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ชื่อโครงการ การสังเคราะห์และพิสูจน์เอกลักษณ์อนุภาคนาโนแคลเซียมซิเตรทสำหรับการนำส่งยาเมทฟอร์มิน

Synthesis and characterisation of calcium citrate nanoparticles for metformin delivery

ชื่อนิสิต นายธัชชาญ ชีพสุมนต์

ภาควิชา เคมี

ปีการศึกษา 2560

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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Synthesis and characterisation of calcium citrate nanoparticles for metformin delivery

by

Mr. Thatchan Cheepsumon

A Report Summitted in Partial Fulfillment of the Requirements
for the degree of Bachelor of Science Program in Chemistry
Department of Chemistry
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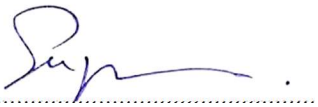
Project Title	Synthesis and characterisation of calcium citrate nanoparticles for metformin delivery
Student Name	Mr. Thatchan Cheepsumon
Advisor Name:	Assistant Professor Rojrit Rojanathanes, Ph.D.

Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in partial fulfilment of the requirements for the Degree of Bachelor of Science.

Project Committees



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(Professor Supason Wanichwecharungruang, Ph.D)

..... Project Advisor

(Assistant Professor Rojrit Rojanathanes, Ph.D)

..... Committee

(Sakulsuk Unarunotai, Ph.D)

This report was approved by Head of Department of Chemistry

..... Head of Department of Chemistry

(Associate Professor Vudhichai Parasuk, Ph.D)

...../...../.....

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ชื่อนิสิตในโครงการ นายธัชชาญ ชีพสุมนต์ เลขประจำตัว 5733103523
ชื่ออาจารย์ที่ปรึกษา ผู้ช่วยศาสตราจารย์ ดร.โรจน์ฤทธิ์ โรจนธเนศ
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บทคัดย่อ

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Project Title: Synthesis and Characterisation of Calcium Citrate Nanoparticles for Metformin Delivery

Student Name: Mr. Thatchan Cheepsumon Student ID 5733103523

Advisor Name: Assistant Professor Rojrit Rojanathanes, Ph.D.

Department of Chemistry, Faculty of Science, Chulalongkorn University, Academic Year 2017

Abstract

In this research, a novel metformin encapsulated calcium citrate nanoparticles were synthesized *via* chemical precipitation method. The products were characterized by thermal gravimetric analysis (TGA) and scanning electron microscope (SEM). The optimum condition was 4:1 mole ratio of calcium citrate to metformin and using absolute ethanol to facilitate precipitation. The maximum loading capacity of metformin is 2.96% . This procedure could be used to formulate metformin encapsulated calcium citrate as novel drug delivery system.

Keywords: calcium citrate, nanoparticles, metformin, drug delivery system

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I would like to express my gratitude to the following people for helping me throughout this project. Without their help, I may not have succeeded this project.

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Thatchan Cheepsumon

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General abbreviation

Met	Metformin
EtOH	Ethanol

International system of units

mg	Milligram
mL	Millilitre
L	Litre
M	Molar
min	Minute
rpm	Round per minute

Chapter I

Introduction

1.1 Background

Diabetes Mellitus is a group of metabolic disorders that high blood sugar levels have prolonged for a period of time. There are three main types of diabetes mellitus, namely type 1 diabetes, type 2 diabetes and gestational diabetes. All types of diabetes mellitus can eventually cause heart disease, stroke, kidney disease, blindness, and nerve damage[1].

Metformin is a biguanide derivative as shown in figure 1-1. It is widely used for clinical practice as a type 2 diabetic drug by oral administration. Metformin acts mostly *via* inhibition of hepatic gluconeogenesis. Its anti-hyperglycemic influence is also mediated by an increase of hepatic insulin sensitivity and absorption of glucose in muscles[2]. Moreover, metformin treatment has been associated with reducing risk of cancer and prolonged aging[2]. On the other hand, there are also several negative side effects of metformin such as nausea, vomiting, upset stomach, diarrhea and weakness. Moreover, oral administration reduces drug absorption process and efficiency of medicine[3].

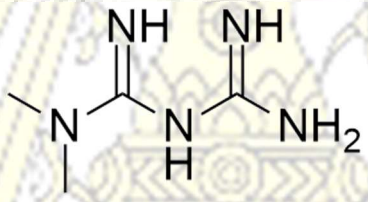

Figure 1-1 Chemical structure of metformin

A drug delivery system with nanoparticles[4] can increase efficiency, reduce side effects and retain drugs for longer times after administration. There are several types of drug delivery system as shown in the table 1-1.

Table 1-1 Advantages and disadvantages of different nanoparticle types[5-8]

Type	Nanocarriers	Pros	Cons
Polymeric NPs	Chitosan	• Biodegradable • Biocompatible • Offer complete drug protection • Controlled and sustained drugs	• Difficult synthesis • Costly formulation
Metallic NPs	Gold Silver	• Simple for diagnosis • Long stability • Avoiding organic solvent • Controlled and sustained drugs	• Less drug loading capacity • Toxic for cell (particle size < 10 nm)
Ionic NPs	Calcium phosphate Calcium carbonate	• Biodegradable • Biocompatible • Easy synthesis • Easy surface modification • Low cost • Potential as delivery system	• Lack of homogeneity • Non-specific target • Decomposition in acid condition (Calcium carbonate)

Maestrelli *et al.*[10] synthesized metformin-loaded chitosomes and niosomes, using different components and procedure incorporate calcium alginate microsphere. It could increase drug adsorption process and decrease side effects of metformin. But the procedures were complicated for preparation and the particles size was in micro range.

Calcium citrate is a calcium salt of citric acid. It is commonly used as a food additive, usually as a preservative. In addition, calcium citrate is also found in some dietary calcium supplements[9]. Moreover, calcium citrate is also biocompatible and slowly degradable[9].

The crystal structure comprises a three-dimensional network in which eightfold coordinated calcium ions are linked by citrate ions, hydrogen bonds among two non-coordinating water molecules and two coordinating water molecules as shown in figure 1-2.

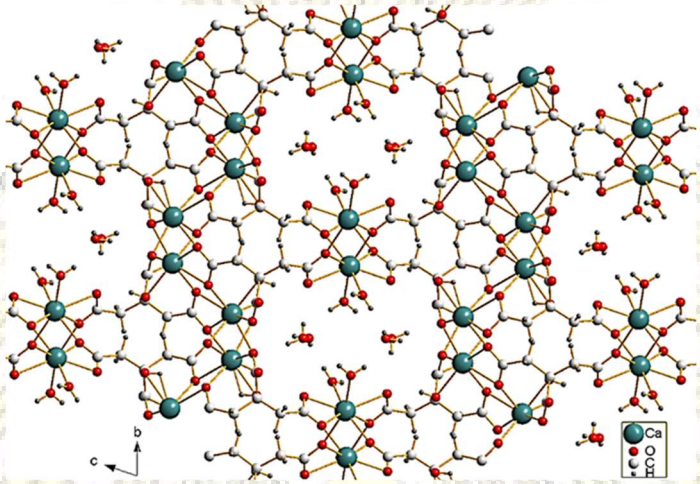

Figure 1-2 Crystal structure of calcium citrate

There have not yet been extensive studies on calcium citrate nanoparticles for drug delivery system. Calcium citrate is a competitor for calcium carbonate and calcium phosphate in terms of bioavailability. In this work, the goal is to synthesize and characterize metformin encapsulated calcium citrate nanoparticles by chemical precipitation, in order to reduce negative side effects, improve delivery efficacy and determine the optimum condition.

