ไมโครเอนแคปซูเลชันแอนโดรกราโฟไลด์ที่ใช้คอนยักกลูโคแมนแนนไฮโดรไลเสต
ด้วยเทคนิคการทำแห้งแบบพ่น

นางสาวสุพิชชา วัฒนะประเสริฐ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
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ปีการศึกษา 2558

MICROENCAPSULATION OF ANDROGRAPHOLIDE USING KONJAC GLUCOMANNAN HYDROLYSATE BY SPRAY DRYING TECHNIQUE

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biotechnology
Faculty of Science
Chulalongkorn University
Academic Year 2015

Thesis Title	MICROENCAPSULATION OF ANDROGRAPHOLIDE USING KONJAC GLUCOMANNAN HYDROLYSATE BY SPRAY DRYING TECHNIQUE
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Field of Study	Biotechnology
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สุพิชชา วัฒนะประเสริฐ : ไมโครเอนแคปซูเลชันแอนโดรกราโฟไลด์ที่ใช้คอนยักกลูโคแมนแนนไฮโดรไลเสตด้วยเทคนิคการทำแห้งแบบพ่น (MICROENCAPSULATION OF ANDROGRAPHOLIDE USING KONJAC GLUCOMANNAN HYDROLYSATE BY SPRAY DRYING TECHNIQUE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ชาลีดา บรมพิชัยชาติกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.จอร์จ เชริดนิกสกี้, ดร.พิลาณี ไวถนอมสัตย์, 98 หน้า.

งานวิจัยนี้มีวัตถุประสงค์ในการพัฒนาสารเคลือบจากผงบุกเพื่อใช้ในการทำไมโครแคปซูล (encapsulation) ของสารออกฤทธิ์ทางชีวภาพจากพืช คือ แอนโดรกราโฟไลด์ โดยวิธีการทำแห้งแบบพ่น (spray drying) โดยมีขั้นตอนการทดลองดังนี้ ขั้นตอนที่หนึ่งการใช้เอนไซม์ mannanase 1,500 Unit เพื่อปรับปรุงคุณสมบัติการไหลของสารละลายบุก 9% (w/w) ให้มีความหนืดที่เหมาะสมสำหรับนำไปทำแห้งแบบพ่น จากผลทดลองพบว่าความเข้มข้นของสารละลายบุก 12% (w/w) เป็นความเข้มข้นที่เหมาะสมที่ทำให้ความหนืดของสาระลายบุกไฮโดรไลเสต (konjac glucomannan hydrolysate, KGMH) มีค่าน้อยกว่า 100 mPa·s และผลการวิเคราะห์องค์ประกอบของสารละลายบุกไฮโดรไลเสตที่ได้ โดยเทคนิค HPLC พบว่า มีปริมาณโอลิโกแซคคาไลด์ (DP4-DP7) เป็นองค์ประกอบมากกว่า 27% ขั้นตอนที่สองคือการศึกษาการใช้สารละลายบุกไฮโดรไลเสตเป็นสารเคลือบโดยวิธีการทำแห้งแบบพ่นและเปรียบเทียบประสิทธิภาพในการทำไมโครแคปซูลโดยใช้สารละลายบุกไฮโดรไลเสตเพียงอย่างเดียว กับการใช้สารละลายบุกไฮโดรไลเสตร่วมกับสารเคลือบอื่น จากผลการทดลองพบว่า ที่อุณหภูมิลมร้อนขาเข้า 170 ℃ และอุณหภูมิลมร้อนขาออก 85±3℃ สารละลายบุกไฮโดรไลเสตที่เตรียมจากสารละลายบุก 12% (w/w) เป็นความเข้มข้นที่เหมาะสมสำหรับการเคลือบและกักเก็บสารแอนโดรกราโฟไลด์ 2% (w/w) และผลการศึกษาประสิทธิภาพในการทำไมโครแคปซูล พบว่า การใช้สารละลายบุกไฮโดรไลเสตเพียงอย่างเดียวให้ประสิทธิภาพดีกว่าการใช้ สารละลายบุกไฮโดรไลเสต ร่วมกับ gamma-cyclodextrin หรือ beta-cyclodextrin ขั้นตอนที่สามคือการศึกษาผลของการใช้สารละลายบุกไฮโดรไลเสตในการผลิตไมโครแคปซูลโดยวิธีการทำแห้งแบบพ่นต่อประสิทธิภาพการกักเก็บสารแอนโดรกราโฟไลด์ และร้อยละผลผลิตของการผลิตไมโครแคปซูล โดยมีปัจจัยที่ทำการศึกษาดังนี้อุณหภูมิลมร้อนขาเข้า 150 ℃, 170 ℃ และ 190 ℃, อุณหภูมิลมร้อนขาออก 75±3 ℃ และ 85±3 ℃ และความเข้มข้นของสารละลายบุกไฮโดรไลเสต 9%, 12%, 15%, 18% และ 21% (w/w) จากผลการทดลองพบว่า ที่อุณหภูมิลมร้อนขาออก 85±3 ℃ ให้ร้อยละผลผลิตของสารไมโครแคปซูลแอนโดรกราโฟไลด์สูงกว่าการใช้อุณหภูมิลมร้อนขาออก 75±3 ℃ อย่างมีนัยสำคัญ และพบว่าการเพิ่มอุณหภูมิลมร้อนขาเข้าสูงขึ้นทำให้ร้อยละผลผลิตของไมโครแคปซูลเพิ่มสูงขึ้นด้วย โดยอุณหภูมิลมร้อนขาเข้า 170 ℃ และ 190 ℃ ให้ร้อยละผลผลิตสูงกว่าการใช้อุณหภูมิลมร้อนขาเข้า 150 ℃ อย่างมีนัยสำคัญ ซึ่งอุณหภูมิลมร้อนขาเข้ามีผลต่อร้อยละผลผลิตของสารไมโครแคปซูล นอกจากนี้พบว่าการเพิ่มความเข้มข้นของสารละลายบุกในการเตรียมสารละลายบุกไฮโดรไลเสตช่วยเพิ่มปริมาณสารแอนโดรกราโฟไลด์ในไมโครแคปซูล โดยการใช้ความเข้มข้นของสารละลายบุก 18% และ 21% (w/w) ให้ปริมาณสารแอนโดรกราโฟไลด์ ในไมโครแคปซูลสูงกว่าการใช้ความเข้มข้น 9%, 12% และ 15% (w/w) แต่สมบัติทางเคมีและทางกายภาพของไมโครแคปซูลที่ผลิตได้ในแต่ละความเข้มข้นไม่แตกต่างกัน จากผลการทดลองสามารถสรุปได้ว่า สภาวะที่เหมาะสมที่สุดในการผลิตไมโครแคปซูลของสารแอนโดรกราโฟไลด์ คือ การใช้ความเข้มข้นของสารละลายบุกไฮโดรไลเสตที่เตรียมจากสารละลายบุก 21% (w/w) ร่วมกับการทำแห้งแบบพ่นโดยใช้อุณหภูมิลมร้อนขาเข้า 190 ℃ และอุณหภูมิลมร้อนขาออก 85±3℃ และขั้นตอนสุดท้ายการทดสอบความคงตัวของไมโครแคปซูลแอนโดรกราโฟไลด์ ในระบบจำลอง (*In vitro*) ทางเดินอาหารของสุกร จากผลการทดลองพบว่า ไมโครแคปซูลแอนโดรกราโฟไลด์มากกว่าร้อยละ 70 สามารถทนต่อระบบการย่อยของสุกรซึ่งจะสามารถเคลื่อนผ่านและถูกดูดซึมในลำไส้ของสุกรต่อไปได้

