# Development of Liposomes Encapsulating *Garcinia mangostana* Fruit Rind Extract for Enhancing of Tropical Delivery System

# การพัฒนาตำรับลิโพโซมที่กักเก็บสารสกัดเปลือกผลมังคุดเพื่อเพิ่มประสิทธิภาพในการนำส่งผ่านผิวหนัง

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**ABSTRACT**

*Garcinia mangostana* fruit rind extract had been macerated with ethanol and formulated in liposomes for topical delivery system by using Tween 80 or cholesterol as vesicle stabilizer. The amount of phospholipid and propylene glycol in the formula were fixed. The concentrations of *G. mangostana* fruit rind extract were varied. From the results, the liposomes consisting of Tween 80 had particle sizes in the same range as cholesterol which were 79.5-90.0 nm. The polydispersity index of liposomes with Tween 80 had increased values when increased the extract concentration while those of cholesterol had no effect. For zeta potential, the liposomes consisting of Tween 80 showed higher negative values than those of cholesterol which were in the range of -38.0 to -39.9 mV. The entrapment efficiency (%EE), we found that the concentration of *G. mangostana* fruit rind extract had no significantly effect on %EE and three formulas showed high loading over 90%. It was found that Tween 80 can be used as vesicle stabilizer in liposomes formation which contains *G. mangostana* fruit rind extract.

**บทคัดย่อ**

สารสกัดเอทานอลของเปลือกมังคุดที่ได้จากการหมักและถูกนำมาเตรียมให้อยู่ในรูปแบบลิโพโซมโดยมีทวีน 80 หรือคลอเรสเตอรอลเป็นสารช่วยเพิ่มความคงตัวของถุงทรงกลม โดยจะทำการคงอัตราส่วนของสารฟอสโฟลิปิดและโพรไพลีนไกลคอล แต่มีการปรับเปลี่ยนความเข้มข้นของสารสกัดเปลือกมังคุดในสูตร จากผลการทดลองพบว่าลิโพโซมที่เตรียมจากทวีน 80 มีขนาดอนุภาคอยู่ในช่วงใกล้เคียงกับที่เตรียมด้วยคลอเรสเตอรอล คืออยู่ในช่วง 79.5-90.0 นาโนเมตร ค่าดัชนีการกระจายตัวของอนุภาคลิโพโซมที่เตรียมจากทวีน 80 มีค่าเพิ่มขึ้นเมื่อมีการเพิ่มความเข้มข้นของสารสกัด ในขณะที่ลิโพโซมที่เตรียมจากคลอเรสเตอรอลมีค่าไม่เปลี่ยนแปลงมากนัก สำหรับศักย์ไฟฟ้าซีตา พบว่าลิโพโซมที่เตรียมจากทวีน 80 ให้ค่าศักย์ไฟฟ้าที่ดีกว่าคลอเรสเตอรอลคืออยู่ในช่วง -38.0 ถึง -39.9 มิลลิโวลต์ ส่วนค่าการเก็บกักสารไว้ในลิโพโซมพบว่า ค่าการเก็บกักสารจะไม่ขึ้นอยู่กับความเข้มข้นของสารสกัดเปลือกมังคุด โดยพบว่าสามสูตรที่เตรียมขึ้นมามีค่าการเก็บกักสารมากกว่า 90 เปอร์เซนต์ จากผลการวิจัยสรุปได้ว่า ทวีน 80 เป็นตัวเลือกที่สามารถนำมาใช้ในการเตรียมลิโพโซมเพื่อเก็บกักสารสกัดเปลือกมังคุด

**Key Words:** *Garcinia mangostana*, Liposomes, Tween 80

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**Introduction**

*Garcinia mangostana* L. is a medicinal plant that has been used in cosmetic for treatment of facial acne (Mahabusarakam et al., 1987). The alcoholic extract of its pericarp has demonstrated antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermis* with MIC 7.81 and 15.63 µg/mL, reapectively (Pothitirat et al., 2010). Currently, formulation development is essential to the delivery of active ingredients into the skin. Lipid-based colloidal system including lipid nanoparticles, microemulsions and liposomes are recognized for topical drug delivery (Souto et al., 2011). We focus on liposomes with a new mixture of forming the lipid bilayer vesicle.

**Objective of the study**

Our objective is to develop liposomes containing *G. mangostana* fruit rind extract with Tween 80 as vesicle stabilizer for topical delivery system.