1.2. Literature reviews

1.2.1 Drug encapsulation

Mizushima *et al.*[11] synthesized encapsulation or adsorption of drug into or on calcium carbonate nanoparticles respectively with chemical precipitation method. From the results, they reported that the mixing speed was a main factor for controlling particle size. The vigorous stirring produced small particles. The particle size of synthesized product and loading efficiency were shown in the table 1-2.

Table 1-2 Particle size of calcium carbonate nanoparticles and their loading efficiency

Mixing speed	Particle size (nm)		Loading efficiency (%)	
	Encapsulation	Adsorption	Encapsulation	Adsorption
Gentle stirring 650 rpm	140.7	170.5	91.1	9.1
Vigorous stirring 1300 rpm	44.8	101.5	90.6	25.7

Loading efficiency of encapsulation was high. While the particle size of encapsulation was small.

The influence of washing on the release of drug was examined as shown in figure 1-3. More adsorbed drug, compared to encapsulated drug, was released after the particles were washed.

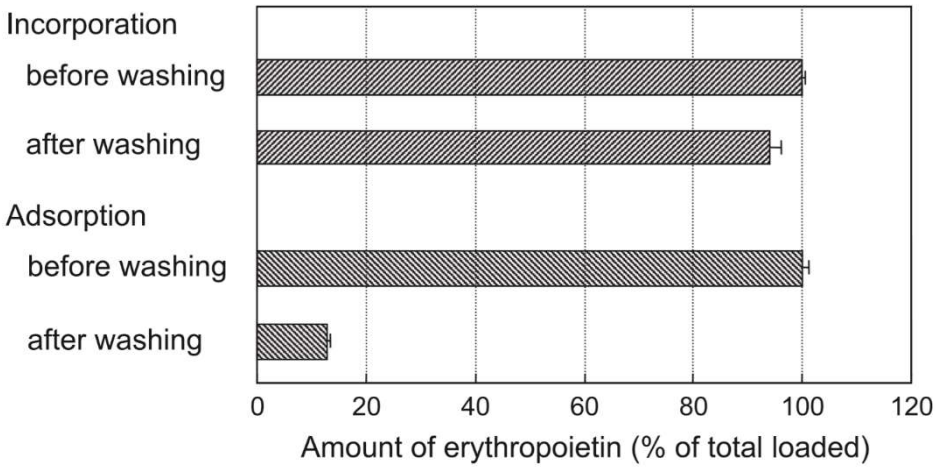

Figure 1-3 Influence of washing on the release of drug from the calcium carbonate nanoparticles

1.2.2 Calcium citrate nanoparticles

Junfeng *et al*.[12] precipitated calcium citrate nanosheets with ratios of alcohol to water solvent system to promote the formation of new bone in animal. The results showed that calcium citrate formed sheets in nanoscale as shown in figure 1-4. When the ratio of water to alcohol was high, calcium citrate nanosheet was thick. When the volume ratio of water to alcohol was reduced, calcium citrate nanosheet was thin.

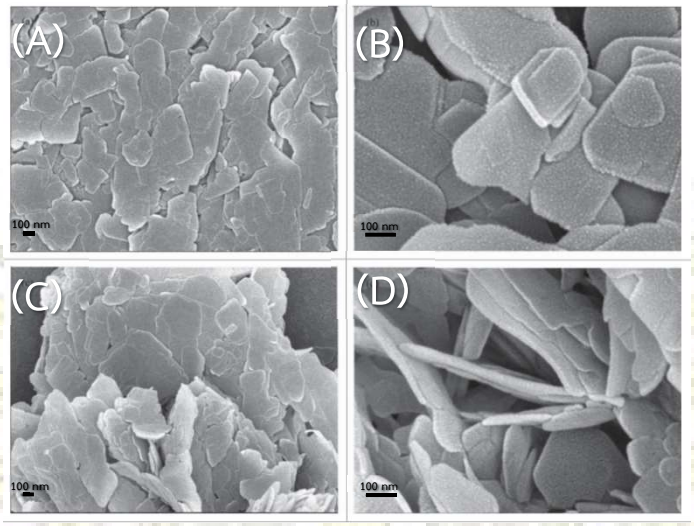

Figure 1-4 SEM micrographs of nano-calcium citrate powder produced at different ratios of alcohol: water: (A) alcohol: water = 2:1, X30000 (B) alcohol: water = 2:1, X100000 (C) alcohol: water = 1:2, X30000 (D) alcohol: water = 1:2, X100000

1.2.3. Potential applications of metformin

Hemmingsen *et al.*[13] compared metformin combined with insulin and insulin alone in clinical trials of patients with type 2 diabetes. The result indicated that metformin combined with insulin led to decreased blood sugar.

Montalvo *et al.*[14] treated metformin with 0.1 %w/w and 1 %w/w in middle-aged mice to prove lifespan. They compared feeding between standard diet and diet with different metformin contents. The result in figure 1-5 indicated that small concentration of drug led to extended lifespan of mice. On the other hand, high concentration of drug was toxic and significantly shortened lifespan of mice.

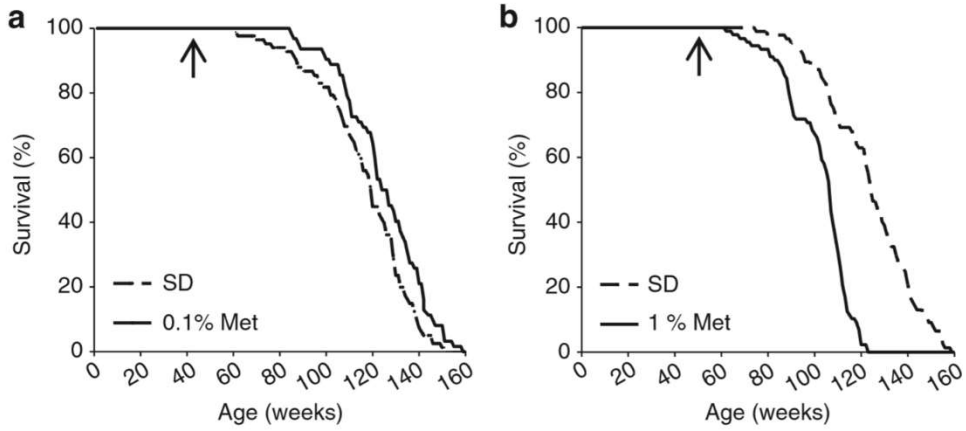

Figure 1-5 (A) survival curve for mice treated with 0.1% metformin (B) survival curve for mice treated with 1% metformin.

