) of VA-loaded microemulsions was dissolved and diluted with isopropanol to an appropriate concentration. The absorbance values of the obtained solutions were then measured in triplicate by ultraviolet (UV) spectrophotometer (Spectronic Genesys 5, Milton Roy, USA) at 325 nm. Isopropanol was used as the blank for zero-setting. The amounts of drug content were calculated by comparing the measured absorbance values with those in the standard curve[23]. Validation of the method was performed to ensure that the calibration curve between 6 and 12 μg/ml of VA in isopropanol solutions was in the linearity range ($r^2 > 0.999$) and the measured absorbance was in the range of 0.2-0.8.

Stability Study of the Microemulsions

For stability study, the VA-loaded microemulsions were stored at room temperature in glass containers with and without aluminum foil wrapping in order to investigate the effects of light. The remained amounts of VA in the samples were performed at 1, 2, 7, 14, 21, and 28 days using UV spectrophotometer as previously described. Each sample was assayed in triplicate.

RESULTS AND DISCUSSION

Phase Diagram

The shaded area assigned on ternary phase diagram shown in Figure 1 presents the microemulsion region of IPP/water/POE-10-OE system. The area outside the shaded indicates a turbid region with multiphase systems, e.g., original emulsions and gels, depending on the concentrations of the compositions in the system. The microemulsion region of IPP/water/POE-10-OE system was 13.69% of total area of the ternary phase diagram. It was noted that the microemulsion region of the present studied was smaller than those obtained from the previous studied systems of the identical surfactant, POE-10-OE since the microemulsion areas of isopropyl myristate/water/POE-10-OE[15] and IPP/water/POE-10-OE:1-butanol[16] were reported to be as 15.31% and 40.82%, respectively. It was in accordance with previous report that larger oil molecules of IPP ($C_{19}H_{38}O_2$) could penetrate into the hydrocarbon portion of the surfactant interface less than smaller oil molecules of isopropyl myristate ($C_{17}H_{34}O_2$)[12]. For the microemulsion systems having 1-butanol, 1-butanol could provide large microemulsion region due to interaction with the surfactant monolayer to increase the flexibility of the interfacial film[24]. However, the use of 1-butanol is not pharmaceutically acceptable.

Characteristics of Samples

All blank and VA-loaded microemulsions were clear yellowish liquids and did not exhibit birefringence under cross-polarized light microscopy. The visual appearance of microemulsions can be quite similar to that of lamellar and hexagonal liquid crystals. Cross-polarized light microscopy is the best way for differentiating them. Under cross-polarized light microscopy, birefringence can be seen for lamellar and hexagonal liquid crystals but no birefringence is found for microemulsions[22]. From miscibility test, it was found that they were immiscible with water, referring to water-in-oil (w/o) type. Transmission electron micrographs of ME-L and ME-H inFigure 2 show the globule structures, indicating the systems of microemulsions. Table 1 exhibits the physicochemical characteristics, i.e., viscosity (η) and correlation coefficient (R_{xy}) between shear rate (x) and shear stress (y) for determination of flow pattern. The apparent viscosity values of the samples were generally low and R_{xy} was nearly or equal to 1 indicating Newtonian flow behavior, as expected for microemulsions[25]. ME-H had higher viscosity than ME-L since it contained higher concentration of POE-10-OE. Incorporation of VA slightly affected the viscosity of the microemulsions; however, it did not change the general flow behavior.

Stability of VA-loaded Microemulsions

After 28 days storage at the room temperature, the appearance of all samples did not obviously change. The obtained samples were physically stable due to thermodynamic characteristics of microemulsions. However, VA-loaded microemulsions were chemically unstable as noted in Tables 2 and 3. The conjugated double bond system in VA structure as illustrated in Figure 3 exhibits an electron-dense region which is attractive to the radicals, resulting in degradation via oxidation[26]. The chemical stability of VA in cosmetic products was found to be depended on several factors such as light, temperature, type of vehicle, initial concentration and available of oxygen[26-29]. As seen in Table 2, ME-L-VA kept in protect-from-light condition had higher chemical stability than those stored in normal condition. Since VA degrades via radical reactions, light is one of catalyzes. In the middle of studied period (Day 7 and 14), ME-H-VA in protect-from-light condition seemed to be more stable than those in normal condition. However, ME-H-VA kept in both studied conditions had similar chemical stability (Table 3). A possible reason involved the interaction between VA and POE-10-OE moieties. High concentration of POE-10-OE could provide high available of oxygen to react with VA, leading to degradation via oxidation. It was found that the concentration of peroxide in the emulsifier affected to the degradation of VA[28]. For ME-H-VA, the effect of available of oxygen on the oxidation might greater than that of light. Therefore, the IPP/water/POE-10-OE system at low concentration of POE-10-OE (20% w/w) was more suitable for incorporating with VA than that at high concentration of POE-10-OE (60% w/w) when the samples were stored in protect-from-light condition.