**Methodology**

**Instrument and reagents**

Egg yolk phosphatidylcholine (EPC) and cholesterol were purchased from Sigma (St. Louis, USA). Tween 80 (Polysorbate 80) was purchased from Croda (East Yorkshire, UK). The ultrasonic processor with timer and pulser was performed on Cole-Parmer EW-04714-50, 115 VAC (Illinois, USA). The zeta potential and particle size measurement was performed with Malvern Zetasizer Nano ZS (Worcestershire, UK). Morphology of the obtained liposomes was observed under a scanning electron microscope (SEM, Hitachi S-3400N, Japan) at an accelerating voltage of 20 kV. All other reagents and solvents were reagent grade and used without further purification. TLC was performed on silica gel $GF_{254}$ (Merck). For column chromatography, silica gel (Merck 230-400 mesh) was used. The entrapment efficiency was performed on Agilent 1260 infinity HPLC system. NMR spectra were recorded with a Bruker Avance ($^1H$, 300 MHz) spectrometer. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. All NMR spectra were obtained in deuterated choloform ($CDCl_3$) and referenced to the residual solvent peak. Mass spectra were obtained from Agilent GC/MS 5975C.

**Plant materials**

The plant material of *G. mangostana* was bought from local drugstore in Nonthaburi province, Thailand. The material was identified by comparison with the specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimen of *G. mangostana* (SRU 049) was deposited at Faculty of Oriental Medicine, Rangsit University, Pathumthani, Thailand.

**Preparation of crude and partial-purified extracts**

The dried fruit rind powder of *G. mangostana* 100 g was macerated with 95% ethanol 400 mL at room temperature for 7 day. The extract was filtered with Whatman No.1 and then evaporated under reduced pressure with rotary evaporator to obtain 9 g of finally crude dark brown extract. The crude extract (2 g) was dissolved in $CH_2Cl_2$ : MeOH (8 : 2) ( 8 ml) . Then the mixture was subjected to silica gel column chromatography and eluted with $CH_2Cl_2$ : MeOH (8 : 2) to obtain partially purified dark brown extract (1.2 g).

**Isolation of α-mangostin**

The crude extract (1.2 g) was dissolved in $CH_2Cl_2$ : MeOH (7 : 3) (5 ml). Then the mixture was subjected to silica gel column chromatography and eluted with mobile phase $CH_2Cl_2$ : MeOH (7 : 3). After that, fractions 12-17 were collected and evaporated to obtain a yellow crystalline solid 212 mg with melting point 180-182 ºC. UV (Abdalrahim et al., 2012) : $\lambda_{max}$ 244, 343 nm, IR (Madihah et al., 2013): (KBr disc) 3256, 2925, 1639, 1460 $cm^{-1}$ $^1H$ NMR (Ly et al., 2009) : (300 MHz, $CDCl_3$) δ[ppm] 1.69 (s, 4H), 1.76 (s, 3H), 1.83(s, 6H), 3.45 (d, *J* = 7.15 Hz, 2H), 3.81(s, 3H), 4.09 (d, *J* = 6.26 Hz, 2H), 5.26 (m, 2H), 6.29 (s, 1H), 6.82 (s, 1H), 13.77 (s, 1H). $^{13}C$ NMR : (75.47 MHz, $CDCl_3$) δ[ppm] 182.0, 161.6, 160.6, 155.8, 155.0, 154.5, 142.5, 137.0, 135.7, 132.2, 123.1, 121.4, 112.2, 108.5, 103.6, 101.6, 93.3, 62.0, 26.5, 25.8, 25.8, 21.4, 18.2, 17.9 and MS (GC/MS): $M^+ = 410$.

**Preparation of *G. mangostana* fruit rind extract liposomes**

As the main vesicle components, test liposomes composed of EPC, cholesterol, and Tween 80 at a weight ratio shown in Table 1.