1.3. Theories

1.3.1 Solubility of ionic compounds (Table)

The molar solubility of a substance is the maximum possible concentration of a solute in a solution, depending on the physical and chemical properties of the solute and solvent as well as on temperature and pH of the solution. The solubility depends on solubility product constant (Ksp) that is the product of concentrations of solvated ions.

$$aA_{(s)} \rightleftharpoons bB^{+}_{(aq)} + cC^{-}_{(aq)}$$

$$K_{sp} = [B^{+}]^{b}[C^{-}]^{c}$$

The molar solubility of calcium citrate can be calculated from its solubility product constant as shown below.

$$Ca_3(C_6H_5O_7)_{2(s)} \rightleftharpoons 3Ca^{2+} + 2(C_6H_5O_7)^{3-}$$

The solubility product constant of calcium citrate is 7.0×10^{-14}.

$$K_{sp} = [Ca^{2+}]^3[C_6H_5O_7^{3-}]^2$$

$$7.0 \times 10^{-14} = (3X)^3(2X)^2$$

where X = molar solubility of calcium citrate

$$X = 1.6 \times 10^{-3}\ M$$

Therefore, the molar solubility of calcium citrate is 1.6×10^{-3} M

The molar solubility and solubility product constant of calcium salt showed in the table 1-3.

Table 1-3 The molar solubility and product solubility constant (K_{sp}) of calcium salt

Compounds	Solubility (g/L)	Molar solubility (M)	K_{sp} (25°C)
Calcium citrate	0.91	1.6×10^{-3}	7.0×10^{-14}
Calcium oxalate	0.0061	4.8×10^{-5}	2.3×10^{-9}
Calcium carbonate	0.0078	7.7×10^{-5}	6.0×10^{-9}
Calcium phosphate	0.000062	2.0×10^{-7}	2.1×10^{-33}

Calcium citrate is very slightly soluble in water (0.1 – 1 g/L). Therefore, the calcium citrate can be synthesized *via* chemical precipitation.

1.3.2 Material characterization

1.3.2.1 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis is a method of thermal decomposition without oxygen gas. The mass of sample is measured as a function of increasing temperature[15]. The results are presented by thermogravimetry curve (TG) and differential thermogravimetry curve. This measurement provides both physical information (adsorption and desorption) and chemical information (chemisorption and thermal decomposition).

The figure 1-6 showed decomposition of calcium carbonate around 600 °C[16] based on the equation below.

$$CaCO_{3(s)} \longrightarrow CaO_{(s)} + CO_{2(g)}$$

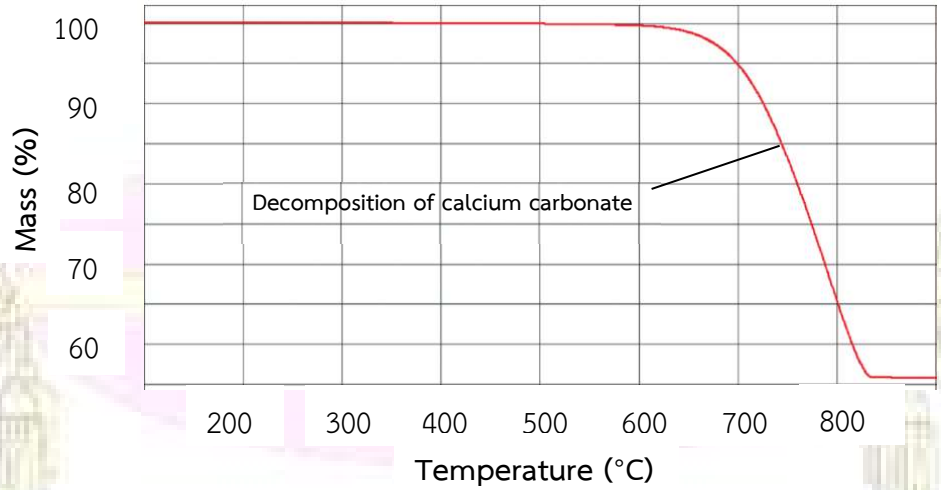

Figure 1-6 TGA curve of calcium carbonate

The figure 1-7 showed that $[Ca_3(C_6H_5O_7)_2(H_2O)_2]\cdot 2H_2O$ dehydrates in two steps. The first mass change around 70 °C was outer water molecules and second mass change around 130 °C corresponded to the loss of inner water molecules. Next, mass change between 350 - 520 °C was decomposition of calcium citrate to calcium carbonate. Lastly, mass change above 600 °C resulted from the decomposition of calcium carbonate to calcium oxide and carbon dioxide[17]. All of decomposition equations are shown below.

$$[Ca_3(C_6H_5O_7)_2(H_2O)_2]\cdot 2H_2O_{(s)} \longrightarrow [Ca_3(C_6H_5O_7)_2(H_2O)_2]_{(s)} + 2H_2O_{(g)}$$

$$[Ca_3(C_6H_5O_7)_2(H_2O)_2]_{(s)} \longrightarrow Ca_3(C_6H_5O_7)_{2\,(s)} + 2H_2O_{(g)}$$

$$Ca_3(C_6H_5O_7)_{2\,(s)} \longrightarrow 3CaCO_{3\,(s)} + 5H_2O_{(g)} + 9C_{(s)}$$

$$CaCO_{3\,(s)} \longrightarrow CaO_{(s)} + CO_{2(g)}$$

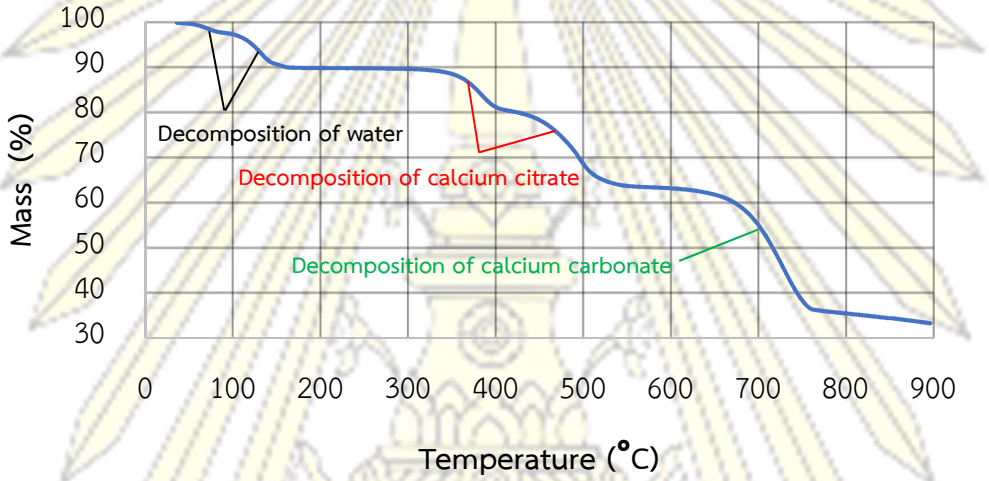

Figure 1-7 TGA curve of calcium citrate

1.3.2.2 Scanning electron microscope (SEM)

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a signal from the surface of solid specimens. This technique provides information about surface morphology[16].

In figure 1-8, Calcium carbonate nanoparticles were synthesized using precipitation method from cockle shells by employing chitosan as precursor. Chitosan is capable of producing inorganic materials such as calcium carbonate with different structures, morphologies and polymorphs.

