ระบบการเก็บกักสารสกัดมังคุดลงในอนุภาคระดับนาโนเมตร

Nanoencapsulation Process for *Garcinia mangostana* Extract

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บทคัดย่อ

มังคุดเป็นพืชที่นิยมปลูกในภูมิภาคเอเชียตะวันออกเฉียงใต้ สารสกัดจากเปลือกมังคุดมีสารออกฤทธิ์ที่สำคัญคือสารกลุ่มแซนโทน ซึ่งมีฤทธิ์ทางการแพทย์ที่หลากหลาย อาทิ ฤทธิ์ในการต้านการเจริญของเชื้อแบคทีเรีย เชื้อราและไวรัส ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านมะเร็งและฤทธิ์ต้านการอักเสบ อย่างไรก็ตามประสิทธิภาพการออกฤทธิ์และการประยุกต์ใช้สารสกัดมังคุดถูกจำกัดเนื่องจากการละลายในน้ำได้น้อยของสารสกัดมังคุด งานวิจัยนี้สนใจแก้ปัญหาดังกล่าวโดยการเก็บกักสารสกัดมังคุดลงในอนุภาคระดับนาโนเมตรที่สร้างจากโพลิเมอร์สองชนิดคือเอทิลเซลลูโลสและเมทิลเซลลูโลส โดยหาความหนืดของเซลลูโลสที่เหมาะสม และหาอัตราส่วนที่ดีที่สุดของโพลิเมอร์ทั้งสอง ในการใช้เป็นเปลือกหุ้มอนุภาค โดยใช้ค่าความจุสารสกัดของอนุภาค และประสิทธิภาพการกักเก็บ เป็นตัวชี้วัด

คำสำคัญ : สารสกัดมังคุด เซลลูโลส อนุภาคระดับนาโนเมตร การเก็บกัก

Abstract

Garcinia mangostana Linn. (mangosteen) is a tropical fruit, cultivated in Southeast Asia. Xanthones, the main biologically active constituents isolated from the pericarp of mangosteen, possess several medicinal and pharmaceutical activities including antioxidant, anticancer, antibacterial, antifungal, antiviral and anti-inflammatory activities. However, therapeutic efficiency and applications of *Garcinia mangostana* extract (GME) are limited by its poor aqueous solubility. Here nanoencapsulation of GME into water dispersible nanoparticles made from ethyl cellulose (EC) and methyl cellulose (MC) was used to solve the problem.The suitable viscosity of EC and the optimization of shell materials based on loading capacity and encapsulation efficiency were carried out to find the best ratio of polymers

Keywords : *Garcinia mangostana* extract, cellulose, nanoparticles, encapsulation,

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Introduction

Mangosteen or *Garcinia mangostana* Linn. is a tropical tree cultivated in Southeast Asian countries such as Indonesia, Malaysia and Thailand. The pericarp of mangosteen has been used as a traditional medicine to treatvarious diseases by Southeast Asians for a long time (Yates and Stout, 1958, Sen *et al*., 1980, Mahabusarakam *et al*., 1987). Various xanthones including α-, β- and γ-mangostins, garcinone E and gartanin, are major compounds found in the extract isolated from pericarp of mangosteen (Pedraza-Chaverri *et al*., 2008). *Garcinia mangostana* extract (GME) exhibits several medicinal activities including antioxidant, antibacterial, antifungal, antiviral anticancer, and anti-inflammatory activities (Chin and Kinghorn, 2008, Obolskiy *et al*., 2009, Bumrungpert *et al*., 2010, Shan *et al*., 2011). However, applications of GME are limited by poor aqueous solubility and low oral bioavailability of the material (Li *et al*., 2011).

Cellulose derivatives especially ethyl cellulose (EC) and methyl cellulose (MC) have been used in pharmaceutical industry for sustained release and taste masking purposes for a long time. EC is a hydrophobic, non-toxic, inexpensive and biocompatible polymer while MC is a safe hydrophilic polymer. Mucoadhesive property of the nanoparticles made from EC and MC has also been reported (Suwannateep *et al*., 2011). Here nanoencapsulation of GME into polymeric nanoparticles fabricated from these two polymers was used to solve the solubility problem of the GME.

The aim of this study is to find the optimum condition for the GME encapsulation process using a blend of EC and MC. Optimization was carried out based on loading capacity and encapsulation efficiency and morphology of the obtained nanoparticles with variables including viscosity of EC and weight ratio of EC to MC.

