ปัจจัยที่มีผลต่อการทำไมโครเอนแคปซูลของสารสกัดขมิ้น

Factors Affecting on Turmeric Extract Microencapsulation

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บทคัดย่อ

ขมิ้นชันเป็นสมุนไพรที่นิยมใช้เป็นเครื่องปรุงแต่งรสและสีของอาหาร ส่วนประกอบหลักถึง 70 เปอร์เซ็นต์ของผงขมิ้นชัน คือคอร์คูมิน ฤทธิ์ทางเภสัชวิทยาของเคอร์คูมินคือฤทธิ์ต้านอาการอักเสบและฤทธิ์ต้านอนุมูลอิสระ อย่างไรก็ตามคอร์คิวมินดูดซึมได้ยาก ทำให้ค่าชีวประสิทธิผลต่ำเมื่อให้ในรูปแบบรับประทาน ในงานวิจัยนี้มุ่งเน้นในการพัฒนาวิธีเก็บกักสารสกัดขมิ้นชันในเอทิล เซลลูโลส พอลิเมอร์ โดยใช้วิธีระเหยตัวทำละลาย และศึกษาปัจจัยที่มีผลต่อการทำไมโครเอนแคปซูล ไมโครสเฟียร์ที่ได้จากการใช้ เอทิล เซลลูโลส ความหนืด 100 เซนติพอยส์ มีขนาดเส้นผ่าศูนย์กลาง 28.67±7.60 ไมครอน ในขณะที่ไม่เกิดไมโครสเฟียร์ เมื่อใช้ เอทิล เซลลูโลส ความหนืด 45 เซนติพอยส์ เปอร์เซ็นต์การกักเก็บสารสกัดขมิ้นชันในไมโครสเฟียร์ ที่ทำจากเอทิล เซลลูโลสความหนืด 100 เซนติพอยส์ ปริมาณ 100 มิลลิกรัม วิเคราะห์โดยตรวจจากเคอร์คูมิน มีค่า 70.45±7.16% แต่เมื่อเพิ่มอัตราส่วนระหว่างสารสกัดขมิ้นกับไดคลอโรมีเทนเป็น 1 ต่อ 400 จะเพิ่มเปอร์เซ็นต์การกักเก็บเป็น 86.80±5.08% การผสมโพลิไวนิล แอลกอฮอร์ ในปริมาณ 0.25% ในวัฏภาคนอก สามารถป้องกันการรวมตัวกันของหยดสารสกัดขมิ้น/เอทิล เซลลูโลส ปริมาตรของวัฏภาคนอกไม่ว่าจะเป็น 400 หรือ 1000 มิลลิลิตร ให้ปริมาณไมโครสเฟียร์ไม่ต่างกัน ส่วนการคนผสมสารละลาย ยิ่งคนนาน (17 ชั่วโมง) ยิ่งเพิ่มปริมาณของไมโครสเฟียร์ที่ได้ ปัจจัยต่าง ๆเหล่านี้สามารถเป็นประโยชน์ต่อการพัฒนาไมโครสเฟียร์ในช่วงขนาดนาโนเมตร ไมโครสเฟียร์ขนาดนาโนเมตรนี้อาจเพิ่มการซึมผ่านของสารสำคัญเคอร์คูมิน ทำให้ฤทธิ์ทางเภสัชวิทยาของขมิ้นชันเพิ่มขึ้นเมื่อให้ทางผิวหนังหรือให้ทางปาก

Abstract

Turmeric is commonly used as a spice and food coloring agent. Major component (70%) of turmeric powder is curcumin. Curcumin possesses anti-inflammatory and antioxidant properties. However, curcumin is not readily absorbed resulting in poor bioavailability when administered orally. In this study, turmeric extract were encapsulated into ethyl cellulose polymer by solvent evaporation technique. Factors affecting on turmeric extract microencapsulation were investigated. Microsphere obtained from 100 centipoises (cps) ethyl cellulose shown diameter of 28.67±7.60 μm, while no solid microsphere obtained from 40 cps ethyl cellulose. Curcumin were used as a marker for turmeric extract entrapment study. The entrapment efficiency of 70.45±7.16% was found in microsphere using 100 mg of 100 cps ethyl cellulose. Increase ratio between turmeric extract and dichloromethane to 1:400 can improve entrapment efficiency to 86.80±5.08%. To prevent coalescence of turmeric extract/ethyl cellulose droplet, continuous phase containing 0.25% polyvinyl alcohol (PVA) is suggested. Either 400 or 1000 mL of continuous phase provide equal amount of microsphere. The longer stirring time (17 hrs), the higher yield of microsphere obtained. These factors could be useful for further development of microsphere in the size of nanometer range. This might enhance the absorption of an active ingredient, curcumin, thus might increase pharmacological effect of turmeric powder when apply either on skin or in oral route.