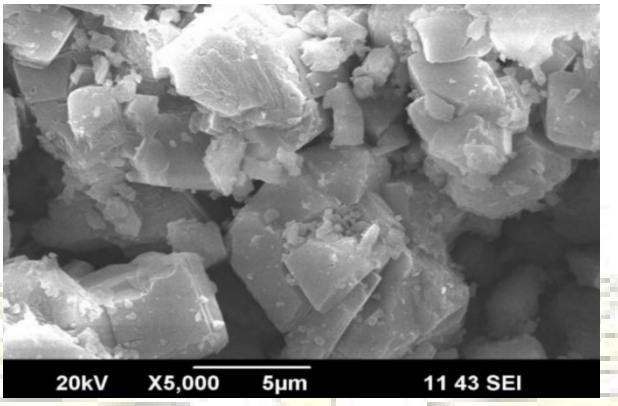

Figure 1-8 SEM image of synthesized calcium carbonate nanoparticles

1.4 Objectives

To synthesize and characterize metformin encapsulated calcium citrate nanoparticles *via* chemical precipitation.

Chapter II

Experiments

2.1 Materials

Analytical grade calcium chloride, trisodium citrate dihydrate and absolute ethanol were purchased from Merck. Metformin Hydrochloride was purchased from TCI. The chemicals information showed in the table 2-1.

2.1.1 Chemicals

Table 2-1 Chemicals information

Chemicals	Molar mass (g/mol)
Calcium chloride	110.98 g/mol
Trisodium citrate dihydrate	294.10 g/mol
Metformin	165.63 g/mol
Ethanol	46.07 g/mol

2.2 Instruments

1. MIKRO 220 Hettich Centrifuge machine
2. Comtherm Designer Series Oven 50 litre 230 Vac
3. Eppendorf Research plus pipette 200-1000 μL
4. Four-decimal place Balance – Denver instruments SI – 234
5. Heidolph duomax 1030 rocking machine
6. NETZSCH TG 209F3 TGA209F3A – 0364 – L
7. Power sonic series 420 ultrasonic
8. JEOL series JSM – 6480LV Scanning electron microscope (SEM)
9. Vortex – Genie II

2.3 Methodology

2.3.1 Synthesis of metformin encapsulated calcium citrate nanoparticles

Mix 2 mL of 1.5 M calcium chloride and 2 mL of 1.5 M trisodium citrate into a 50 mL tube. Then, 2 mL of various metformin solution was added, under constant vortex motion. Excess absolute ethanol was added dropwise until white precipitation was formed. The nanoparticles were separated from colloidal dispersion by centrifugation at 4000 rpm for 5 minutes and washed with DI water five times. The white nanoparticles were dried overnight in an oven at 80°C, Yielding white powder as synthesized products.

2.3.2 The effects of metformin hydrochloride concentration

The reaction procedure followed 2.3.1 with various concentration of metformin according to the table 2-2.

Table 2-2 Various concentration of metformin encapsulated

Condition	1	2	3
Met (M)	0.125	0.250	0.500

2.3.3 The effects of water-ethanol solvent system

The reaction procedure followed 2.3.1 except the ratio of ethanol to water according to the table 2-3.

Table 2-3 Various the ratios of ethanol to water

Condition	4	5	6	7
Ethanol (%V)	25	50	75	100

2.3.4. Characterization techniques

2.3.4.1 Thermogravimetric analysis

The sample prepared from 10 mg of nanoparticle. The analytical temperature was increased from 50°C to 800°C with 10°C per minute.

2.3.4.2 Scanning electron microscopy

The nanoparticles were dispersed in water to the concentration of about 5 mg/mL. Then, the dispersion was dropped onto a glass slide.

Chapter III

Results and discussion

Chemical precipitation based on procedure developed by Mizushima *et al.*[11] was used in this research to synthesize metformin encapsulated calcium citrate nanoparticles. The chemical reaction involved in the synthesis following the equation below. According to previous studies by our research group, the 1:1 calcium ion to citrate ion mole ratio yielded spherical particles in range of 100-250 nm. Therefore, this research used the ratio according to the previous studies.

$$3CaCl_{2(aq)} + Na_3(C_6H_5O_7)_{(aq)} \longrightarrow Ca_3(C_6H_5O_7)_{2(s)}$$

In this research, the effects of metformin concentration and ethanol to water ratio were investigated. Products from condition 1, 3, 4, 6 and 7 were characterized with thermogravimetric analyzer and scanning electron microscope.

3.1 Effects of metformin concentration

Comparing the mass loss between unloaded calcium citrate and metformin loaded. Thermograms confirmed that metformin was successfully encapsulated within calcium citrate. Figure 3-2 showed the product from condition 1 indicating that there was 2.96 % mass loss at 310 °C. Ramachandran and Ramukutty[19] reported % mass loss of pure metformin at 227.08 °C[19]. The appearance of metformin decomposition temperature was higher than pure metformin maybe due to stabilization of calcium citrate. While figure 3-3, There was 0.34 % mass loss of metformin. The decomposition of metformin was very small mass change in TG curve that could not observe but DTG curve could observe decomposition of metformin at 310 °C.