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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5373919523 : MAJOR BIOTECHNOLOGY
KEYWORDS: MICROENCAPSULATION, ANDROGRAPHOLIDE, KONJAC GLUCOMANNAN, HYDROLYSATE, SPRAY DRYING TECHNIQUE

SUPICHAR WATTANAPRASERT: MICROENCAPSULATION OF ANDROGRAPHOLIDE USING KONJAC GLUCOMANNAN HYDROLYSATE BY SPRAY DRYING TECHNIQUE. ADVISOR: ASST. PROF. CHALEEDA BOROMPICHAICHARTKUL, Ph.D., CO-ADVISOR: GEORGE SRZEDNICKI, Ph.D., PILANEE VAITHANOMSAT, Ph.D., 98 pp.

This research aimed at developing a suitable coating material from konjac glucomannan (KGM) for encapsulating a plant bioactive compound, andrographolide, using spray drying. There are three steps of study. In the first step, a suitable process for modifying the rheological properties of KGM solution by enzymatic treatment was developed. Mannanase (1500 units of enzyme) per 9% (w/w) of KGM solution was used in the study. The concentration of KGM solution was varied from 9 to 18% (w/w). It was found that 12% (w/w) was the optimum KGM concentration to obtain the konjac glucomannan hydrolysate (KGMH) with the viscosity less than 100 mPa·s. High Performance Liquid Chromatography (HPLC) analysis of hydrolyzed KGM showed that more than 27% of DP4-DP7 oligosaccharides were obtained. The second step, the solution was then used as coating material in spray drying with inlet air temperature of 170 °C and outlet air temperature of 85±3 °C. It was found that KGMH which was prepared from 12% (w/w) KGM was suitable for coating 2% (w/w) andrographolide. Its efficiency of encapsulation was also higher than using KGMH combined with gamma-cyclodextrin or beta-cyclodextrin. To produce a bioactive microcapsule, effect of spray drying temperature (outlet air temperature, 75±3 °C and 85±3 °C and inlet air temperature 150 °C, 170 °C and 190 °C) and concentration of the KGM (9%, 12%, 15%, 18% and 21% (w/w)) on encapsulation efficiency and yield of andrographolide microcapsules was studied. In the third step, the results indicated that andrographolide microcapsules produced at outlet air temperature at 85±3 °C had significantly ($p \leq 0.05$) higher % encapsulation and % product yield than those at 75±3 °C. Increasing inlet air temperature to 170 °C and 190 °C resulted in a higher significantly ($p \leq 0.05$) % encapsulation yield than at 150 °C. Therefore, the effect of inlet air temperature and concentration of KGM can effect with encapsulation efficiency of microcapsules. The 18% and 21% (w/w) concentration of KGM can enhance the retention of andrographolide in the microcapsules more than those of 9%, 12%, 15% (w/w). It was found that increasing the concentration of KGM during enzymatic hydrolysis had the same effect as increasing the inlet temperature to 170 °C and 190 °C. Physical and chemical properties of the microcapsules did not differ significantly ($p > 0.05$). The best condition is to use 21% (w/w) of KGM along with an inlet air temperature of 190 °C and outlet air temperature of 85±3 °C. It provided the best retention efficiency and yield of andropraphólide. *In vitro* testing of release of andrographolide in a simulation model of piglet's digestive system showed that more than 70% of andrographolide remained in the microcapsules and can be further released to the target site.

Field of Study: Biotechnology
Academic Year: 2015

Student's Signature .....
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Co-Advisor's Signature .....
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ACKNOWLEDGEMENTS

I would never been able to finish my dissertation without guidance of my advisor, co-advisor, committee members and also the financial support from various sources of funding. The love and care from my family and friends have encouraged me to move forward. I would like to express the deepest appreciation to all them.

First of all, I would like to express my deepest gratitude to my advisor, Assistant Professor Dr. Chaleeda Borompichaichartkul who give me a chance for new experience and devoted her efforts to make me a good researcher. She is a role model for a good teacher and researcher and inspired me on creating my own pathway. Without her guidance, continuous help and patience while correcting my thesis, this dissertation would not have been possible. I would also like to give special thanks to my co-advisor, Dr. George Srzednicki and Dr. Pilanee Vaithanomsat for useful comments and suggestions.

I also thank my examination committee, Associate Professor Dr. Saiwarun Chaiwanichsiri, Assistant Professor Dr. Pasawadee Pradipasena, Associate Professor Dr. Surachai Pornpakakul and Associate Professor Dr. Somkiat Prachayawarakorn for their valuable suggestions.

I would like to thank Chulalongkorn University for providing scholarship for my studies and funding this research. The financial support from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), Chulalongkorn University for Special Task Force for Activating Research (STAR): Dehydration of Food and Biomaterials, Huachiew Chalermprakiet University for giving the time and opportunity to learn and study in this research.

A special thanks to my family L.T. CDR. Pichitporn Wattanaprasert (my father), Mrs. Kamonlak Wattanaprasert (my mother) and Mr. Arkom Wattanaprasert (my brother). They are supporting me and encouraging me with their best wishes. Finally, I am deeply grateful to Phattanit Tripetch, Rarisara Impaprasert, Supattra Muangham and all friends at the Department of Food Technology and Department of Biotechnology Chulalongkorn University for their help, encouragement and for giving me useful suggestions.