คำสำคัญ: ขมิ้นชัน, เคอร์คูมิน, เอทิล เซลลูโลส, ไมโครเอนแคปซูล

Keywords: turmeric, curcumin, ethyl cellulose, microencapsule

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INTRODUCTION

Turmeric (*Curcuma Longa, Linn*, Zingiberaceae) has been used for centuries in Asian traditional system of medicine, as a spice and a natural food color. Curcumin, a yellow odorless pigment that is obtained from rhizomes of turmeric are known as the biologically active component of turmeric powder. Curcumin has been reported to possess anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-bacterial, and anti-tumor features (Surh et al. 2001; Sun et al. 2002). Colonic mucosal cyclooxygenase and lipoxygenase activities were suppressed by dietary curcumin, which appears to account for its chemopreventive effects on colon carcinogenesis. It was found to suppress the generation of reactive oxygen species (ROS) including superoxide and hydrogen peroxide in peritoneal macrophages (Ruby et al. 1995). Curcumin was also shown to prevent accumulation of advanced glycosylation which complicated diabetes mellitus (Sajithlal et al. 1998). Furthermore, chemopreventive action of dietary curcumin on skin carcinogen in mice was reported (Limtrakul et al. 2001), which also leads to an indication for consumption of curcumin to protect skin from radiation burn. However, curcumin is not readily absorbed and not easily soluble, resulted in poor bioavailability when administered orally (Ravindranath and Chandrasekhara 1980). The reports showed that about 60% of the dose was absorbed but not detectable in urine and that about 30-80% of curcumin disappeared from in vitro rat intestinal tissue upon application (Ravindranath and Chandrasekhara 1981).

Microencapsulation is a technology devoted to entrapping solids, liquids, or gases inside one or more polymeric coating (Mathiowitz 1999). The releasing of active ingredient was controlled by coating polymer. Encapsulation is a fairly standard practice in the food, consumer product, and cosmetics industries. Particle size of 10-500 micron facilitates the absorption of active ingredient leading to elevate bioavailability. Choosing the appropriate polymer system is essential for microencapsulation. Ethyl cellulose is a water insoluble and hydrophobic polymer. Ethyl cellulose often used as enteric coat polymer for drug release control in colon since it resisted for gastric erosion (Hu et al. 1998; Biju et al. 2004) The amylase-ethyl cellulose coated tablet retarded ranitidine release until the pellet had reached the colon. Thus the absolute bioavailability increases up to 5.5% (Basit et al. 2004). The aim of this study is to investigate factors that affect tumeric powder extract microencapsulation by using ethyl cellulose as a coating polymer. This system could be applied for oral consumption and might be useful for health concerns.

METERIALS AND METHODS

Materials

Turmeric powder extract or turmeric extract (70% curcumin) was purchased from Thai-China Herb Ltd. (Bangkok, Thailand). Curcumin standard and poly(vinyl alcohol) (PVA) MW of 200,000 were purchased from Sigma (St. Louis, USA). Ethyl cellulose (EC) 45 and 100 centipoises (cps.) were purchased from Colorcon (Rama Production, Bangkok, Thailand). Dichloromethane was purchased from Merck (Darmstadt, Germany).

Microparticle preparation

Turmeric extract is encapsulated into EC coating polymer by solvent evaporation technique adapted from Mathiowitz (Mathiowitz 1999). Briefly, solution of turmeric powder extract (25 mg) and polymer (100, 250, or 500 mg EC) in 15 mL dichloromethane (DCM), that is called drug/matrix dispersion phase was gradually drop into aqueous continuous phase containing 400 or 1000 mL of 0.25, 2.5, 5, or 10% PVA, which acts as self-emulsifier and stabilizer. The mixture was homo genized (Ystral, model 3002 X10/10, Dottingen, Germany) at speed of 100 rpm for 30 minutes. Then, solvent, dichloromethane, was extracted to PVA and evaporated by continuous stirred over time (300 rpm, room temperature, 5 or 17 hrs). The particle was collected by centrifuge at 10,800 rpm and was washed several times with water. The pellet was dry in freeze-dryer. Figure 1 illustrated process steps in turmeric powder extract microsphere encapsulation.