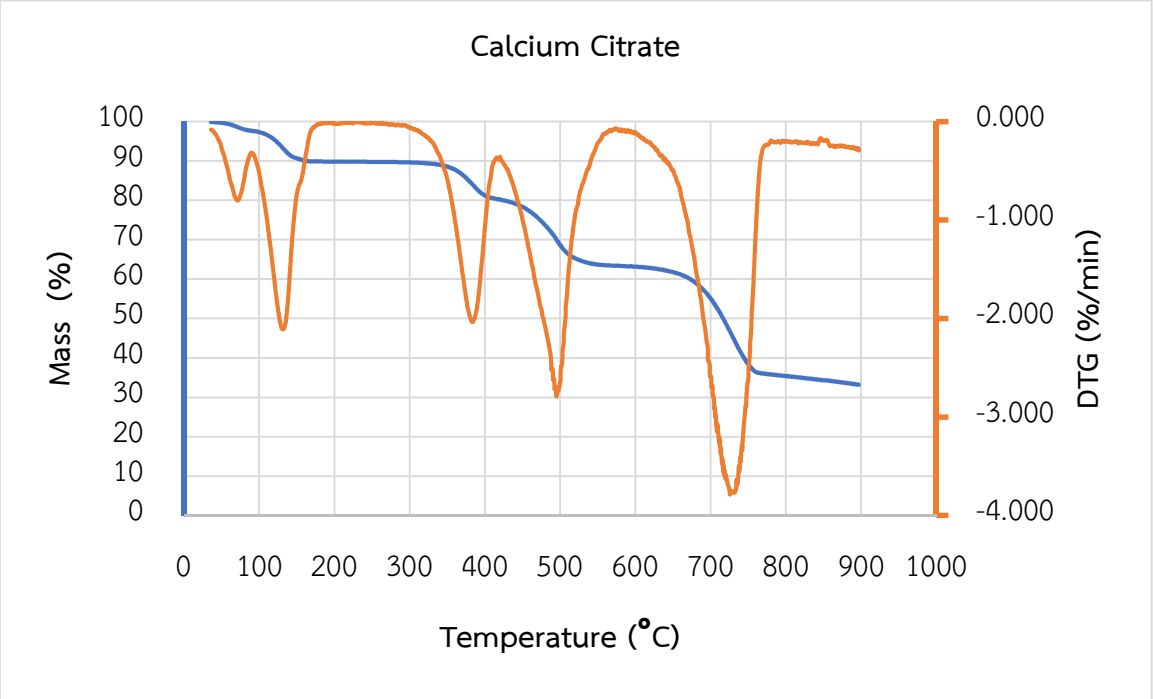

Figure 3-1 TG and DTG curve of unloaded calcium citrate

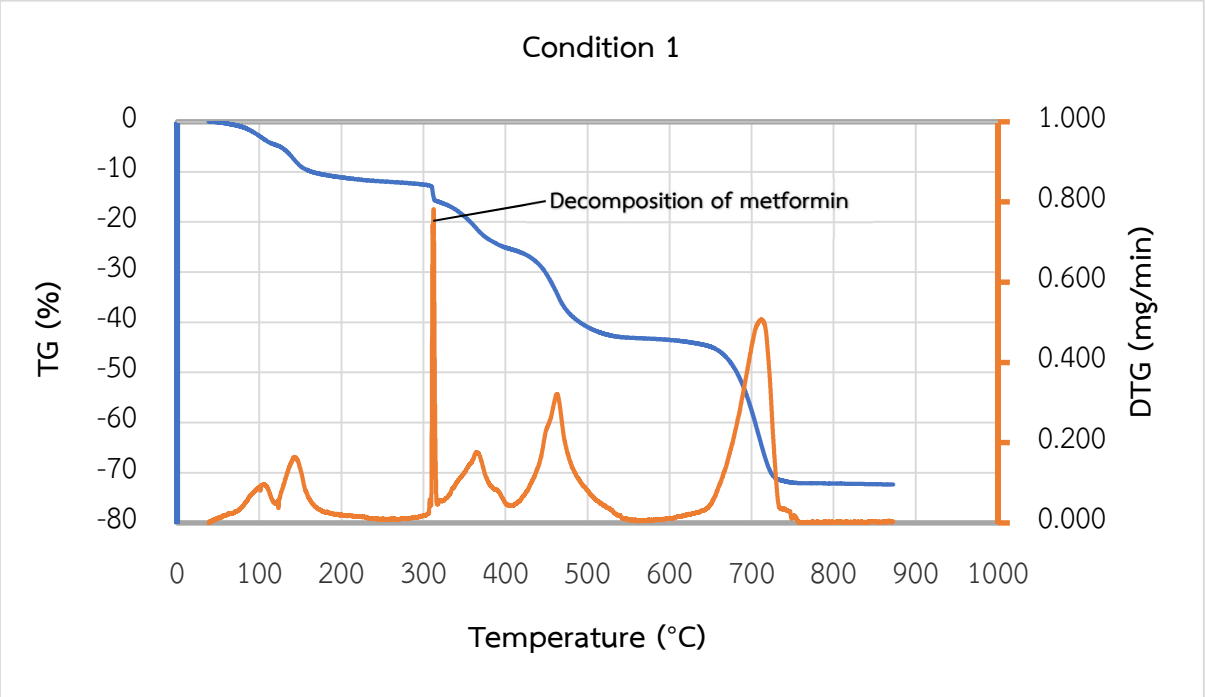

Figure 3-2 TG and DTG Curve of 4:1 mole ratio of calcium citrate to metformin

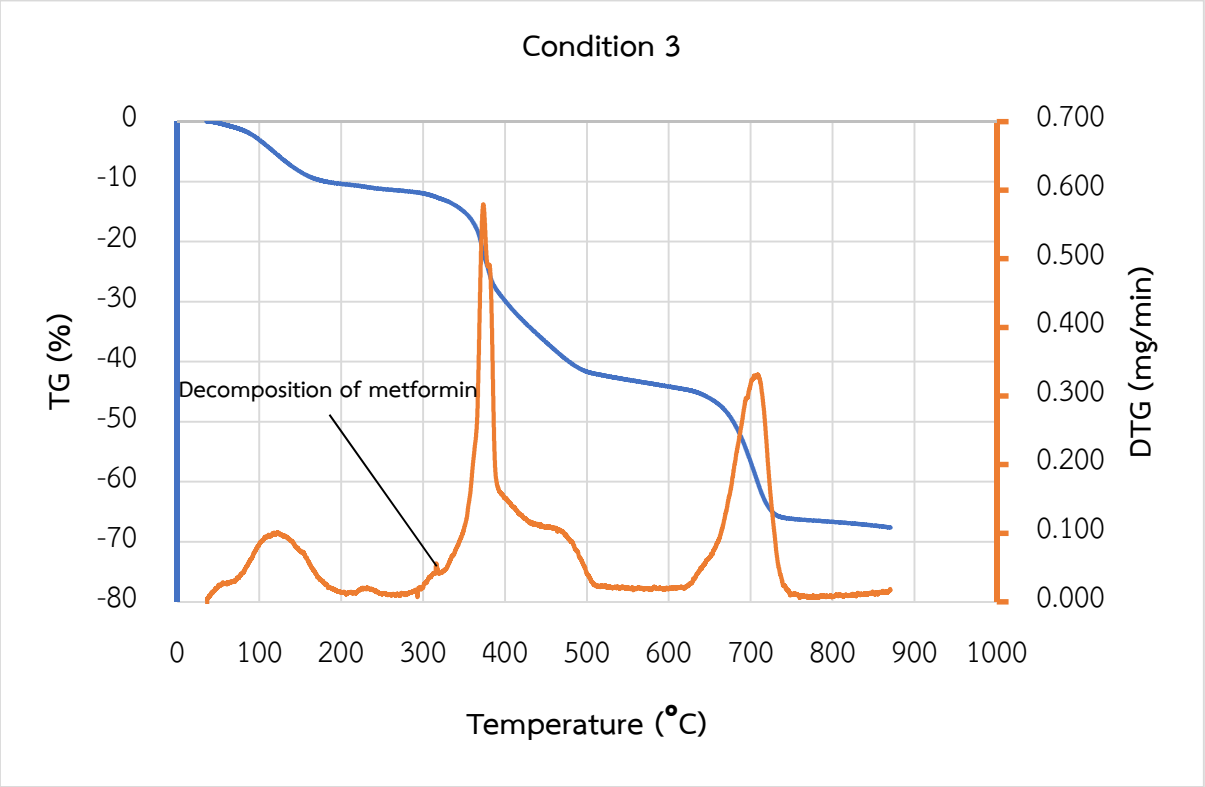

Figure 3-3 TG and DTG curve of 1:1 mole ratio of calcium citrate to metformin

SEM image of the product from condition 1 showed the particles appeared in rice grain shape with diameter 2 micrometres. While SEM image of the product from condition 3 showed the particles appeared in cubic shape with diameter 2 micrometres. Gorner *et al.*[20] mentioned that high amount of drug increase viscosity which became more difficult for dispersion of particles and led to increase particle size. The different morphologies between condition 1 and condition 3 were probably caused by metformin.