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CHAPTER 1

INTRODUCTION

1.1 Research background

Andrographolide is found as the main component in the leaves of *Andrographis paniculata*. It is a bicyclic diterpenoid lactone and is widely used as traditional Thai medicine for the treatment of gastrointestinal tract, upper respiratory infections, fevers, chronic dysentery and diarrhea, inflammations, and sore throat and infectious diseases (Calabrese *et al*., 2000; Iruretagoyena *et al*., 2005). In the livestock industry, andrographolide can be used for treatment of diarrhea in piglets. However, andrographolide is not stable against light and it's not stable at pH 2, 7, 9 and 10 (Cheung *et al*., 2001). Therefore, it should be coated by a protective material preventing its deterioration. Such form of preservation should also be compatible with its use in animal nutrition. Therefore, microencapsulation could be one of the methods that can stabilize the bioactive compounds against light and increase their water solubility (Tungprapa, 1999). The advantages of microencapsulation have been reported. It can reduce the reactivity of core materials with regard to outside environment, decrease evaporation or transfer rate of core materials, control the release of core materials, comfort the handling of core materials and mask the taste of core materials (Versic, 1988). A plethora of techniques have been used, including centrifugal extrusion, fluidized bed, spray chilling and spray drying (King, 1995) Spray drying is widely used in the microencapsulation for food industry due to its relatively low cost, high productivity, increased microbiological stability of food and pharmaceutical products and diminished risk of chemical and biological degradations

(Gharsallaoui *et al*., 2007). Besides, spray drying has been used to produce agricultural materials and pharmaceutical products. However, the efficiency of microencapsulation depends on wall material composition. Wall materials can be selected from natural and synthetic polymers such as carbohydrate or protein based materials (Gharsallaoui *et al*., 2007).

Konjac glucomannan (KGM) is a natural polysaccharide isolated from the tuber of *Amorphophallus* spp. plants found in Asian countries such as China, Taiwan, Japan and Thailand (Tatirat and Charoenrein, 2011). KGM is widely used in food, pharmaceutical, chemical industry and biotechnology (Zhang *et al*., 2005). KGM can be used as gelling agent, thickener, film former, emulsifier and coating material (Yu *et al.*, 2007; Zhang *et al*., 2005). Yang *et al*. (2009) used KGM as a material for encapsulating the bioactive compounds by spray drying process. They found that KGM showed a good emulsifying capability and protection for volatile aroma components of encapsulated sweet orange oil. Adamiec *et al*. (2012) also found that KGM has a good ability for coating antimicrobial Kaffir lime leaves oil extract during spray drying-microencapsulation. However, KGM has a very high apparent viscosity, so that there is a need to reduce its viscosity to appropriate level before encapsulation and spray drying.

Therefore, two main goals of this research were (1) to optimise enzymatic process for the modification of konjac glucomannan to be used as natural coating material and (2) to optimise suitable microencapsulation process of andrographolide using spray drying technique. The outcome of this research would have potential to be developed for use in the industry.

1.2 Objectives

1.2.1 To devise suitable enzymatic hydrolysis conditions for the reduction of viscosity of KGM solution in order to be used as wall material for microcapsules containing andrographolide

1.2.2 To evaluate appropriate operating condition for producing microcapsules containing andrographolide suitable for spray drying

1.3 Scope

The thesis research is divided into four main parts

1.3.1 Optimization of enzymatic hydrolysis of KGM solution

- Type of enzyme, incubation temperature, the enzyme concentration, speed of stirrer and KGM solution concentration

1.3.2 Selection a suitable coating material for andrographolide

1.3.3 Optimization of the encapsulation process for andrographolide via spray drying

- Outlet air temperature
- Inlet air temperature
- KGM concentration and inlet air temperature

1.3.4 Investigation of the mechanism of release of andrographolide from microcapsules during *in vitro* study

1.4 Hypothesis

Konjac glucomannan can be used as an appropriate natural coating material for microencapsulation of andrographolide using spray drying technique.

1.5 Expected outcomes

The production protocol of a konjac glucomannan solution with suitable viscosity to be used as a coating material for microencapsulation process of andrographolide using a spray dryer.

CHAPTER 2
LITERATURE REVIEW

2.1 Andrographolide

Andrographis paniculata Nees, also known as “Kalmegh” or “King of Bitters”, belongs to the Acanthaceae family. From the ancient times, it has been known as a treatment for diseases of gastro-intestinal tract, upper respiratory infections, herpes, fever and sore throat. The active compound, andrographolide extracted from *Andrographis paniculata*, is classified as diterpenoid lactone (Zhu, 1998). The chemical structure of andrographolide is shown in the Figure 2.1. The andrographolide is known as a bioactive compound. Diterpene lactones, with structure that is related to extract from *Andrographis paniculata*, comprise neo-andrographolide, deoxy-oxoandrographolide and deoxyandrographolide, dideoxy-andrographolide, andrographiside and its 14-doexy analogue, deoxymethoxy-andrographolide and deoxyandrographiside. Besides, some parts of the diterpene lactones and flavonones derivatives were also included with oroxylin and wogonin before it was detached from its origin of *Andrographis paniculata* (Zhu, 1998). In addition, andrographolide was recognized for its ability to prevent the proliferation of tumor in different types of human cancers (colon cancer cells). In immune cells, it prevented of the proliferation human peripheral blood lymphocytes (Kumar *et al*., 2011). As for the *in vitro* antibacterial activity of andrographolide, it has been found to be active against *Salmonella*, *Shigella*, *Escherichia coli*, and *Staphylococcus aureus* at the concentration of 25 mg/mL (Singha *et al*., 2003). This study also found the importance of the antibacterial activity in the aqueous phase from which

the andrographolide was extracted. Furthermore, the result was also compared to the rough aqueous extraction of leaves that exhibited important antimicrobial activity against the gram-positive *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, and gram-negative *Pseudomonas aeruginosa*. The ethanol extract of andrographolide was also found to have a crucial activity against the entero-hemorrhagic strains of *Escherichia coli* (Zaidan *et al.*, 2005).

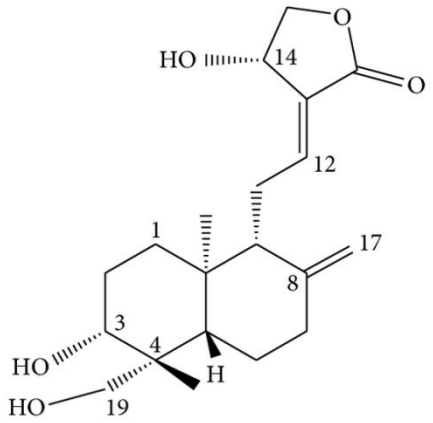

Figure 2.1 Molecular structure of andrographolide (Jayakumar *et al*., 2013).