**Table 1** Composition of mangosteen fruit rind extract liposomes

| No. | Name of chemicals | Formula 1 (M1) | Formula 2 (M2) | Formula 3 (M3) | Formula 4 (M4) |
|---|---|---|---|---|---|
| 1 | EPC (mg) | 300 | 300 | 300 | 300 |
| 2 | Propylene glycol (mL) | 7.50 | 7.50 | 7.50 | 7.50 |
| 3 | Tween 80 (mg) | 25.0 | - | 25.0 | - |
| 4 | Cholesterol (mg) | - | 25.0 | - | 25.0 |
| 5 | Mangosteen extract (mg) | 40.0 | 40.0 | 60.0 | 60.0 |
| 6 | Water (mL) | 12.14 | 12.14 | 12.11 | 12.11 |

All liposomes were prepared by modified ethanol injection method (Amnuaikit, Boonme, 2014). Briefly, the lipid constituents were weighed and propylene glycol was added to dissolve all ingredients. The mixture was heated at 60°C until dissolved. Then it was added into the water phase with stirrer to obtain white cloudy yellow-brown solution. The size of the vesicles was then reduced by ultrasonic probe for 15 min. After that each formula was subjected to particle size, zeta potential, entrapment efficiency measurements and morphology observation.

**Entrapment efficiency**

Five hundred microliters of Sephadex G-50 swelled in deionized water was filled in the 1.0 mL-syringe barrel which contained a small cotton ball to support the gel. Then the syringe barrel was inserted into a test tube and spun at 3,000 rpm for 3 min to remove excess water from the gel. Then the syringe barrel containing Sephadex gel was transferred into a new test tube. Four hundred microliters of the liposomal preparation was applied to the Sephadex bed and spun at 1,500 rpm for 3 min. Two hundred microliter of water was added to the syringe barrel.

The system was spun again at 3,000 rpm for 3 min (Torchilin, Weissig, 2003). The separated material was added with 0.20 mL of Triton-X100 to break the liposomes and transfer into 10.0 mL volumetric flask. The volume of mixture was adjusted with mobile phase of HPLC to determine the content of α-mangostin. For the analysis of total content of α-mangostin, 0.40 mL of the liposomal preparation was mixed with 0.20 mL of Triton-X100 to break the liposome and transfer into 10.0 mL volumetric flask. The volume of mixture was adjusted with mobile phase of HPLC and subjected to analyze for α-mangostin. HPLC condition composed of column Ace C-18 (0.46 X 15 cm.), mobile phase (acetronitrile : 1% acetic acid, 80 : 20), flow rate 1.0 mL/min, UV detector at 254 nm, and injection volume 10 µL.

**Results**

The purification of *G. mangostana* crude extract with silica gel column chromatography to partially purified extract was uncomplicated process and disposed of undesirable material that could give awful color to the product. The partially purified extract of *G. mangostana* was standardized for α-mangostin by using isocratic HPLC condition. The purity of isolated α-mangostin was more than 95%.

For the preparation of *G. mangostana* liposomes, the modified ethanol injection method was chosen to prepare nanoparticles. In this experiment, egg yolk phosphatidylcholine (EPC) was used with either cholesterol or Tween 80 as vesicle stabilizer. All formula was conducted in the same method to obtain slightly cloudy yellow solutions (figure 1). The particle size, polydispersity index, zeta potential and entrapment efficiency were measured with zetasizer Nano ZS as shown in Table 2.

