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Research Article

PREPARATION OF SOLID LIPID NANOPARTICLES CONTAINING MANGOSTEEN PERICARP EXTRACT

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ABSTRACT

Objectives: The aim of this study was to develop solid lipid nanoparticles (SLNs) containing mangosteen pericarp extract (MPE) to achieve enhanced photoprotection and to provide an alternative to synthetic sunscreens in the market.

Materials and Methods: The MPE was prepared using the maceration method, and evaluated for sun protection factor (SPF) value using an ultraviolet (UV)-Vis spectrophotometer. SLNs were prepared through ultrasonication method. Blank-SLNs were formulated using stearic acid (SA) or palmitic acid (PA) as solid lipids at a concentration of 3%. Tween® 80 or polyvinyl alcohol (PVA) was employed as a surfactant with a concentration ranging from 1 to 2%. The obtained blank-SLNs were investigated for their physical characteristics, (i.e., morphology, particle size, polydispersity index [PDI], and zeta potential values). The blank-SLNs with suitable physical characteristics were selected to encapsulate MPE and evaluated for the physical characteristics.

Results: The MPE was a brownish viscous substance with an SPF value that ranged from 3.09±0.005 to 27.20±0.05 at a concentration ranging from 0.02 to 0.1 mg/ml. Based on the physical characteristics, the blank-SLNs employing PA or SA with 1% of PVA were selected. The MPE-SLNs were spherical, with a particle size that ranged from 443.51±6.50 to 533.52±16.15 nm; PDI ranged from 0.35±0.008 to 0.459±0.02, and zeta potential value ranged from 18.32±1.37 to −19.03±0.64. The entrapment efficiencies of MPE-PA-SLNs and MPE-SA-SLNs were 83.24±1.37% and 84.17±0.411%, respectively.

Conclusion: The results indicated the promising potential of MPE as a UVB photoprotector. The MPE-SLNs were also successfully formulated, but, further study is needed to confirm the potential of MPE-SLNs to be used as a sunscreen, and their stability during storage.

Keywords: Mangosteen pericarp extract, Solid lipid nanoparticles (SLNs), Ultraviolet radiation, Sun protection factor.

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INTRODUCTION

Sunlight is a form of electromagnetic radiation which is divided into three regions, i.e., ultraviolet (UV) (UV, 200–400 nm), visible (400–780 nm), and infrared light (>780 nm). UV light comprises the most harmful sunlight wavelengths, and its intensity has increased in recent years [1]. The bad effects of UV radiation on the human skin include chronic effects (photoaging, genetic mutations, and DNA damage, which ultimately causes skin cancer) and acute effects (photosensitivity, photoallergy, and sunburn or erythema) [2].

Various methods are used for protecting skin from these harmful effects of UV radiation. Use of photoprotective clothes, sunglasses, and hats complemented with the use of sunscreens during the highest UV radiation hours are key principles of photoprotection [3]. In recent years, the use of sunscreen product has become more popular, but previous studies have reported that chemical sunscreens possessed potential toxicity to humans. On the other hand, physical sunscreens offer greater protective action, but their opacity, viscosity, and greasiness have limited their usage [4].

Natural extracts have recently been considered as alternative sunscreen agents due to their potency in absorbing UV light [5]. Previous findings showed that 2 mg/ml of *Dracocephalum moldavica* L. or *Viola tricolor* L. leave extracts containing polyphenolic compounds such as rutin, apigenin, luteolin, and violanthin have sun protection factor (SPF) values of 24.79 and 25.69, respectively [5]. Another study reported that 0.1 mg/ml of Sri Lankan mangosteen extract containing flavonoids and polyphenols had an SPF value of 15.96 [6]. In other words, plant extracts show promising potential as an alternative to synthetic sunscreens.

Mangosteen is one of the tropical fruits that can be easily found in the rainforests of Thailand, Malaysia, and Indonesia. Mangosteen fruit pericarp contains α-mangostin as the major compound and more than 40 other xanthones [7]. α-Mangostin, a polyphenolic xanthone, contains chromophore, which absorbs light in a UVB region, and shows maximum absorption peaks at 244 and 317 nm. The absorption wavelength of 244 nm represents the π→π* transition of the aromatic structure while the peak at 317 nm is related to n→π* transition of carbonyl structure [7].