2.2 Pharmacological effects of andrographolide

Andrographolide has been verified to be the best option to battle with diarrhea and other bacterial infection symptoms. Andrographolide, as mentioned in the section 2.1, has the ability to cure the diarrhea. It can be categorized in the same group as loperamide, which is known as an anti-diarrhea medicine that is used with labile *Escherichia coli* enterotoxin-induced diarrhea in animal models (Gupta *et al*., 1990). Chiou *et al*. (1998) found that andrographolide enhances the average daily feed intake of lactating sows and the little weight gain of piglets. The livestock industry in the 21st Century has been demanding supplementation of herb to feed animals. Consumers

nowadays tend to look after their health more and more. One of the concerns of the consumers is about the purchase of a product such as meat without antibiotic residues, or growth hormones in the piglets. The use of antibiotics in the feed can also affect the consumers and their health. This fact also relates to quality of the pork meat for export. Feeding studies have shown that feeding the andrographolide (1,000 ppm) to use that are to breast feeding their piglets from the birth up to a week before it is in the process of weaning can increase the weight of the piglet and the milk consumption by the pig during their breast feeding time in comparison to the ordinary food that is given to the pig. Dosage of andrographolide has been developed into different dosage forms. In herb injection dosage form, andrographolide is also commercially available for medical use as an antipyretic agent (Table 2.1). The andrographolide is rarely applied in pharmaceutical formulation. Meanwhile, several serious allergic or toxic effects to humans after injection of this formulation were reported (Zhu, 1998).

Table 2. 1 Dosage of andrographolide for models

Products name	**Dosage**	**Models**	**References**
Andrographolide	10 mg/kg	Human	(Calabrese *et al*., 2000)
Andrographolide	500 mg/kg	Mice	(Singha *et al*., 2003)
Andrographolide	25-75 µM	Platelets	(Lu *et al*., 2011)
Andrographolide	1,000 ppm	Piglet	(Detpiratmongkol *et al*., 2014)

2.3 Physicochemical properties and problems of andrographolide

Andrographolide is known as a substance composed of a α, β-unsaturated γ-lactone ring and connected to a decalin ring system via an unsaturated C_2 moiety. The molecular formula of andrographolide is $C_{20}H_{30}O_5$, with the molecular weight of 350.45. Andrographolide is a colorless, neutral and crystalline substance, recrystallized from ethanol or methanol as plate with the dimensions of about 0.3 × 0.4 × 0.3 mm. that the crystal formation of andrographolide is monoclinic. Andrographolide shows a major absorption band at 223 nm and has a melting point of 230-231 ^{o}C. The infrared spectrum of andrographolide led to the absorption at 1727 cm^{-1}, where it is concurred to α, β-unsaturated γ-lactone and 1672 cm^{-1} corresponding to conjugated C=C (Cava *et al*., 1962). O'Neil (2013) mentioned that andrographolide is soluble in water and acetone, methanol, chloroform and ether. The latest study on andrographolide was conducted to test the solubility in water of andrographolide, showing a value of approximately 60 mg/L in water at 25 ^{o}C. Throughout the study of andrographolide, it has been noted that it is a crucial component in *Andrographis paniculata* and has valuable therapeutic properties. However, the drawback is that it has poor solubility. This also led to the limitation of its bioavailability. The pharmacokinetic study consisting in injecting andrographolide *Andrographis paniculata* into a rat at a dose of 20 mg/kg of its bodyweight showed that andrographolide was fully absorbed into blood. However, the substance also led to a four-fold decrease when a 10-times higher dose was selected (Panossian *et al*., 2000) Moreover, Yu *et al*. (2007) conducted a kinetic study on andrographolide and found that the substance proved to be unstable in alkaline or very strong acidic pH. Meanwhile Wongkittipong *et al*. (2004) pointed that the degradation of

andrographolide is related to the initiating of the lactone ring by hydrolysis. The oral formulation of andrographolide are always characterized by the bitter taste of the substance. The application of andrographolide is regulated because of the poor stability of pharmaceutical dosage forms. Thus, there is a need for strategy to overcome the problem of andrographolide involved in the drug release behavior *in vitro*. Moreover, pure form of andrographolide is unstable to light and pH. Microencapsulation is one of methods that can help solving the problem of stabilizing andrographolide against influence of light and heat and increasing water solubility as well as making it into powder form which is convenient for use and storage.

2.4 The microencapsulation by spray drying process

The microencapsulation by spray drying process can be initiated by preparing the material and methods to condition the substance as a raw material to be used in the machine. Figure 2.2 shows the drying process that will help making the particles in order to maintain its chemical properties without losing its quality through exposure to heat (Gharsallaoui *et al*., 2007; Risch and Reineccius, 1998).

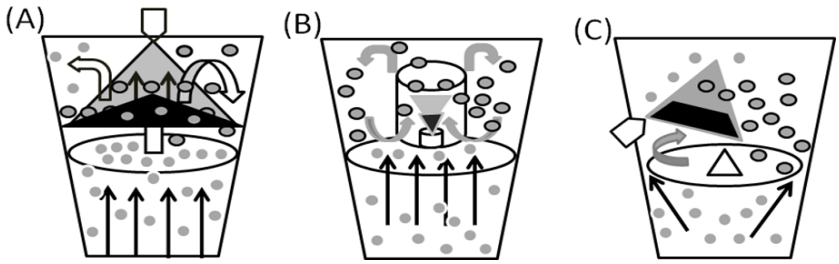

Figure 2.2 Schematics of a fluid-bed coater (A) Top spray; (B) Bottom spray; (C) Tangential spray Modified from (Jyothi *et al*., 2010).

2.5 Principle of the spray drying process

Gharsallaoui *et al*. (2007) and Masters (1991) explained the principle of the spray drying process machine in six main points;

2.5.1 The process of atomization

The droplet will be injected into the nozzle of the spray drying machine and turned into the spray. The process will create the transformation of the heat and the droplet of the particle to the appropriate level. The particle will have a larger surface area and will be available for the heat, and evaporate a larger amount of moisture. The appropriate condition of the spray drying process will depend on the natural causes and the stickiness of the droplet that is introduced into the machine. This is an important part for the process since the result will affect the final product. Furthermore, the process will be related to the amount of the substance injected into the dryer. The more substance is used, the larger the size of the particle. In addition, various types of nozzles can be used in the process.

The study of Gharsallaoui *et al*. (2007) used "Rotary atomizers", a nozzle that is designed to spray by using the inner core rotation like using the wheel. The droplet will be transferred into the core of the wheel and spinning out at the circumference with a very high speed. Once the droplet is out from the wheel and turning into the spray in the drying chamber, it will be controlled by the speed of the wheel. The particle diameter can vary from 30 – 120 micrometers.