Factors critical for microencapsulation

From methods to prepare microsphere described above, each factor has been varied while others were kept constant. These factors are including viscosity and amount of ethyl cellulose, concentration and amount of PVA, stirring time, and ratio of curcumin to dichloromethane. The processes that fail to make solid microsphere did not go for the further evaluation. The effectiveness of the system was measured by particle size analysis and entrapment efficiency. Particle size was measured by light microscope (Nikon®, Japan) with calibrated scale. Entrapment efficiency was measured as described below. All experiments were performed in triplicate (n=3).

Entrapment efficiency

Five mg of the microsphere was dissolve in 5 mL methanol. The suspension was sonicated for 5 minutes and then centrifuged for 10 minutes at 10,800 rpm. The supernatant was subjected to analyze for curcumin by spectrometry at wavelength of 420 nm. The amount of curcumin from turmeric powder extract has been calculated from standard curve of standard curcumin versus absorption value at 420 nm. Figure 2 showed spectrum and maximum absorption wavelength of standard curcumin. To make sure that there was no peak from methanol and other ingredients at 420 nm, each material in microsphere are dissolved in methanol and scan for their maximum absorption wavelength. There was no peak interference from these materials at 420 nm (data not shown).

Statistical analysis

SPSS program (Kruskal Willis test) was used for data analysis. Data were expressed in term of mean±standard deviation (SD.) and were significantly difference when *p-value* less than 0.05.

RESULTS AND DISCUSSION

Factors affecting turmeric powder extract microencapsulation

1. Viscosity of ethyl cellulose

Viscosity of ethyl cellulose (EC) related to the strength of microsphere wall. As shown in Table 1, there was solid microsphere formed when using 100 cps ethyl cellulose while no such particle obtained by using 45 cps EC. In the later case, there were coalescences of drug/matrix (turmeric extract/polymer) droplet, followed by separation of droplet dispersion phase. Molecular weight of 100 cps EC

is higher than those of 45 cps EC, thus higher viscosity of drug/matrix dispersion phase occurred. Such increase in drug/matrix dispersion viscosity, typically caused by higher molecular weight of the matrix material (polymer), may be desirable to strengthen the microsphere wall and prevent it from coalescence.

2. Amount of ethyl cellulose

Amount of ethyl cellulose did not significantly affect on weight and size of microsphere, or entrapment efficiency (Table 2). Size of microsphere obtaining from 500 mg EC is fairy large (55.12 ±9.54μm). This finding came along with another study that increasing viscosity of the drug/matrix dispersion just simply increase amount of polymer yields larger microsphere (Freitas et al. 2005). Generally, high viscosity dispersion phase also improves entrapment efficiency, since it restrict the migration of the drug in the continuous phase, and thus prevent drug loss. The theory may be trustful for hydrophilic drug such as protein but may not suitable for hydrophobic drug like turmeric extract since the data shown that the highest entrapment efficiency was found in microsphere obtaining from 100 mg EC (70.45฿7.16 %). This may be caused by higher weight of microsphere obtaining from 100 mg EC (28.30฿8.06 mg). However, to confirm this notice, more study should be performed.

3. Concentration of PVA

The result on concentration of PVA related to particle forming was shown in Table 3. When concentration of PVA exceeds 0.25% w/w, turmeric extract/EC droplet coalesced and then dispersion phase separated out of the aqueous phase. PVA is commonly used as stabilizer and self-emulsifier in continuous phase. PVA was adsorbed on the surface of newly formed droplets, thus preventing coalescence (Freitas et al. 2005), the higher concentration of stabilizer, the larger excess of material that adsorbed on the surface of the droplet (Yang et al. 2001). However, it did not come along with our finding. The rationale could be explained from solvent evaporation steps in microsphere preparation illustrated in Fig 1. There were two mechanisms occurred at the same time, (1) the adsorption of PVA on the surface of the droplet; and (2) the partition of solvent to the surface of the microsphere wall and then evaporate. In our case, the excess adsorption of PVA on the surface of the droplet might interrupt the partition rate of solvent into continuous phase. Moreover, excess PVA, at concentration of 2.5% or over, increased viscosity of continuous phase and might slow down the solvent evaporation rate. Briefly, the first mechanism dominated the second one. In these cases, coalescence might possibly happen. Increasing the stirring speed might resolve this problem, as it increases shear forces and turbulence to the system, and thus repel excess adsorbed PVA. Hence, more concentration of PVA could be used.