Solid lipid nanoparticles (SLNs) have been shown to act as a promising carrier system for sunscreen preparations. The previous study reported that smaller particle sizes of SLNs scatter the light, and result in higher sunscreen activity compared to conventional formulations. In addition, SLNs possess a slower release rate of organic sunscreens than nanosuspension and conventional o/w emulsion [8,9]. Thus, the sunscreens are retained on the skin surface longer and provide longer protection against UV radiation [9,10]. In addition, SLNs may protect labile active compounds from degradation caused by the external environment (e.g., water). SLNs are physically stable. Moreover, SLN preparations may avoid the use of organic solvents and are easy to scale up [9].

The efficacy of sunscreen is determined by the SPF value, which is defined as the ratio of the minimal erythema dose of UVB radiation measured on the skin with or without the presence of a sunscreen agent [11]. The FDA and COLIPA provide a recommended *in vivo* testing protocol to measure the SPF value of products on human volunteers [12]. Although it is an established and recommended method by FDA and COLIPA, it has several

disadvantages such as being time-consuming, expensive, and potentially harmful to human volunteers. On the other hand, the measurement of SPF by *in vitro* testing has advantages such as being less expensive, safe for humans and having the ability to provide preliminary data for further development of an effective sunscreen. Based on economical, practical and ethical considerations, *in vitro* determination of SPF is a more suitable method and should be used more often than *in vivo* method [12]. This research is designed with the objective of preparing SLNs containing mangosteen pericarp extract (MPE) to be used as an alternative to synthetic sunscreens in the market.

MATERIALS AND METHODS

Materials

Fresh fruit of *Garcinia mangostana* (purchased in June 2017, in Bangkok, Thailand); ethanol, methanol, ethyl acetate, AR grade, and high-performance liquid chromatography (HPLC) grade (RCI Labscan, Thailand); polyvinyl alcohol, JP-18FT (Japan VAM and POVAL Co., Ltd., Japan); palmitic acid (PA) (Namsiang Company Limited, Thailand, lot no 1021L736980); α-mangostin (Wuhan Chemfaces Biochemical Co., Ltd., China, lot no CFS201702A015), glyceryl trimyristate (The Sun Chemical Co., Ltd., Thailand, lot no 606184); glyceryl behenate (P.C. Intertrade Co., Ltd., Thailand, lot no 149295); Tween® 80 (Croda Singapore PTE LTD, Singapore); and glyceryl palmitostearate (Gattefosse, Germany); cetyl palmitate, stearic acid (SA) (Emery Oleochemicals, Malaysia) were used.

Preparation of MPE

The mangosteen fruit pericarps were cut into small pieces approximately 1×1 inch in size and dried at the temperature of 45±0.5°C in a hot air oven. The dried fruit pericarps were ground into powder using a botanical grinder. The mangosteen pericarp powder was macerated with ethyl acetate at room temperature for 48 h. The MPE was concentrated using a rotary evaporator (Rotavap or Buchi R-200, Switzerland) and was kept in a desiccator for further studies [13].

Characterization of MPE

The validated HPLC method was employed. Characterization of the MPE such as the presence of α-mangostin in the MPE, peak purity index of the MPE peak, purity percentage of the MPE, and determination SPF value, was performed.

α-Mangostin was analyzed utilizing a validated HPLC method using Shimadzu LC-10AD VP (Shimadzu, Japan). The column was a BDS Hypersil C18, 5 µm (4.6×250 mm) at ambient temperature. The mobile phase was a methanol: water mixture (87:13 v/v) with a flow rate of 1.0 ml/min. The detection wavelength was set at 244 nm. The MPE was dissolved by the addition of ethanol at a concentration of 80 µg/ml and then filtered through a 0.45 µm membrane filter [14,15].

The presence of α-mangostin in the MPE was identified by comparing the HPLC retention time and spectral match factor of the MPE with that of the α-mangostin reference standard. Peak purity index of the MPE was determined by comparing spectra collected during chromatographic separation. Purity percentage of the MPE was calculated from the concentration of α-mangostin in the MPE.

The SPF value of the MPE was determined using a UV-Vis spectrophotometer. An MPE solution in ethanol in a concentration range of 0.02–0.1 mg/ml was prepared. The absorption spectra were taken from 290 to 320 nm, and ethanol was used as a blank. The SPF value was calculated using the Mansur's equation [16,17].

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs\ (\lambda)$$

Where,
EE: Action spectrum of erythema [17];
I: Spectrum of solar intensity [17];
Abs: Sunscreen product absorbance;
CF: Correction factor (=9.37). The calculation used a standard sunscreen formulation containing 1% octyl methoxycinnamate presented an SPF value of 1.5.