Materials and Methods

Materials

GME with 56% α-mangostin was from the Tipco Group Public Company Limited (Bangkok, Thailand). Ethyl cellulose (EC, viscosity 4 cP, 10 cP, 46 cP, 100 cP and 250-300 cP; ethoxy content 48%), methyl cellulose (MC, viscosity 400 cP; 1.60–1.90% degree of methoxy substitution) and dialysis cellulose membrane (M.W. 12,400 Da) were purchased from Sigma-Aldrich (Steinheim, Germany).

Preparation of GME-encapsulated cellulose derivative nanoparticles

GME was encapsulated into cellulose derivative nanoparticles using solvent displacement method. Briefly, EC and GME were dissolved in ethanol while a blend of EC and MC was dissolved in 80% (v/v) ethanol. The mixture was placed into a dialysis bag and dialyzed against distilled water to obtain dispersion of GME-encapsulated nanoparticles. The effect of EC viscosity was study using EC of five different viscosity values (4 cP, 10 cP, 46 cP, 100 cP and 250-300 cP). The best EC was chosen to blend with MC at weight ratios of 2:1, 1:1, and 1:2.

Characterizations

Morphology characterizationof the obtained products was carried out by scanning electron microscopy (SEM) (JSM-6400, JEOL, Ltd., Japan) and transmission electron microscopy (TEM) (JEM-2100, JEOL, Ltd., Japan). Size and zeta potential of particles were measured by dynamic light scattering technique (DLS) using a Mastersizer S and Zetasizernanoseries (Mulvern Instruments, Worcestershire, UK).

Loading capacity and encapsulation efficiency

Each aqueous suspension of GME-loaded nanoparticles, GME-EC and GME-ECMC (5 ml), was centrifugally filtered through Amicon Ultra-15 membrane (MWCO 100,000). The obtained solid on the filter was soaked in 5 ml ethanol for 3 h to extract GME from the nanoparticles. UV/Vis spectrophotometry was used to measure amount of GME in the ethanolic extract at 317 nm with the aid of a calibration curve. The encapsulation efficiency (%EE) and loading capacity (%Loading) were calculated using equation (1) and (2) as follows:

$$\%EE = \frac{\text{Weight of GME found in the filtered particles}}{\text{Weight of GME initially used}} \times 100 \quad (1)$$

$$\%Loading = \frac{\text{Weight of GME found in the filtered particles}}{\text{Weight of the filtered particles}} \times 100 \quad (2)$$

Results and Discussion

GME-loaded nanopart[illegible] solvent displacement method (displacing ethanol with water) in which the GME was encapsulated into EC/MC nanoparticles via self-assembling process (Suwannateep *et al*., 2011). During the dialysis process, ethanol was displaced with water slowly, and therefore, the water insoluble EC chains slowly self-assembled themselves in such a way that the hydrophilic hydroxyl moieties were in contact with water while the methylene moieties oriented themselves away from the water environment, forming water dispersible nanoparticles with hydrophobic core. At the same time, the hydrophobic GME extract tried to be away from water molecules and therefore moved to the inside of the particles. If MC chains were present, entanglement of the EC and MC also occurred during particle formation, leading to the shell material of EC and MC mixture. More MC chains were present at the outer surface of the particles since the polymer is water soluble. Some MC chains may also be left out in the water medium.

The effect of EC viscosity

Viscosity of EC is related to the length of polymer chain. Among the five tested EC of different viscosities, 4cP EC, the EC withlowest viscosity, gave thelowest %EE and lowest %Loading (Figure 1). EC with viscosity of 10, 46, 100 and 300 cP gave comparable loading and encapsulation efficiency (Figure 1). Thus, EC with viscosity 250-300 cP was used in the next experiments. We speculated that too short EC chain could not effectively trap GME to the inside of the particles since the steric and entanglement among polymer chains were not enough.

Optimal Ratio of EC to MC was 1:1

To increase stability, mucoadhesion and water dispersibility of the nanoparticles, methylcellulose (MC), a hydrophilic, water-soluble polymer, was blended with EC. Here the amount of MC in the polymer blend was optimized based on %loading and %EE. The results revealed that for maximium loading and EE, the MC content should not exceed 50% (w/w) (Figure 2). Encapsulation using EC/MC polymer blend with the MC content of