4. Amount of PVA

Either 400 mL or 1000 mL of 0.25% PVA lead to solid microsphere formed as shown in Table 4. There was droplet coalesced observed when using 36 mL 0.25% PVA. This indicated amount of PVA or continuous phase affecting solvent evaporation step. In this process, the amount and composition of the continuous phase are chosen so that the entire volume of the disperse solvent can be dissolved. In our study, 36 mL of continuous phase was inadequate to extract all of solvent since it could not maintain a high concentration gradient for the solvent between the microsphere and the continuous

phase leading to droplets coalesce. As noticed from Table 4, size of microsphere was identical for those obtained from 400 or 1000 mL PVA. This suggested continuous phase volumes did not significantly influence microsphere size.

5. Stirring time

Evaporating dichloromethane leaves ethyl cellulose to slowly form hard-shell cover turmeric extract droplet and then become solid turmeric extract microsphere. Under the same stirring speed (300 rpm) at room temperature, 5 hours-stir results in lower yield than 17 hours-stir (Table 5). The longer stirring time, the higher yield of microsphere was formed. Increase stirring rate might shorter stirring time since it facilitates solvent evaporation.

6. Ratio between dichloromethane and curcumin

Dichloromethane often use as solvent in evaporation technique since it is immiscible to continuous aqueous phase, easily evaporated (boiling temperature <40 ^{o}C), and dissolve most of the hydropholic active ingredient. Ratios of DCM and turmeric extract affect on the entrapment efficiency but not on size of the particles (Table 6). There was no significantly difference between entrapment efficiency of microsphere obtained from 1:400 and 1:600 turmeric extract: DCM. The ratio was limited by solubility of turmeric extract in dichloromethane. The highest ratio of turmeric extract to DCM was 1:400 w/v. For hydrophilic drug, the higher target load of bioactive material is likely to decrease the encapsulation efficiencies of proteins and peptides in (poly(lactic-co-glycolic acid) and increase the 24-h (burst) drug release (Freitas et al. 2005). In our study, turmeric extract is hydrophobic material, so that the chance of drug loss from internal phase to external phase is low since curcumin has more solubility in DCM than in water. Therefore, the highest turmeric extract loaded depends on the solubility of curcumin in the solvent.

CONCLUSION

The important issue to be concern in microencapsulation is the solubility of the active ingredients in solvent, which related to entrapment efficiency as well as solubility of solvent in aqueous phase, which related to rate of evaporation. Turmeric extract is water insoluble material. It is not difficult to get the high entrapment efficiency even though turmeric extract migrate to the surface of droplet since it prefers dichloromethane over water. The task is the beginning step, which is the forming of solid microsphere. From our study, factors affecting this step are molecular weight and amount of EC, the concentration of PVA, and the amount of continuous phase. The next step is reducing size of microsphere. Surprisingly, the mention factors did not significantly affect the size. Many studies reported on factors affecting microencapsulation of hydrophilic active ingredient, but not so many reported on factors affecting hydrophobic active ingredient. Further experiments should focus on stirring time and stirring speed as well as geometry and number of impeller in order to reduce particle size of microsphere to nanometer range. This would be greatly benefited to the absorption of microsphere into either skin or GI tract. Therefore the pharmacological effect of the major ingredient of turmeric extract, curcumin, could be enhanced.

ACKNOWLEDGEMENT

The authors would like to thank National Research Council of Thailand for research grant and Center of Research and Development in Herbal Health Products, Khon Kaen University for laboratory equipment.

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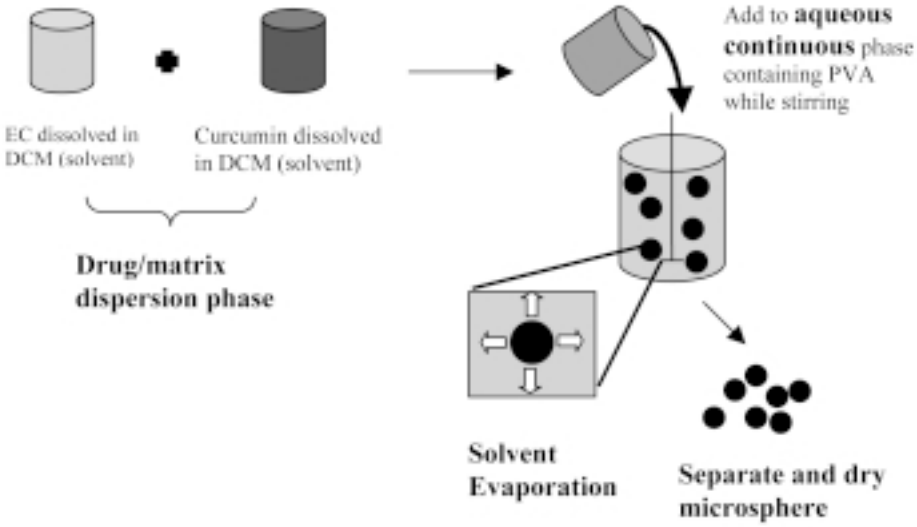