Selection of solid lipids

Lipids such as glyceryl palmitostearate, glyceryl behenate, glyceryl trimyristate, SA, PA, and cetyl palmitate were screened for their potential to solubilize MPE. The 1.5 g of MPE was accurately weighed in screw-capped test tubes. The 3 g of solid lipid was separately heated above its melting point. The molten lipid was added into the MPE. The mixture was stirred continuously until the clear and homogeneous dispersion of the MPE was obtained. The lipids giving a homogeneous mixture of both molten and solidified stages were selected for further studies [18].

Preparation and characterization of blank-SLNs and MPE-SLNs

In this study, ultrasonication technique was used for the preparation of aqueous SLNs dispersion. The oil and aqueous phases were separately prepared. The oil phase consisted of molten solid lipid, and the aqueous phase consisted of hydrophilic surfactant and water. The type of surfactant was varied (i.e., tween® 80 and polyvinyl alcohol and their concentrations were varied in a range of 1–2%). Solid lipid (3 g) was melted at 5°C above its melting point. Under stirring at 14,000 rpm using a high-speed stirrer, the oil phase was dispersed in a hot aqueous phase at the same temperature for 5 min. The obtained hot pre-mix emulsion (100 g) was sonicated using an ultrasonicator with an amplitude of 80% for 15 min to form hot nanoemulsion, which was quickly poured into cold water with a 1:2 ratio of nanoemulsion to cold water to obtain SLN dispersion. The preparation of MPE-SLNs followed the same procedure as blank-SLNs preparation, with the addition of MPE (1.5 g) in the molten lipids.

The physical characteristics such as morphology, particle size, size distribution (polydispersity index [PDI]), and zeta potential were investigated using a scanning electron microscope (SEM) and a photon correlation spectroscopy (PCS), respectively. Formulations with particle sizes between 300 and 500 nm and PDI value ≤0.5 were selected for further studies. The nanoparticles were collected using ultracentrifuge Hitachi CP-NX 100 at 25°C, 18,000 rpm for 15 min [19].

Entrapment efficiency

Entrapment efficiency percentage was evaluated using the validated HPLC method. To calculate the entrapment efficiency, 1 g of SLNs dispersion containing MPE was put into an ultracentrifuge assembly. The encapsulated MPE was separated by ultracentrifugation at 25,000 rpm at 25°C for 15 min. The precipitated pellets containing MPE were dissolved by the addition of ethanol. The clear solution was analyzed for entrapped α-mangostin content at a wavelength of 244 nm. All analyses were done in triplicates. The entrapment efficiency was calculated by equations below [19]. MPE-free SLN will be used as a blank.

$$EE\% = \frac{\text{mass of the MPE in nanoparticle}}{\text{theoritical mass of MPE used in nanoparticle preparation}} \times 100$$

Data analysis

The data of particle size, size distribution (PDI), zeta potential, entrapment efficiency percentage, and SPF value were statistically analyzed using ANOVA, and a significant difference ($p<0.05$) was indicated. The data were subjected to multiple comparisons by Tukey test to evaluate the difference.

RESULTS AND DISCUSSION

Preparation of MPE

The MPE was obtained through maceration process of 450 g of the dried fruit pericarp with ethyl acetate. The extract was a brownish, viscous liquid. The yield percentage obtained was 9.85% ±0.5%, n=3. The yield percentage in this study was higher than that reported by

Ruamkittham, with a yield percentage of 7.47%, which might be due to different environmental factors such as provenance, soil condition, and time of harvest [20].

Characterization of MPE

The HPLC analytical method was successfully validated in the concentration range of 10–60 µg/ml. The recovery percentage ranged from 99.83% to 100.49% with an R.S.D. value below 2%. Chromatogram of MPE showed similar retention time to that of standard α- mangostin with retention time of 8.8 minutes. The major peak was identified as α- mangostin with spectral match factor of 999.987. Peak purity index of the major peak was 0.99. In other words, there was no interference from coeluting analytes. The percentage of α-mangostin in MPE was calculated to be 44.30±3.45%.

The SPF is a quantitative measurement of the UVB protective ability of sunscreen. Table 1 shows that MPE is suitable to be used in sunscreen products. In this study, the final concentration of MPE in the product was targeted at 0.06 mg/ml to obtain an SPF value around 15 [21].

Selection of solid lipid

Selection of lipids was performed to dissolve 1.5 g of MPE in 3 g of solid lipids. Amongst the solid lipids which investigation, SA, and PA demonstrated the effectively solubilizing potential of MPE and yielded a brown transparent mixture at both molten and solidified stages. No separation was found after the solidification of the molten lipids. However, MPE was not dissolved in diglycerides (cetyl palmitate) or triglycerides (glyceryl palmitostearate, glyceryl behenate, and glyceryl trimyristate)

As regulated by the US FDA, all six lipids under this investigation are generally recognized as safe lipids. The MPE was dissolved in PA (hydrophile-lipophile Balance [HLB]=15.6) and SA (HLB=15) which are saturated fatty acids but was not dissolved in long-chain diglycerides and triglycerides (HLB in the range of 2–10) due to the less lipophilic nature of the ethyl acetate extract. Based on the above result, the HLB value of the obtained MPE was appeared to be around 15 [22,23].