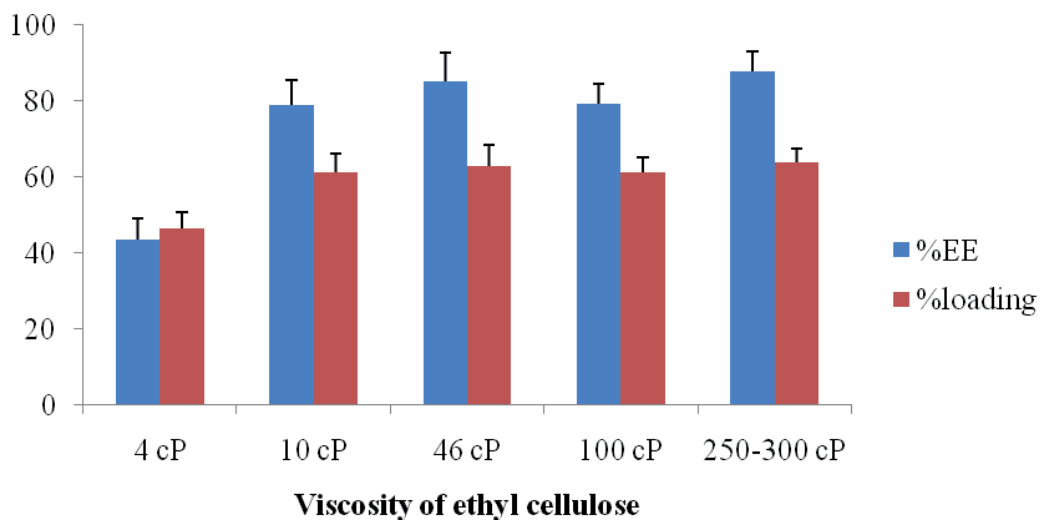

Figure 1 %EE and %loading obtained from GME nanoencapsulation using EC of different viscosities. The process was carried out at EC:GME weight ratio of 1:2.

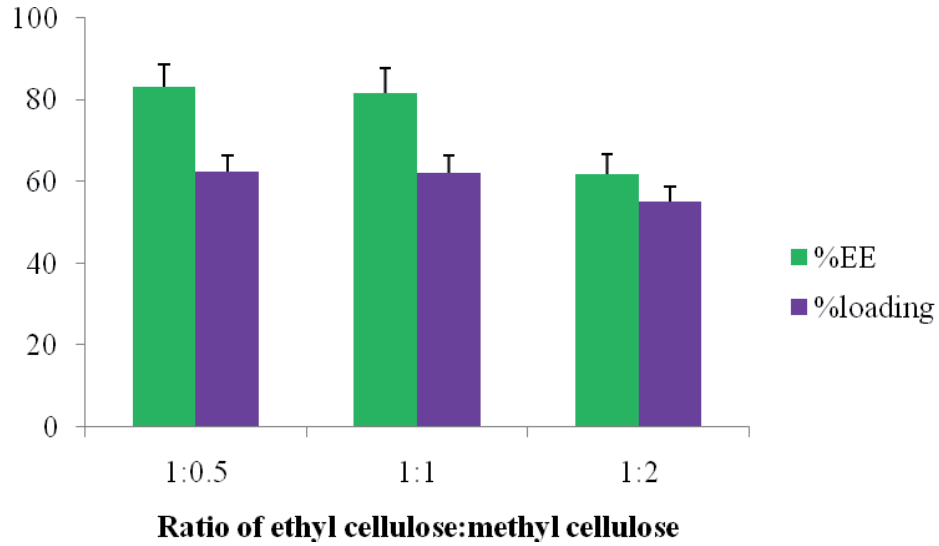

Figure 2 %EE and %loading of GME obtained from the nanoencapsulation process using the blend of EC (250-300 cP) and MC at various ratios of EC to MC. The experiment was carried out at polymer:GME weight ratio of 1:2.

25% and 50% (w/w) gave comparable loading capacity and EE. However, when the MC content was 66% (w/w), the loading capacity and EE dropped significantly and precipitation of unencapsulated GME was also observed. We speculated that with too high percentage of MC, there were not enough hydrophobic EC polymeric chains to hold hydrophobic GMEresulting in dropping of EE and loading capacity. It should be kept in mind that unlike EC, MC cannot indendentlyform into particles, so it is more likely to be used as an additive for the wall materials, not the main structural material. From our results, the optimum ratio of EC:MC for GME encapsulation was 1:1.