2.5.2 Liquid-hot air contact

The droplet that has been sprayed will make a contact with the hot air that is coming through the drying chamber. It also leads to the contact between the heat and the particle that will result in the dehydration of the droplet. The contact between the heat and the droplet particle can be classified into three systems;

• Co-current flow dryer: the heat will run in the same direction with the sprayed droplet. The area that has the highest temperature will contact with the most hydrated area of the sprayed droplet. Once the contact is made, the temperatures will slowly decrease. This method is widely used with the products that need heat for rapid dehydration.

• Counter-current flow dryer: The hot air will travel opposite with the sprayed droplet. Hence, the temperature of the droplet particle, which started at low temperature, will be increased dramatically that will reach its peak at the highest temperature. The driest part of the particle will firstly contact the highest temperature. This method is suitable products that do not lose their quality at high temperature.

• Mixed flow dryer: This system is a mixture between the two above mentioned system, where the sprayed droplet and the hot air will move both ways.

2.5.3 Evaporation of droplet water

The evaporation of droplet water is related to the transferring of the heat and the mass transfer through the interface of the hot air and the sprayed droplet. Once the hot air gets in contact with the sprayed droplet, it will the transferring the heat into the droplet particle. Furthermore, the moisture inside the particle will be moved to the surface and evaporate. The moisture in the particle also moves towards the interface and is evaporated toward the surface of the sprayed droplet gets and

the evaporation rate of water decreases. The dehydration will also increase the thickness of the surface of the particles. Finally, an outer surface of the particle will be strengthened and shrink and become the shell of the inner particle.

2.5.4 Product recovery and air cleaning

The dry product that falls into the bottom of the drying chamber will be transferred by the hot air to the cyclone to separate the product from the hot air. Thus, the hot air will be moved into the upper part of the cyclone through the air filter, while the product will be reflected by the cyclone and finally moved to the collecting container.

2.5.5 Factors that affect the product's appearance

Masters (1991) stated that there were numerous factors that affected the product based on the spray drying process. These factors include;

- Power used for the drying process. Once the power is increased for the spray drying process, the pressure or the speed is increased leading to increased spinning of the wheel. This will increase the density of the spray.
- Characteristics of the starting droplet; if a droplet - which is formed by the spray dryer has a high stickiness, high density, or has a very low temperature before entering the dryer, it will result in a larger particle.
- Amount of the droplet intake. Increasing the amount of droplet intake will also create a larger particle and higher humidity of the product.
- Types of atomizer. The rotary atomizers will give a smaller and more uniform particle.
- Air flow. The air flow is another main factor affecting the residence time of the droplets in the drying chamber. If the air flow is low, the time that the

droplet will be in the drying chamber is likely to be longer. The droplet will have more time to make a contact with the heat and decrease the humidity of the product.

• The drying air temperature; the drying air temperature can also affect the final product. A higher drying air temperature leads to faster dehydration and shorter residence time and lower density. If the heat escapes from the outlet, the humidity will be a lower. The exit air temperature will lead to agglomeration of the dried product.

• Ratio between the solution and the solute substance. The preparation of the liquid sample before feeding into spray dryer is a crucial step that can affect the final product. Furthermore, the ratio of the component, the types of the substances and the concentration of the solution will also affect the capability of maintaining the core material. Coating material is known as a solution covering the main substance to be kept safely from the outer environment.

2.5.6 Wall material

Wall is known as a solution covering the main substances protected from the outer environment (see Table 2.2). Wall material also helps increasing the concentration of the liquid for the spray drying process and is able to modify its form depending on the temperature or the other process conditions. The main substances that are widely used to create microcapsules include belonging to polymer that came from carbohydrate. Consequently, the wall is necessary for the stability of the microcapsule.

Table 2. 2 Experimental conditions for microencapsulation of food ingredients by Spray drying process

Encapsulated ingredient	**Wall material**	**Air inlet and outlet temperature (ºC)**	**References**
L-Menthol	Gum Arabic Modified starch	180, 95-105	(Soottitantawat *et al.*, 2005)
Fish oil	Starch derivatives Glucose syrup	170, 70	(Drusch *et al*., 2007)
Short chain fatty acid	Maltodextrin	180, 90	(Teixeira *et al*., 1995)
Kaffir Lime Oil	Konjac glucomannan	180, 80	(Adamiec *et al*., 2012)

The selection of the wall material must be carefully considered. Gharsallaoui *et al*. (2007); Risch and Reineccius (1998) suggested that the best wall must be able to maintain the film setting up or creating a film that can cover the main substance. Hence, it must be flexible and strong, able to stick with the kept substances without making any reactions, maintain its low thickness when using on a high solution and not consuming the humidity once it is made into a hard product.

The wall also has to be of high stability and able to protect the main substances from outside environment including oxygen, sunlight, heat or any other chemical substances. It also has to be able to release the main substances without any smell or taste and be of a low cost. With these parameters, it is crucial to explore the methods that can affect the process of creating microcapsules through the spray drying process, which becomes the main factor of this study. Furthermore, the study also needs to be conducted based on the most appropriate concentration of

chemical that can be used to make a microcapsule along with other factors including the inlet air temperature, outlet air temperature and the ratio of the thickness of the wall to the main substances (Soottitantawat *et al*., 2005). Bioavailability of microencapsulated ingredients enabling the release of bioactive component at a specific site in the gastrointestinal tract after ingestion is shown in Figure 2.3.

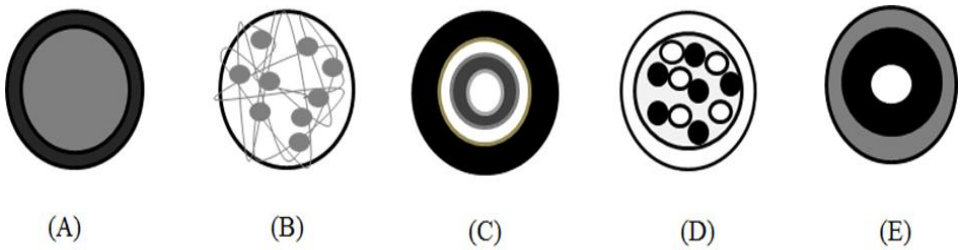

Figure 2.3 Morphologies of microcapsules single core and shell capsule (A); dispersed core in polymer gel (B); multilayer capsules (C); dual core capsule (D) and single core multi shell capsule (E)
Modified from Augustin and Hemar (2009)

2.5.6.1 Entrapment in polymer

One of the most well-known biopolymers used for encapsulation matrix is sodium alginate, which has a characteristic of forming hydrogels upon cross-linking. Alginate polymer is frequently selected for the controlled release in drug formulating process. The hydration of an alginate matrix can also bring a gelatinous layer, where it can be maintained as a drug diffusion barrier. Hence, the water-insoluble drug can also be released through the erosion of the outer gel layer where it leads to the well-hydrated process. Liew *et al*. (2006) explained that within the hydrated surface layer of the matrix, the core itself will be maintained as dry and become a non-releasing station of the drug and polymer.