Preparation and characterization of blank-SLNs

The selection of surfactant used in this study was based on the required HLB values of solid lipids (PA and SA) which are 15–15.6 [22,23]. Therefore, tween® 80 and polyvinyl alcohol (PVA) were chosen due to their HLB value of 15 and 18, respectively [24,25]. Other studies have reported that the ultrasonication method resulted in smaller particle size and PDI than the solvent injection method or high-pressure homogenization method [26]. Thus, ultrasonication was selected in this study.

The physical appearances of blank-SLNs were white milky dispersion. The blank-SLNs containing PVA were more viscous than the blank-SLNs containing tween® 80. The physical characteristics of blank SLNs are shown in Table 2.

The morphology of blank-SLNs was investigated using SEM. Fig. 1a shows the spherical shaped and non-smooth surface of nanoparticle topography. It was found that different lipid types affected the particle size and PDI of blank SLNs. When the same type and concentration of surfactant were employed, SA (C18)-SLNs had bigger particle size than PA (C16)-SLNs ($p<0.05$). The particle size and PDI of blank SLNs for both lipids using tween® 80 were larger than those produced by PVA ($p<0.05$). The particle size and PDI of both lipids using PVA were decreased by increasing surfactant concentration. On the other hand, an increase in particle size and PDI was obtained by increasing surfactant concentration from 1 to 2% using tween® 80. The zeta potential value of blank-SLNs for both lipids using tween® 80 was lower ($p<0.05$) than those SLNs containing PVA.

The shorter hydrocarbon chain length of PA leads to smaller particle size in comparison to longer hydrocarbon chain length of SA [27]. In addition, the higher melting point of SA (69.6°C) compared to that of PA (62.9°C) [28] results in a higher viscosity of the dispersed phase and leads to larger particle size [27].

Surfactant plays an important role in an emulsion. It helps the stabilization of the system and controls the particle size. High surfactant concentration decreases the surface tension of the lipid droplet, stabilizes the droplet surface during homogenization, and results in smaller particle size and lower PDI [29]. Likewise, low surfactant concentration may be not sufficient to stabilize the system and results in aggregation and larger droplet size [29].

Table 1: *In vitro* assessment of the SPF of the MPE

MPE concentration (mg/ml)	SPF value[a]
0.02	3.09±0.0051
0.04	9.22±0.0091
0.05	12.16±0.01
0.06	14.86±0.007
0.08	20.99±0.01
0.1	27.20±0.05

MPE: Mangosteen pericarp extract, SPF: Sun protection factor, [a]Mean±SD, n=3

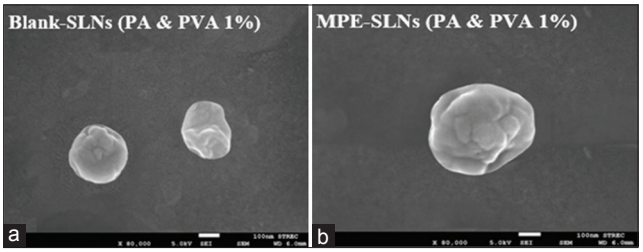

Fig. 1: Scanning electron micrographs of (a) blank PA-SLNs (magnification ×80,000; scale 100 nm); (b) MEP-PA-SLNs (magnification ×80,000; scale 100 nm)

Table 2: Formulations of blank SLNs

Code	Lipid 3% (w/w)	Surfactant (% w/w)		Particle size[a]	PDI[a]	Zeta potential[a]
F1	Stearic acid	Tween® 80	1	584.33±8.39	0.53±0.02	−28.5±1.18
F2			1.5	818.97±0.04	0.69±0.04	−27.9±0.48
F3			2	1397.3±620.2	0.98±0.02	−27.9±1.04
F4		PVA	1	382.61±5.82	0.15±0.01	−15.76±0.79
F5			1.5	341.53±3.46	0.07±0.007	−16.55±0.21
F6			2	336.1±1.94	0.05±0.009	−16.65±0.53
F7	Palmitic acid	Tween® 80	1	555.3±11.12	0.48±0.01	−30.4±1.83
F8			1.5	765.32±0.02	0.59±0.02	−31.01±1.48
F9			2	985.71±15.28	0.855±0.08	−29.33±2.064
F10		PVA	1	306.5±0.01	0.09±0.01	−15.75±0.37
F11			1.5	304.87±1.55	0.06±0.007	−14.92±0.50
F12			2	301.68±1.99	0.04±0.005	−15.21±0.19