Optimal ratio of blended polymer to GME was 1:1

Performing the encapsulation process at higher GME to polymer weight ratio resulted in significant drop inencapsulation efficiency and %loading as shown in Figure 3. Product obtained from the process performed at the GME to polymer weight ratio of 2:1 gave the loading

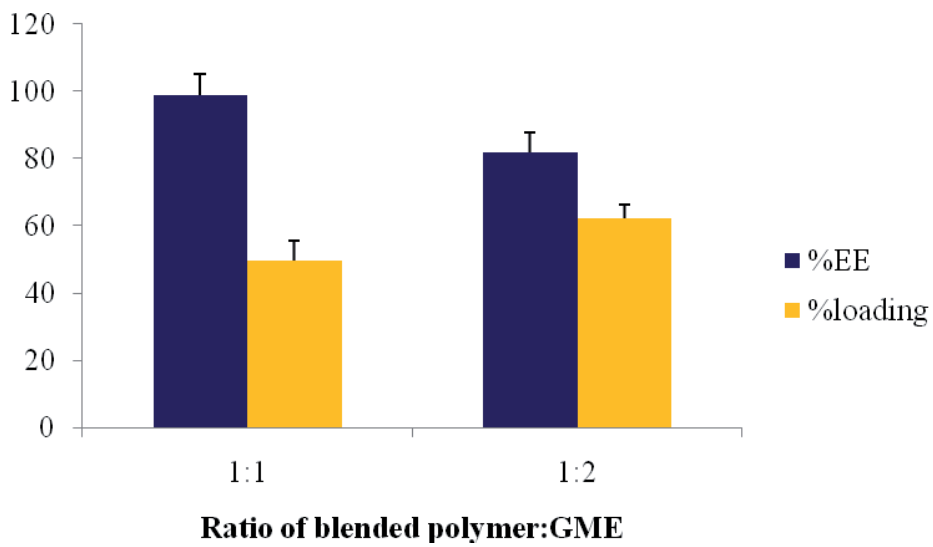

Figure 3 %EE and %Loading obtained from GME encapsulation using two different weight ratios of polymer:GME.

Table 1 Characterization of GME-loaded particles made from the1:1 blend of EC and MC, and prepared at thepolymer to GME weight ratio of 1:1.

Factors	Values
Hydrodynamic diameter (nm)	625.4 ± 19.6
Polydispersity index (PDI)	0.305 ± 0.041
Zeta potential (mV)	-3.6 ± 0.2
%EE	98.94 ± 6.26
%Loading	49.73± 5.89

of 62.06 ± 4.32 while %EE of the process was only 81.84 ± 6.35, comparing to the process carried out at GME to polymer weight ratio of 1:1 which gave %loading and %EE of 49.73 ± 5.89 and 98.94 ± 6.26, respectively. Thus, the process at the GME to polymer weight ratio of 1:1 was chosen as the best system.

Particle characterization

GME-loaded nanoparticles possessed a spherical shape (Figure 4A and 4B) with hydrodynamic size around 625.4 ± 19.6 nm and zeta potential of -3.6 ± 0.2 mV (Table 1). It was obvious that the encapsulated GME dispersed well in water while the unencapsulated GME showed poor water dispersibility (Figure 4C). With the loading of almost 50% (w/w), the GME-loaded particles showed excellent water dispersibility because all the hydrophobic GME molecules were at the inside of the nanoparticles. The outer surface of the particles probably contained hydrophilic hydroxyl moieties which interacted well with water.

Conclusion

GME was [redacted] nanoparticles of the polymer blend of EC and MC, with high encapsulation efficiency (98.94 ± 6.26) and loading capacity (49.73 ± 5.89) via solvent displacement method. The optimum condition for GME encapsulation was at 1:1 (w/w) polymer to GME, and the best wall material was a blend of EC (250-300 cP) and MC at 1:1 weight ratio. The water dispersible GME-loaded nanoparticles were spherical with hydrodynamic diameter around 625.4 ± 19.6nm.

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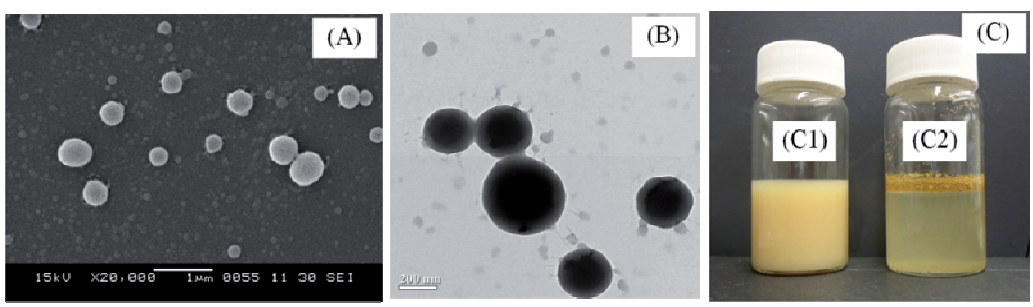

Figure 4 A) SEM and B) TEM photographs of GME-loaded nanoparticles, and C) photographs of GME-loaded nanoparticles in water (C1) and aqueous suspension of GME in water (C2).

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