Condition 1

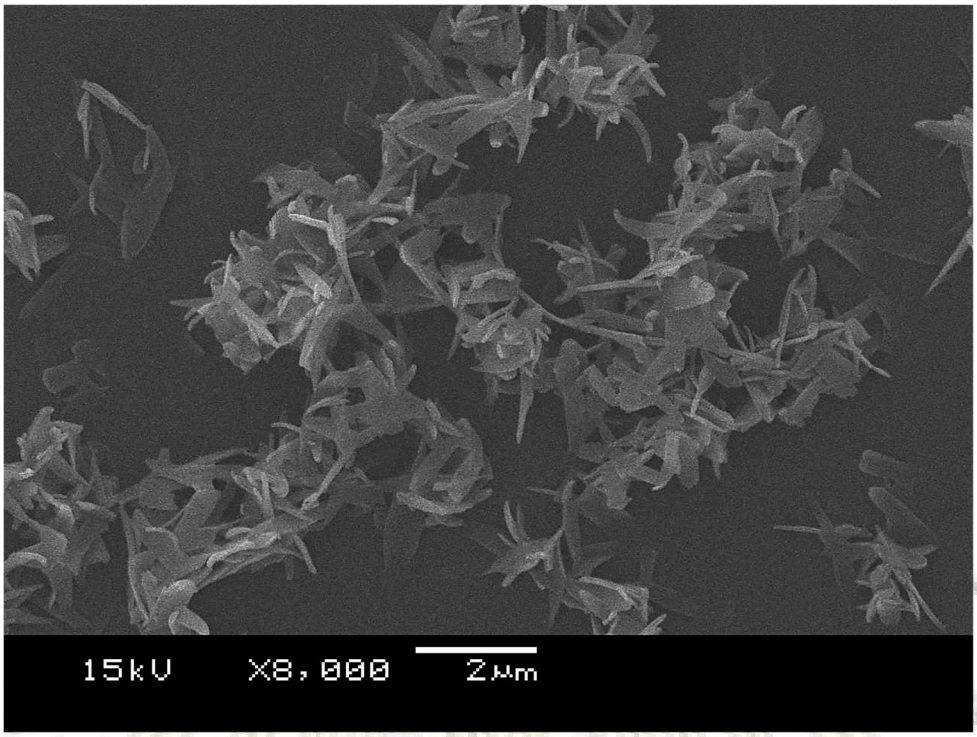

Figure 3-4 SEM image of 4:1 mole ratio of calcium citrate to metformin

Condition 3

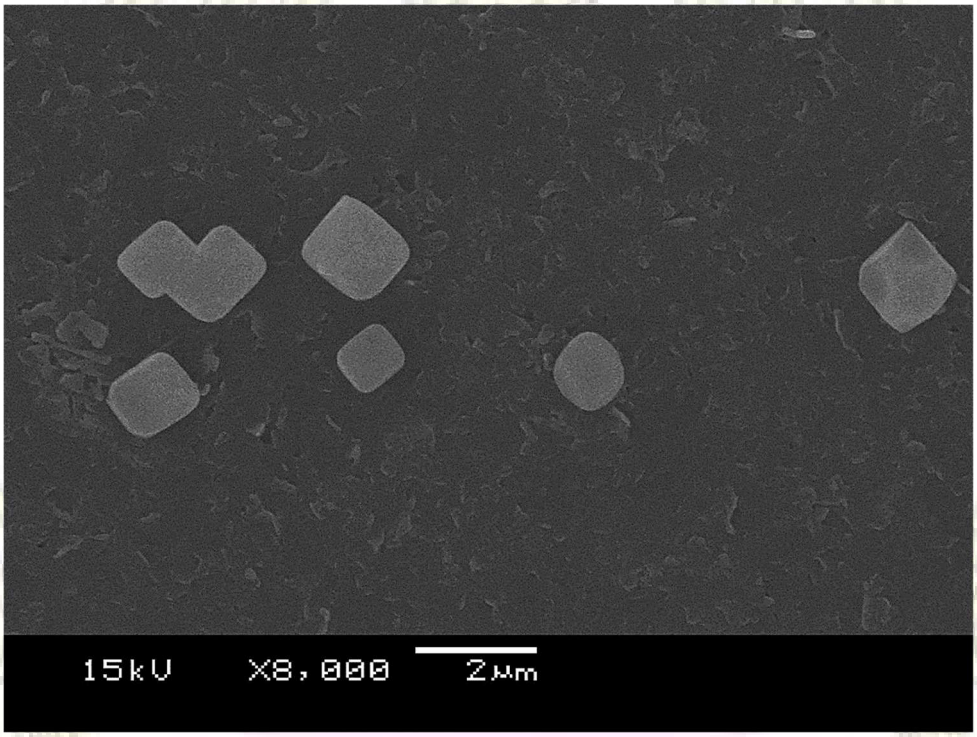

Figure 3-5 SEM image of 1:1 calcium citrate to metformin

3.2 Effects of ethanol concentration

Ethanol to water ratio was one of possible factors that could affect the formation of metformin encapsulated calcium citrate due to their solubility decreased. Products of condition 4 and 6 exhibited no mass loss around 310 ℃ may be due to there are too little of ethanol. While condition 7 exhibited 1.14 % mass loss of metformin. Therefore, the optimum condition was used absolute ethanol to encapsulate metformin.