Shariff *et al*. (2007) conducted a test on andrographolide entrapment into cross-linked alginate through the isotropic gelation of sodium alginate with calcium ions. The result proved that the micropellets of andrographolide - with the sodium alginate- led to a less bitter taste. But the study also showed that only 15% of andrographolide in micropellets were released during 4 hours at pH 1.2 and pH 4.0 buffer solutions in the whole study.

This showed that sodium alginate as wall material for andrographolide was unsuccessful to the solubility of the drug under the experiment condition chosen by the researchers.

2.5.6.2 Structural features of cyclodextrins

Cyclodextrin (CD) or cycloamylose are manufactured by degradation of starch to the transferase of cyclodextrin glucosyl. The degradation of starch is also included with an intramolecular reaction to create a circular ring structure. The CD can be described in various types including α-, β- and γ-CD, which hold units of glucose of 6, 7 and 8 accordingly. The molecular structure and the characteristics of CD are shown in Figure 2.4 and Table 2.3. The secondary hydroxyl groups of CD are listed on one of the two edges of the ring, while main hydroxyls will be placed on the other, a C_1 to C_4 bond of glucopyranose units. The non-bonding electron pairs of the glycosidic oxygen bridges will be led to the inside of the cavity, in which they bring a high electron density environment that leads to positive Lewis-base features. The C-2 hydroxyl group, which is selected as of one glucopyranoside units, will create a hydrogen bond with the C-3-OH group of the adjacent glucopyranoside unit. In the cyclodextrin molecule, a comprehensive secondary belt will be formed through the hydrogen bonds, in which it will lead to a

rigid structure. The secondary belt, as mentioned earlier, can lead to the structure of hydrogen bonding, where it can be explained by its lowest solubility of β-cyclodextrin as compared to other three cyclodextrins. In α-CD molecule, the separated position of one of the glucopyranose units has an incomplete hydrogen-bond belt. It also is more soluble than the other two cyclodextrins. To identify the structure of CD, it can be noted that the CD are toroidally shaped molecues that lead to the rigid cyclic oligosaccharides combining to the D-glucopyranose units, where it is connected by α-(1, 4) glycosidic bonds.

Table 2. 3 Characteristics of the native cyclodextrins β CD or γ CD

Cyclodextrin	**β CD**	**γ CD**
Molecular weight	1135	1297
Number of glucose units	7	8
Internal diameter (nm)	0.78	0.95
Outer diameter (nm)	1.53	1.69
Height (nm)	0.78	0.78

Source: Brewster and Loftsson (2007)

2.5.6.3 Mechanism of complexion between CD and insoluble compound

The inclusion complexion is the combination with two or more molecules; one molecule is defined as a “host”, where the other is defined as a “guest”. Both molecules are also linked totally or partly through physical forces without a participation of the covalent bonding. The interior cavity of CD, that is created to be hydrophobic, can allow the inclusion of non-polar molecules to build up the complexes. Meanwhile, this will leave the CD to render the hydrophilic exterior that leads to the solubility in water (Dinh *et al.*, 1999). Frömming and Szejtli (1993) explained that the process also depended on factors including the cavity diameters of the CD, the guest molecules that came with an adequate size, the shape and polarity that is selected to create an inclusion complex. Apart from the size, the substituents of the CD can lead to the creation of methyl hydroxyethyl, hydroxyproply group, which could impact to the complexation since there is a high percentage that might block the opening of CD (Mura et al., 2002). Furthermore, the inclusion of a guest into a cavity of CD is proven to be a replacement of the input water molecules by the less polar guest, while the complexion process becomes energetic so as to interact with other nonpolar guest molecules, in which it has an improperly solvated hydrophobic cavity (Frömming and Szejtli, 1993). The process of complexion is shown in Figure 2.4. Meanwhile, the water molecules in CD cavity are proved to be unable to fill the tetrahedral hydrogen to bond into the capacity. They can be noted as molecules of enhanced energy or enthalpy. During the process of complexion, the conditions become energetically unfavorable to the polar-apolar interactions during the combination of the included water molecule and the CD cavity, The CD-ring strain is

releasing during complexion and van der Waals interaction becomes a formation by the combination of the guest molecule and the CD. Frömming and Szejtli (1993) concluded that the guest, at one point, has a formation of hydrogen that is bonding between the guest and the host.

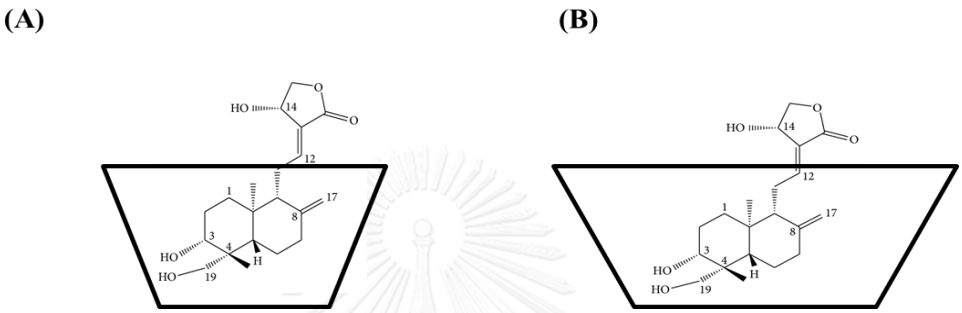

Figure 2.4 Molecular structure of β CD + andrographolide (1:1) (A) and γ CD + andrographolide (1:1) (B) Modified from Zhou *et al.* (2009)

2.6 Konjac glucomannan

Konjac glucomannan (KGM) is a natural polysaccharide isolated from the tubers of *Amorphophallus* spp., plants found in Asian countries such as China, Taiwan, Japan, and Thailand (Tatirat and Charoenrein, 2011). KGM is widely used in the food, pharmaceutical, chemical and biotechnology industries (Zhang *et al.*, 2005). KGM consists of β-(1, 4)-linked D-glucose and D-mannose units with a 1.0 : 1.6 molar ratio of glucose: mannose and a low degree of acetyl groups at the C-6 position (Figure 2.5). The molecular weight of the polymer is 10^5-10^6 g/mol (Cescutti *et al.*, 2002). In addition, KGM has desirable properties of good film-formation, a high water-solubility, edibility, and biodegradation (Tobin *et al.*, 2012). It has a tendency to create a fine dense network upon drying (Zhang *et al.*, 2005). In addition, the