Figure 1 Schematic overview over the principal process steps in microsphere preparation by solvent evaporation. EC = ethyl cellulose, DCM = dichloromethane

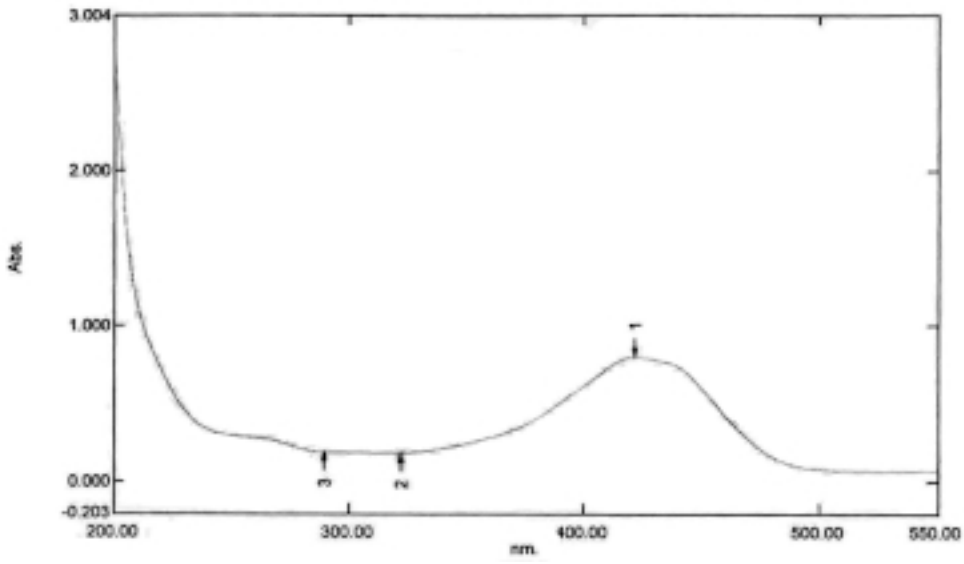

Figure 2 Scanning chromatogram of standard curcumin in methanol by spectrophotometer showing the maximum absorption wavelength of 420 nm (peak number 1). Peak number 2, 3 are unidentified.

Table 1 Effect of viscosity of ethyl cellulose on size of turmeric extract microsphere.

Viscosity of ethyl cellulose (cps)	Solid microsphere forming	Size (μm)
45	No	n/a
100	Yes	28.61±7.60

n/a = no available

Table 2 Effect of amount of ethyl cellulose on entrapment efficiency of turmeric extract microsphere.

Amount of ethyl cellulose (mg)	Weight of microsphere (mg)	Size (μm)	Entrapment efficiency (%)
100	28.30±8.06	27.81±1.15	70.45±7.16
250	20.15±6.43	26.81±1.59	49.15±2.28
500	20.70±0.85	55.12±9.54	49.67±15.56

Table 3 Effect of concentration of poly(vinyl alcohol) (PVA) on size of turmeric extract microsphere.

Concentration of PVA (%w/w)	Solid microsphere forming	Size (μm)
0.25	Yes	28.61±7.60
2.5	No	n/a
5	No	n/a
10	No	n/a

n/a = no available

Table 4 Effect of the amount of poly (vinyl alcohol) on entrapment efficiency of turmeric extract microsphere.

Amount of 0.25% PVA (ml)	Solid microsphere forming	Size (μm)	Entrapment efficiency (%)
36	No	n/a	n/a
400	Yes	28.61±7.60	70.45±7.16
1000	Yes	27.35±6.10	69.05±8.67

n/a = no available

Table 5 Effect of stirring time on yield, shape and color of turmeric extract microsphere.

Stirring time (hour)	Yield*	Shape and Color of microsphere
5	<10	Round, yellow
17	>100	Round, yellow

* Number of particle under light microscope in 1 frame

Table 6 Effect of turmeric extract: dichloromethane ratio on entrapment efficiency.

Turmeric extract: dichloromethane (w/v)	Size (μm)	Entrapment efficiency (%)
1:400	25.00 ± 0.50	86.80 ± 5.08
1:600	28.61 ± 7.60	70.45 ± 7.16
1:700	23.29 ± 5.57	38.6 ± 9.89*
1:1000	25.07 ± 13.17	43.47 ± 7.80*

* Significantly difference