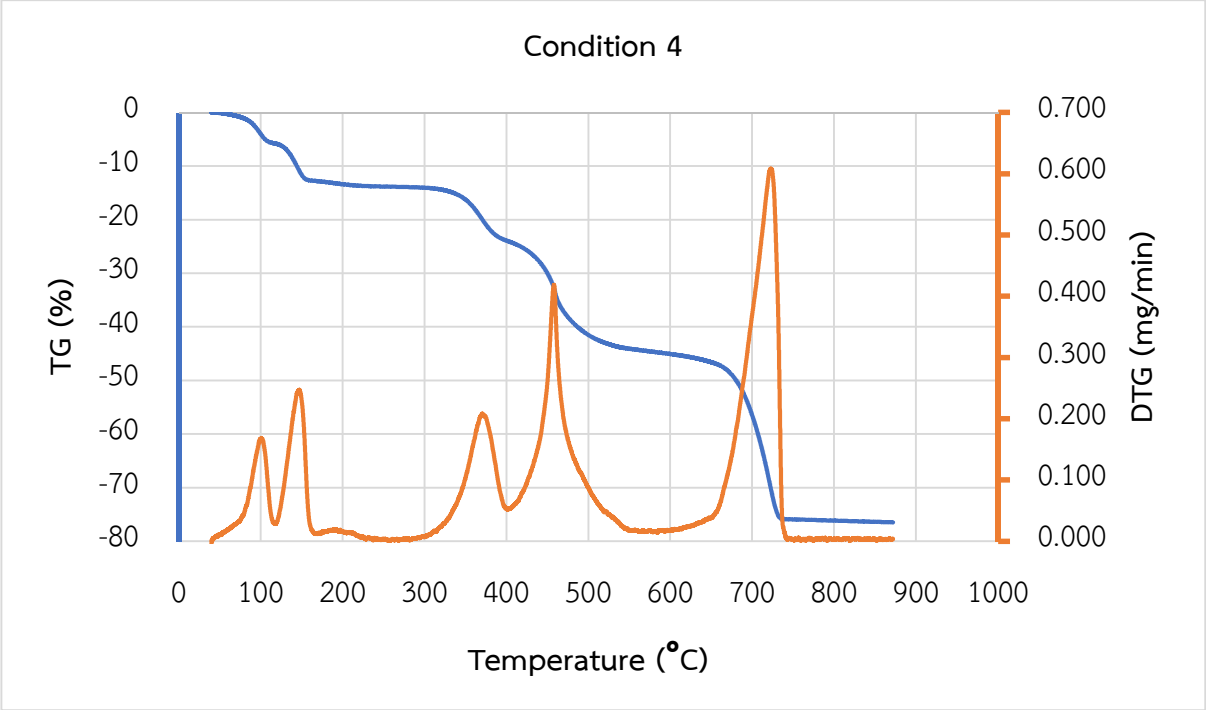

Figure 3-6 TG and DTG curve of 1:3 volume ratio of ethanol to water

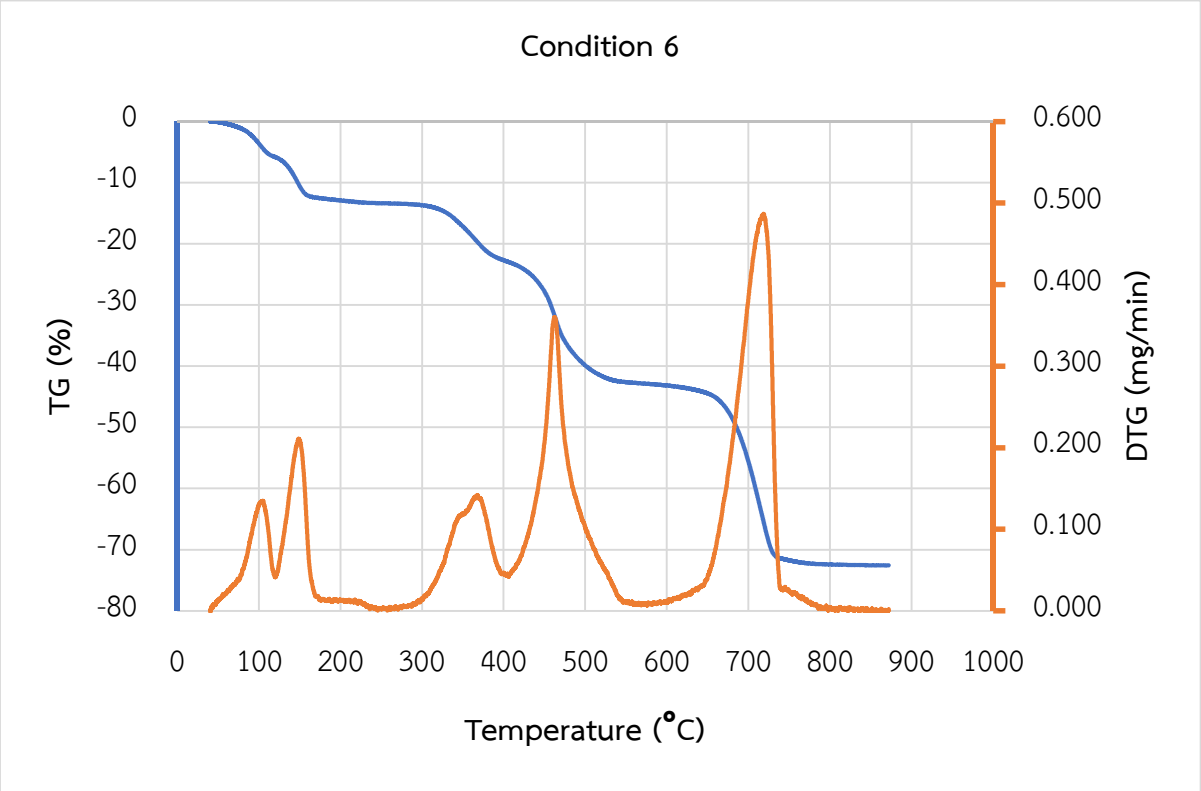

Figure 3-7 TG and DTG curve of 3:1 volume ratio of ethanol to water

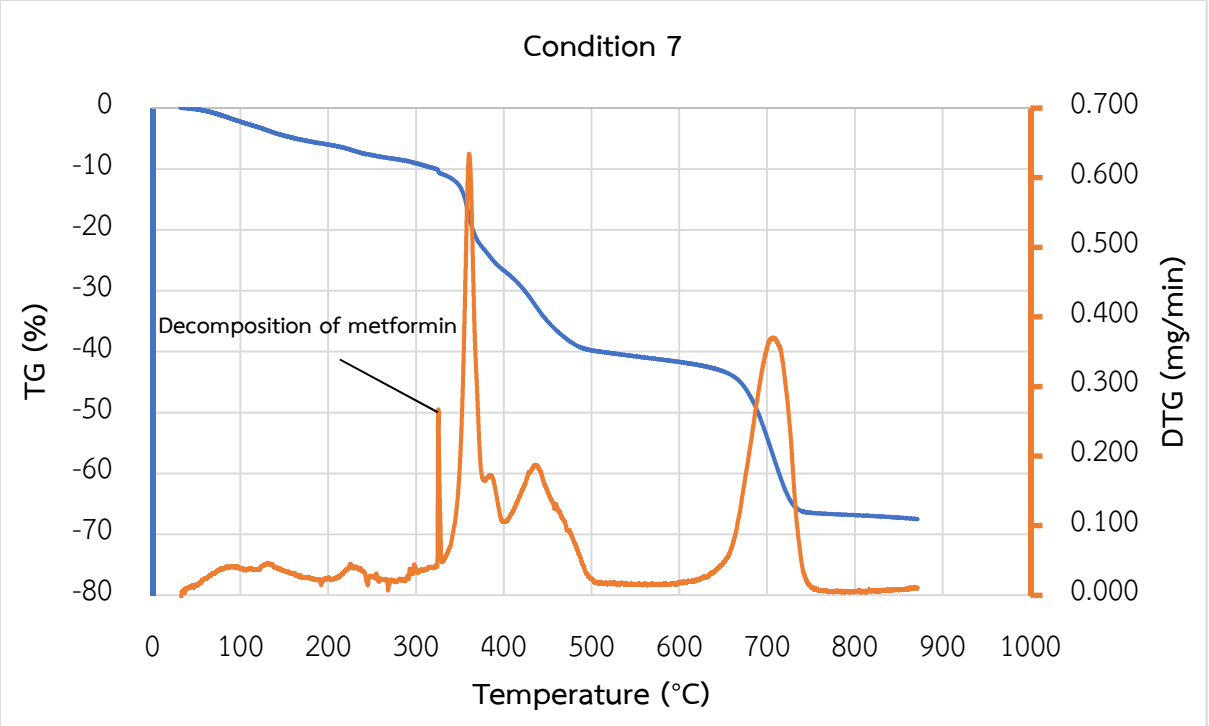

Figure 3-8 TG and DTG curve of absolute ethanol

SEM image in condition 4, 6 and 7 indicated that the morphology of particles tended to increase particles size maybe due to the ethanol reduced dispersion particles in water.