microencapsulation using KGM as the coating material has been reported to be able to protect volatile compounds and increased the encapsulation yield (Yang *et al.*, 2009), as well as antimicrobial compounds (Adamiec *et al*., 2012). As KGM is viewed as a suitable material for the encapsulation of bioactive compounds using spray drying process for its above mentioned characteristics, the use of KGM is limited by the fact that it has a very high apparent viscosity when dissolved in water even at low concentration (more than 2% (w/w)). Therefore, its viscosity needs to be reduced to an appropriate level (< 100 mPa·s) before using it in the spray drying and encapsulation process. There is a study on using, β-mannanase to hydrolyse the (1, 4)-β-D-mannopyranosyl linkages of glucomannans. The β-mannanases that were obtained from *Bacillus* sp. under the optimal conditions were capable to hydrolyse KGM at 50 ºC, pH 5.5 for 24 hours, hydrolyse more than 90% of polysaccharides into oligosaccharides and some monosaccharides (He *et al*., 2001). Glucomanno-oligosaccharides, produced from the mannanase hydrolysis of KGM, were found to be a prebiotic affecting the growth of probiotics such as *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Lactobacillus acidophilus*. They became better substrates than prebiotic (oligo-fructose) for the growth of lactic acid bacteria (Al-Ghazzewi and Tester, 2012). However, there is no research on the optimization of KGM concentration that would be used in mannanase enzymatic degradation for lowering viscosity of KGM solution suitable for microencapsulation using spray drying.

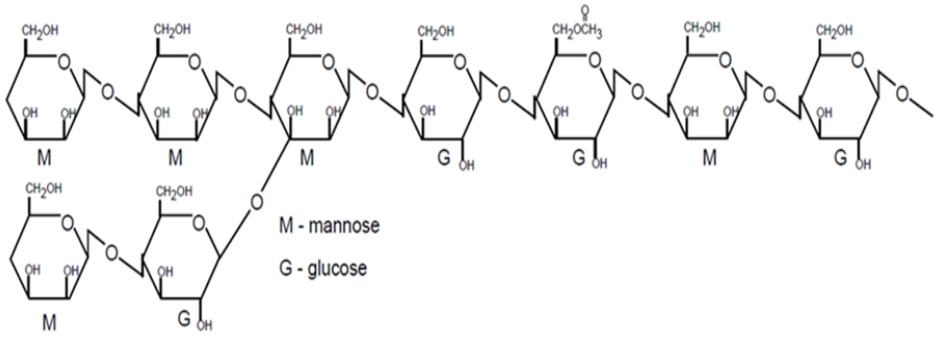

Figure 2.5 Molecular structure of konjac glucomannan (Yui *et al*., 1992).

2.6.1 Characteristic of konjac powder

Konjac powder itself has different characteristic from other powders that are made from plants. Konjac powder is a polymer that is soluble in water. However, a special technique is to solubilize the konjac powder. A crucial factor in the process of solubilization the konjac powder in the fact that stirred all the time until the konjac powder is completely dissolved. Otherwise, if the konjac powder is not stirred all the time, it will become solid. Furthermore, the konjac powder solution also gives different characteristics than other polysaccharides:

- The stickiness and the expansion of the konjac powder increase the water absorption.
- When it is used in the solution, the particle will absorb and expand into high viscosity solution. It can be expanded 27 – 40 times of its usual size.
- The higher the viscosity of the KGM powder, the higher the expansion rate will be. The ability to absorb water by KGM powder will depend on the time and temperature (Cescutti *et al*., 2002).

- If the temperature is rising, it will also directly affect the absorbtion process and causes the viscosity of the solution to be rapidly changed. The study of Tye (1991) shows that at the temperature of 50 ^{o}C, the glucomannan can expand itself in the best condition. Moreover, the increase of the viscosity in glucomannan also affects its ability to expand. It can be argued that the viscosity of konjac powder will depend on the composition of konjac powder as a raw material and the manufacturing process along with the extraction of the konjac powder. In addition, the creation of a gel from the konjac powder is an irreversible process and can be conducted under alkaline condition. The alkaline solution will create a deactivation reaction, where there will react with the acetyl group in glucomannan release out. As a result, it will be a gel formation network by the hydrogen linkage. Moreover, the konjac gel is also stabilized under the alkaline condition. From the study, it is noted that the least concentration of the konjac powder solution that can transfer into a gel formation is 0.5%. The alkaline solution substances that are widely chosen include potassium carbonate, calcium carbonate and calcium hydroxide. Furthermore, Tye (1991) explained that the konjac powder can also be used with gum or other powders. For example, when it is used with kappa carrageenan, the gel itself can become thermo-reversible. Using the konjac powder with other substances also leads to new types of gel that can be used in various applications, e.g., the creation of stabilizer gel.

2.6.2 The use of konjac glucomannan powder

Since glucomannan is considered to be unable to be digested by human digestive system, it is known that as a food that does not give any energy. People who consume glucomannan will be less hungry. Therefore, glucomannan is widely known as a health supplement. The food chemical codex of the United States of America gave an approval for using glucomannan as an ingredient in the food industry. It is also frequently used as a gel, emulsifier, and film making. KGM is used in the food industry to increase edible fiber content and improve the mouth feel for the consumers. It is also used in the drinks to increase the bulk or in the livestock as a the moisture enhancer. The gel from the KGM will tenderize the meat. Tian *et al*. (1999) conducted a research on the KGM powder by modifying the konjac into KGM ester to maintain the characteristic of emulsifier and use as the emulsifier substance in oil-in-water emulsion. Yang *et al*. (2009) conducted an experiment by using KGM powder for coating some of the fruits including cantaloupe and apple. The result showed that the coating can help maintaining the flow of oxygen and other gases including carbon dioxide, oxygen and steam, which control the change and ripening process of the products. Zhang *et al*. (2005) explained that the KGM powder performed well in coating, where it gave a solid and flexible coating to the product. Once it is used with polyacrylamide, gelatin, chitosan and sodium carboxymethyl cellulose, it can transform into a transparent coating. Furthermore, there are reports about using the KGM powder for the encapsulation process. Nussinovitch and Solomon (1998) stated that glucomannan from the konjac is considered to be one of the hydrocolloid substances that can be used in the microencapsulation as a coating material or containing a liquid substance including enzymes, biological agents,

pharmaceutical agents and immunological agent. The coating process can lead to the maintaining of the liquid without breaking up, even when it is in the condition of -20 to 90 degree Celsius. Yang *et al*. (2009) conducted an experiment by manufacturing microcapsule that contain the sweet orange oil by using the coating materials including gum Arabic, maltodextrin, modified starch, sodium octenyl succinate and KGM powder. Hence, the experiment also used tween 80 as emulsifier that led to the homogenized process. Then, the emulsion that came from the drying process with the inlet air temperature of 160 ^{o}C was selected. The results show that the microcapsules of the sweet orange oil that were using gum Arabic, KGM and sodium octenyl succinate had a higher encapsulation yield, maintained the flavor better than other substances. It also gave higher encapsulation yield than using the maltodextrin.