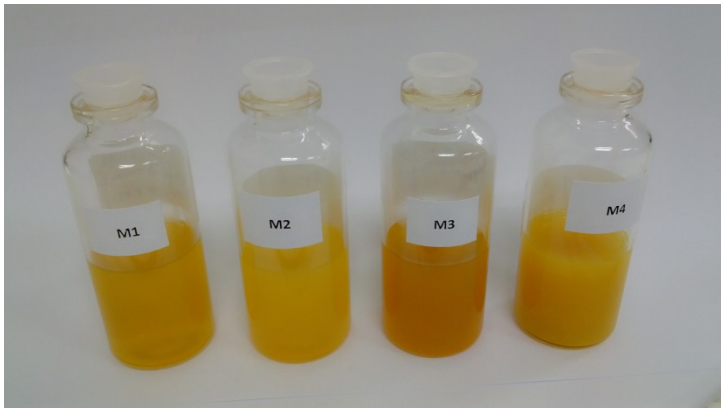


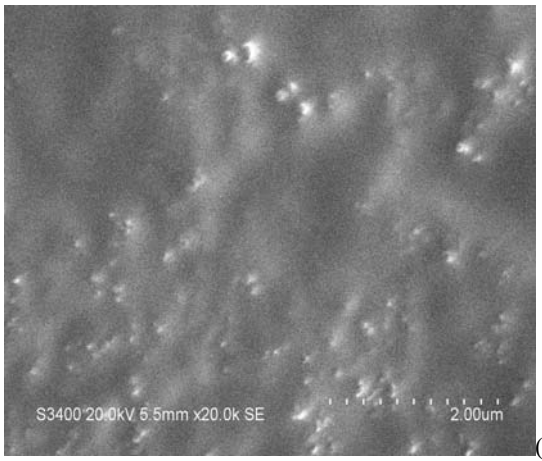

**Figure 1** Liposomes of *G. mangostana* crude extract.

**Table 2** PS, PI, ZP, and EE% of oxyresveratrol liposomes

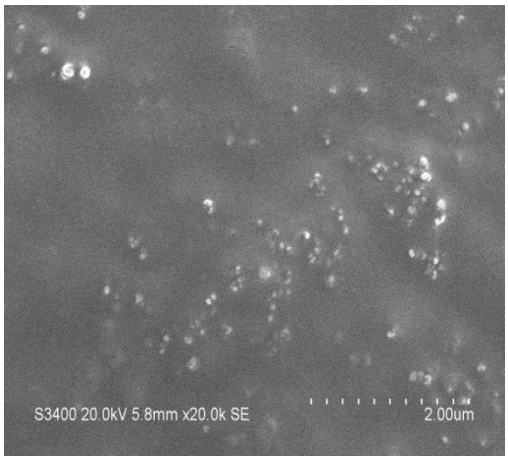
| Formulation | PS ± SD (nm) | PI ± SD | ZP ± SD (mV) | EE% ± SD |
|---|---|---|---|---|
| M1 | 90.00 ± 0.01 | 0.252 ± 0.010 | -38.07 ± 0.49 | 93.43 ± 0.33 |
| M2 | 85.76 ± 0.66 | 0.216 ± 0.010 | -30.73 ± 1.48 | 91.32 ± 1.26 |
| M3 | 81.70 ± 0.58 | 0.343 ± 0.010 | -39.97 ± 0.38 | 95.65 ± 2.78 |
| M4 | 79.50 ± 0.74 | 0.228 ± 0.006 | -29.60 ± 0.87 | 83.79 ± 1.47 |

Abbreviation: PS, particle size; PI, polydispersity index; ZP, zeta potential; EE%, entrapment efficiency

The scanning electron microscope (SEM) photographs in Figure 2 showed a round vesicle of the liposomes preparing with cholesterol (M1) and Tween 80 (M2) in Figure 2.




(M1)




(M2)

**Figure 2** Scanning electron microscope (SEM) pictures of liposomes M1 and M2 at 2 µm.

**Discussion and Conclusions**

From our study, content of EPC and propylene glycol were fixed but varied in the amount of *G. mangostana* extract. For the size of nanoparticles, the particle size of liposomes loaded *G. mangostana* extract with Tween 80 was less than 100 nm. It was close to those consisting of cholesterol. This means that *G. mangostana* extract at this range of concentrations had no effect on size of the vesicle. Next, the polydispersity index (PI) value is a value calculated for size distribution. The higher value means that the sample has broad size distribution. The PIs of liposomes consisting of Tween 80 were increased when increasing the concentration of *G. mangostana* extract. However, this phenomenon could not be observed in the liposomes containing cholesterol. This might be that *G. mangostana* extract could interact more with Tween 80 than cholesterol due to its solubilize property. Next, Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles, and is one of the fundamental parameters known to affect stability. For zeta potential, liposomes with Tween 80 had more negative values than those from cholesterol and the concentration of *G. mangostana* extract had no effect on these values. For entrapment efficiency,

the quantity of material entrapped inside liposomes, M1-M3 showed high loading over 90% while M4 enclosed with 83.79%. Tween 80 was chosen to stabilize the vesicle by using long hydrocarbon chain acting as lipophilic part while the polyethoxylated sorbitan part pointed into the aqueous core of liposomes and also arisen at the surface of the vesicle (Sinko, 2006). For the physical stability, M1, M2, M3 were stable more than 1 month at room temperature storage. From the results, Tween 80 was an alternative choice for making small vesicle liposomes. It can be obtained easily from cosmetic retailer and can be stored at room temperature.

Mangosteeen fruit rind extract has α-mangostin as a major ingredient that possesses anti-*P. acne* activity. It was encapsulated in liposomes consisting of either Tween 80 or cholesterol as vesicle stabilizers. Their particle sizes were in the range of 49-510 nm with highly negative value of zeta potential around 28-34 mV. Further investigation on formulation and skin penetration are in progress in our laboratory.

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