The Miglyol 812/water/POE-10-OE microemulsions at the surfactant concentration of 23% w/w were reported to be used as the vehicles for VA; however, the chemical stability of the products was not determined yet[14]. Although IPP/water/POE-10-OE microemulsions at the surfactant concentration of 20% w/w was appropriate for VA incorporation, their chemical stability was too low. Other strategies are required to increase stability of VA-loaded microemulsions such as addition of antioxidants.

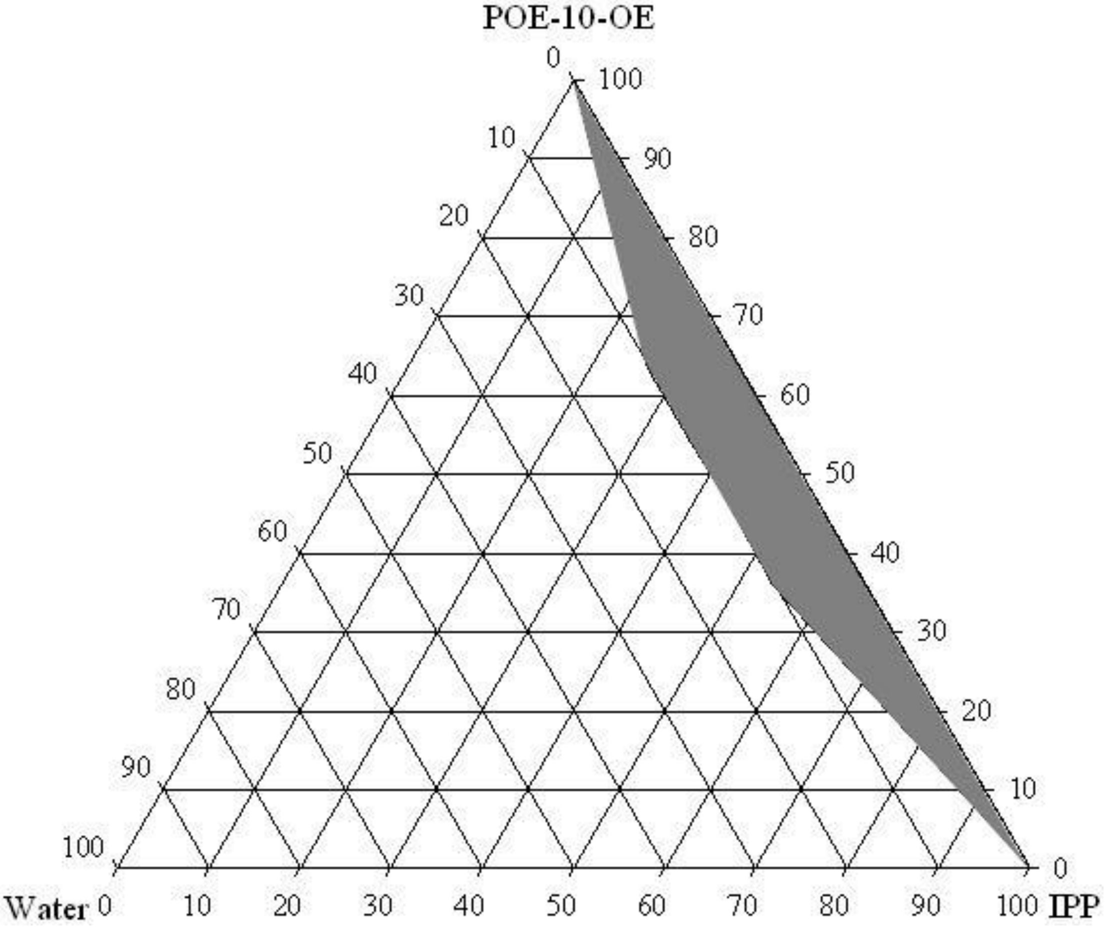

Figure 1. Ternary phase diagram of IPP/water/POE-10-OE system. The shaded area presents the microemulsion region.