[a]Mean±SD, n=3. PVA: polyvinyl alcohol, SD: Standard deviation, PDI: Polydispersity index, SLNs: Solid lipid nanoparticles

Table 3: The physical properties of SLNs containing mangosteen pericarp extract

Code	MPE	Before centrifugation		After centrifugation		Zeta potential[a]	EE %[a]
		Particle size[a]	PDI[a]	Particle size[a]	PDI[a]		
MPE-SA-SLNs	1.5 g	533.52±16.15	0.459±0.02	612.74±7.02	0.63±0.01	−19.03±0.64	84.17±0.41
MPE-PA-SLNs		443.51±6.50	0.35±0.008	568.15±6.68	0.44±0.03	−18.32±0.68	83.24±1.37

[a]Mean±SD, n=3. MPE: Mangosteen pericarp extract, SLNs: Solid lipid nanoparticles, SD: Standard deviation, PDI: Polydispersity index

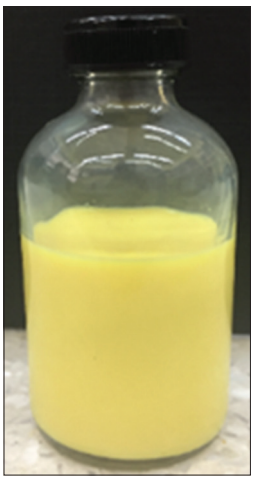

Fig. 2: Mangosteen pericarp extract - solid lipid nanoparticles

Previous studies reported the same cases, in which higher surfactant concentration yielded bigger particle size, which may be related to the depletion-flocculation mechanism of surfactant. This happens due to the formation of micelles at high concentration of surfactant in the continuous phase that increases the local osmotic pressure and attracts the droplet to come close enough to merge their adsorbed layers. Ultimately, the aggregation took place and increased the particle size [30].

Zeta potential indicates a repulsive force between nanoparticles to prevent the aggregation of nanoparticles in the process of emulsion stabilization. All the blank-SLNs were found to be negatively charged due to fatty acid residues of PA and SA [27]. Blank-SLNs using tween® 80 had lower zeta potential value than those SLNs using PVA due to the existence of oleic acid traces in tween® 80 [13].

The blank-SLNs with particle sizes below 300–500 nm were selected to encapsulate the MPE to prevent the penetration of nanoparticles into the deeper layer of the skin. Based on the trial experiment, the higher concentration of PVA forming a thicker film when applied on the skin resulted in unpleasant feeling during application. Therefore, the blank-SLNs with 1% PVA, the lowest concentration, were selected (F4 and F11).

Preparation and Characterization of MPE-SLNs

The physical appearance of SA-SLNs containing MPE and PA-SLNs containing MPE (Fig. 1b) was yellow fluid dispersion as shown in Fig. 2 (Table 3).

The particle size and PDI of SLNs containing MPE were found to be larger than blank SLNs ($p<0.05$). SA-SLNs containing MPE had a larger particle size and PDI than PA-SLNs containing MPE ($p < 0.05$). There were no significant differences ($p>0.05$) of zeta potential and entrapment efficiency percentage between SA-SLNs containing MPE and PA-SLNs containing MPE. After centrifugation, the particle size and PDI were larger when compared to with that of before centrifugation ($p<0.05$).

The incorporation of MPE into molten lipids probably increased the viscosity of the dispersed phase, led to less emulsification capability of the system and resulted in larger particle size of SLNs containing MPE [27]. The particle size and PDI of SA-SLNs containing MPE were larger than that of PA-SLNs containing MPE because the hydrocarbon chain length of SA (C18) was longer than that of PA (C16) [27]. In addition, centrifugation causes the collision of the particles under high velocity leading to particle agglomeration and larger particle size.

CONCLUSION

The *in vitro* test performed by a UV-Vis spectrophotometer verified the photoprotective ability of MPE, which was also successfully entrapped into SLNs using PA or SA as solid lipids and PVA as a surfactant. SEM showed the spherical morphology of MPE-SLNs. Further investigation is needed to confirm the promising ability of SLNs containing MPE to be used as an alternative to synthetic sunscreens in the market, and its stability during storage conditions.

CONFLICTS OF INTEREST

All authors have none to declare.

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