Condition 4

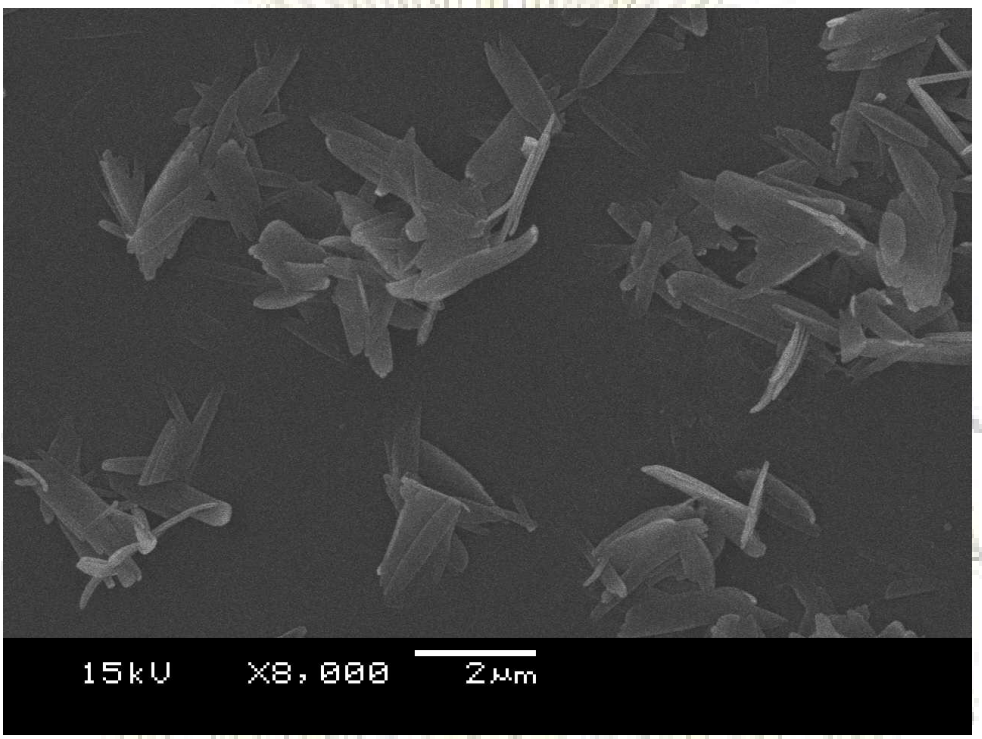

Figure 3-9 SEM image of 1:3 volume ratio of ethanol to water

Condition 6

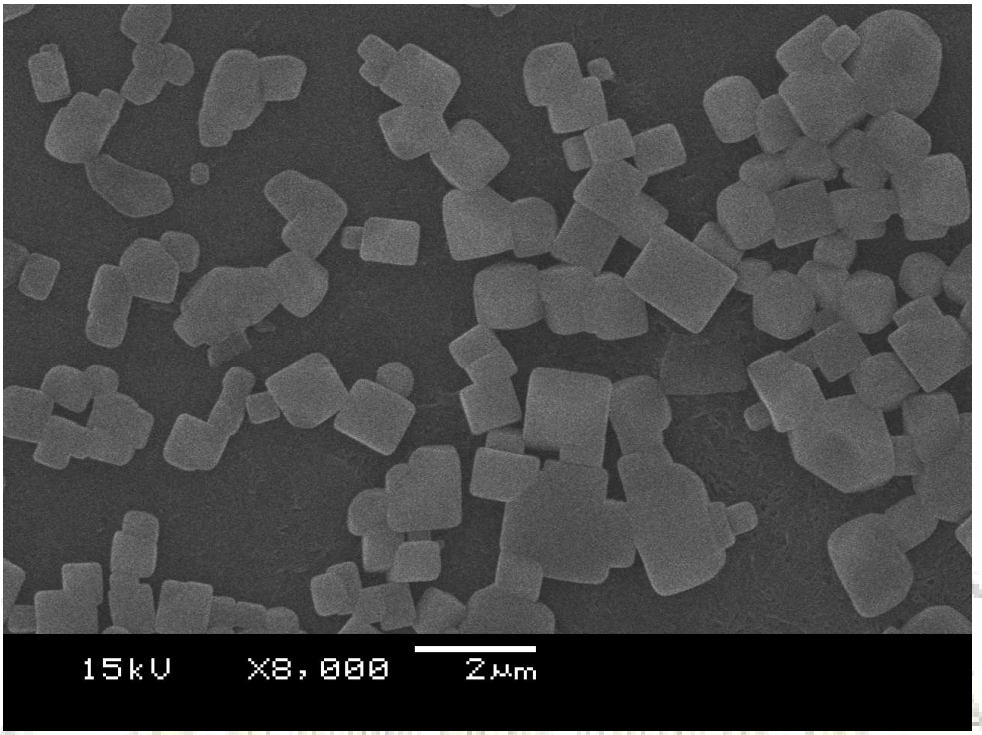

Figure 3-10 SEM image of 3:1 volume ratio of ethanol to water

Condition 7

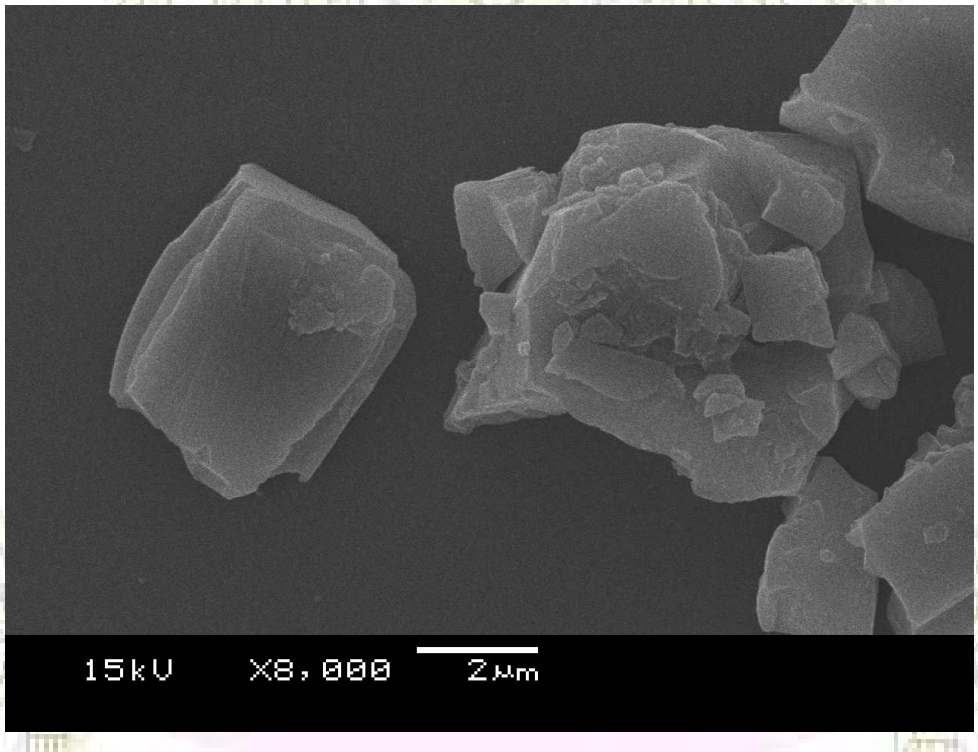

Figure 3-11 SEM image of absolute ethanol

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Chapter IV

Conclusions

In this research, metformin encapsulated calcium citrate was prepared *via* chemical precipitation method. The optimum condition was 1:0.25 ratio of calcium citrate to metformin and enhancing precipitation rate by absolute ethanol. Consequently, the maximum loading capacity of metformin was 2.96%. The synthetic particles appeared in rice grain shape with diameter 2 micrometresu and tended to agglomerate by increasing metformin concentration and ethanol to water ratio. However, various amounts of calcium ion and citrate ion should be investigated further.

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Vitae

Mr. Thatchan Cheepsumon was born on October 18th, 1995 in Chonburi, Thailand. He graduated from Chonradsadornumrung school in 2013 and continued to study in bachelor of Chemistry at the Faculty of Science, Chulalongkorn University, Bangkok, Thailand. The current address is 26 My home 101, Soi Punnawithi 11, Sukhumvit 101 Road, Bangchak Sub District, Prakanong District, Bangkok 10260.