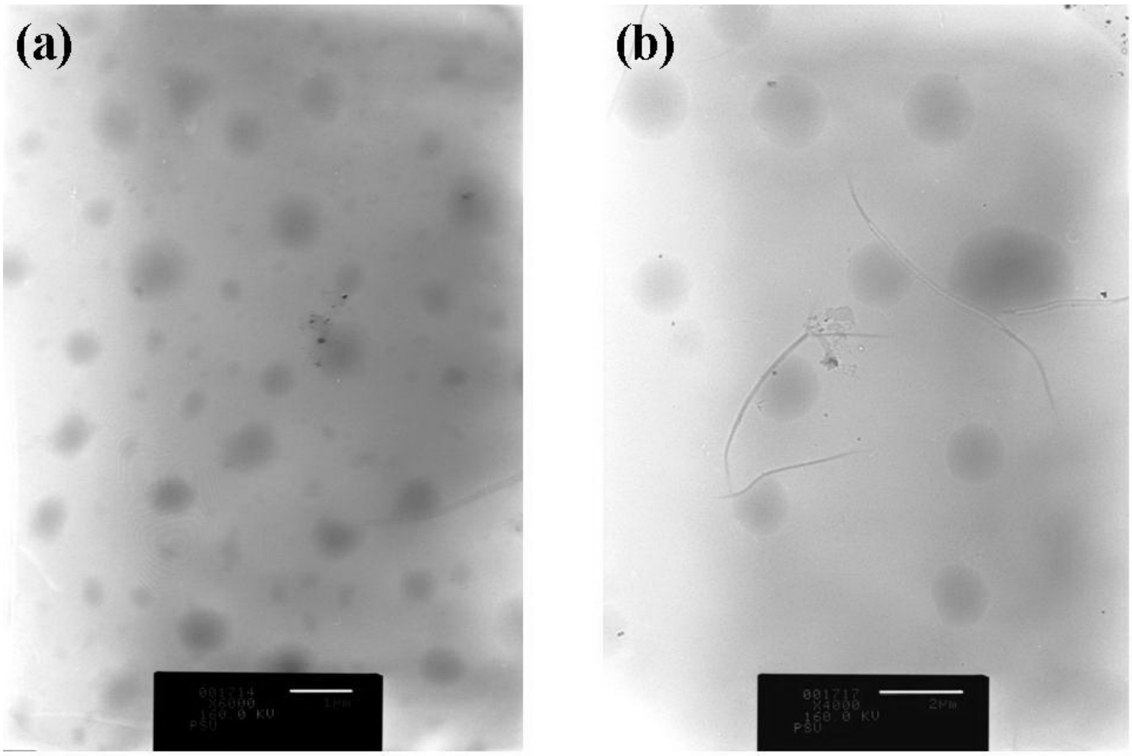

Figure 2. Transmission electron microphotographs of (a) ME-L (the scale bar represents 1 μm and x6,000) and (b) ME-H (the scale bar represents 2 μm and x4,000).

Table 1. Physicochemical characteristics of samples; *n*=3

Sample	η (cP)	R_{xy}
ME-L	14.91±0.08	1.0000
ME-L-VA	15.62±0.05	0.9999
ME-H	47.89±0.20	1.0000
ME-H-VA	48.92±0.18	1.0000

Table 2. Remained amount of VA in the ME-L-VA during the stability study; *n*=3

Time (days)	Normal condition		Protect-from-light	
	Amount of VA (% w/w)*	% Remained amount*	Amount of VA (% w/w)*	% Remained amount*
0	1.13	100.00	1.13	100.00
1	0.92	81.47	1.00	87.93
2	0.85	75.02	0.90	79.28
7	0.83	72.97	0.88	78.00
14	0.69	61.22	0.73	64.22
21	0.49	43.15	0.65	57.11
28	0.34	30.38	0.57	50.17

*The SD values were lower than 0.002; therefore they did not reported in the Table.

Table 3. Remained amount of VA in the ME-H-VA during the stability study; *n*=3

Time (days)	Normal condition		Protect-from-light	
	Amount of VA (% w/w)*	% Remained amount*	Amount of VA (% w/w)*	% Remained amount*
0	1.01	100.00	1.01	100.00
1	0.97	96.44	0.99	97.91
2	0.95	94.47	0.92	90.97
7	0.67	66.29	0.86	84.86
14	0.62	61.66	0.73	71.97
21	0.44	43.50	0.47	46.98
28	0.32	31.67	0.32	31.72

*The SD values were lower than 0.002; therefore they did not reported in the Table.

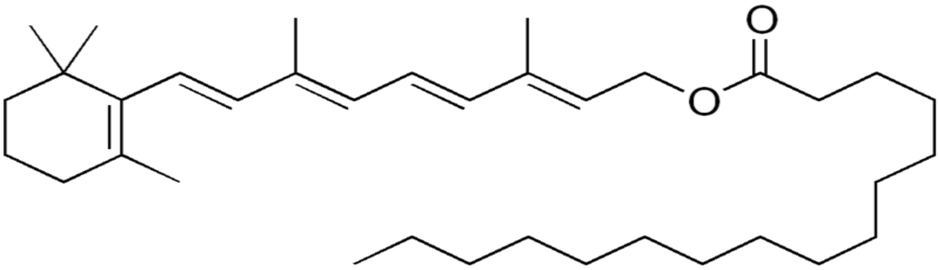

Figure 3. Structure of VA.

CONCLUSIONS

The results indicated that the system composing of IPP, water and POE-10-OE was able to provide 13.69% of microemulsion region in the ternary phase diagram. Incorporation of VA into the studied microemulsions did not alter the original characteristics. The IPP/water/ POE-10-OE microemulsions at the surfactant concentration of 20% w/w was more suitable for VA incorporation than those at the higher surfactant concentration of 60% w/w since more chemical stability was observed in the storage of protect-from-light condition. However, some strategies for increasing the chemical stability of VA-loaded microemulsions are required in order to protect the oxidation of VA.

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