KGM as a material for encapsulation of bioactive compounds however, has a very high apparent viscosity. The viscosity need to be reduced to appropriate level before encapsulation and spray drying. In process of modification of rheological properties of KGM by enzymatic hydrolysis to reduce the viscosity of KGM solution, Yang *et al*. (2009) applied cellulase to hydrolyse KGM at 50 ^{o}C and found that the viscosity can be reduced to 200 mPa·s. He *et al*. (2001) reported that β-mannanases could hydrolyse 1,4-β-D-mannopyranosyl linkages of glucomannans. The conditions required for β-mannanases from *Bacillus* sp. to hydrolyse glucomannan in konjac flour were 50 ^{o}C, pH 5.5 for 24 h. Under these conditions, they found that more than 90% of polysaccharides were hydrolysed into oligosaccharides and a little monosaccharide was found. Glucomanno-oligosaccharides produced from mannanase hydrolysate affect the growth of probiotics such as *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Lactobacillus acidophilus* KGM derived

oligosaccharides better substrates than oligofructose for the growth of lactic acid bacteria (Al-Ghazzewi and Tester, 2012). All enzymes selected to study the hydrolysis activity of KGM were for the carbohydrate type of substrate. Therefore, the target of each enzyme was to break 1, 4 glycosidic linkage that is also found in the KGM structure and the conditions of hydrolysis by cellulase, pectinase and mannanase. Each enzyme has a specific ability to hydrolyse KGM solution.

Enzyme modification techniques can be used for modification of viscosity of KGM solution. The technique was described to decrease the viscosity of polysaccharide solution by enzyme hydrolysis like using mannanase in the processing of plant fiber source. Enzymatic hydrolysis leads to produce the oligosaccharides in KGM solution that are raw materials in food industry (Table 2.4) (Al-Ghazzewi and Tester, 2012; Chen *et al*., 2013).

Table 2. 4 Characteristics of the enzyme hydrolyses of substrate solution

Enzyme	Target of enzyme	References
Mannanase	1,4-β- linked mannose-residues	(Al-Ghazzewi and Tester, 2012)
Pectinase	1,4-α-D-galactosiduronic	(Rexová-Benková *et al*., 1983)
Cellulase	1,4-β- linked glucose-residues	(Al-Ghazzewi and Tester, 2012)

All enzymes that were selected to study the hydrolysis of konjac glucomannan are acting on the carbohydrate type of substrate. Therefore, the target of each enzyme is to break 1, 4 glycosidic linkage that is also found in the konjac glucomannan structure and the condition of hydrolysis by cellulase, pectinase and mannanase. Each enzyme showsas a specific activity in hydrolysis of KGM solution. In order to achieve the goals of this research the optimization of enzymatic process for modification of

KGM is to be applied KGM is a natural coating material and a suitable microencapsulation process of andrographolide spray drying technique is to be devised.

2.7 The digestive system and control release of the pig

Kidder and Manners (1978); Low and Partridge (1980) had descried a pig's digestive system as follows. Like most of the animal, a pig's digestive system functiones like those of other monogastric mammals. However, as the time goes by, the changes of size of digestive system in pigs are affected by its habitual dietary routine. A pig has always been known as an omnivorous creature, but it is also an animal that is consuming plant material which largely effect on its die. A pig has also an enormous capacity in its digestive system for consuming enzyme degradable carbohydrates, particularly on its upper part of the gastrointestinal tract. Hence, the ecosystem affects its enormous intestine, which discreetly ferments and the fibrous material.

2.7.1 Gastric secretion

Hydrochloric acid (HCl) is seen as a secreted solution in the parietal cells, which most of the time have been concealing inactive zymogens to prevent the self-digestion of the cells. The zymogens, which have been recognized as a group of proteins, are mostly engaged in the lumen at an acidic pH below 5 or by active pepsin A. When a pig reaches its age, the digestive system will produce pepsin A, which is predominately known as gastric protease, followed by gastricsin. Furthermore, it also

has a solid proteolytic activity at pH 2.5–3 (Kidder and Manners, 1978; Low and Partridge, 1980).

2.7.2 Bile secretion

Bile is known as fluid with high pH (6.8 – 7.0). It contains salts, phospholipids and cholesterol, lead to the containment of lipid at 0.6 – 0.7%. Furthermore, the liquid also comprises sodium, potassium, chloride, bicarbonate, mucus and bile pigments. However, the bile pigments are known as a waste product in the fluid. Bile salts and phospholipids are known as major substances for the digestive system of the pig, whereas the result of molar ratio of total phospholipid to total bile salts is 1:10.1. This can be expain how the bile salt can be emulsified and absorbed the lipid (Kidder and Manners, 1978; Low and Partridge, 1980).

2.7.3 Small intestinal digestion and absorption

The luminal stage of carbohydrate digestion will mostly apply to the starches. The enzyme that could impact on the digestive system α-amylase, which is secreted by the pancreas. Furthermore, starch hydrolysis products including maltose, isomaltose, maltotriose, α-limit dextrins and dietary disaccharides (sucrose and lactose) are consumed during the membranous stage, where the enzymes will be digested through the structural part of the intestinal surface membrane.

Before every absorption process, the polysaccharides will be hydrolyzed to monosaccharide, while the oligosaccharides will show their ability to distribute along the small intestine. The activity of lactase and trehalase also reaches its peak through the proximal part of the intestine (at 10-20% of the intestinal length). Hence, the activity of the maltases also reaches their peak through the mid-way along with the

small intestine, while the monosaccharaides will be absorbed with the functional transport of enabled diffusion (Kidder and Manners, 1978; Low and Partridge, 1980).

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

Konjac glucomannan (food grade) (KGM) was obtained from the Yunnan Genyun Konjac Resource Corp., Kunming (Yunnan, PR China).

Andrographolide 98% extracted from stem leave of *Andrographis paniculata* P.E. from Zhijiang Minglou, Hangzhou Economic Technological Development Area, Zhejiang, Hanghou Joymore Technology Co., Ltd. (China)

3.1.2 Chemicals and reagents

Table 3. 1 Chemicals for enzyme hydrolysis process

Name	Optimal activity	Company	Country
Celluclast® 1.5 L (Food grade)	(pH 5.0, 50 ºC)	Novozymes	Denmark
Pectinex Ultra SP-L (Food grade)	(pH 5.0-7.0, 50-60 ºC)	Novozymes	Denmark
Mannanase (Food grade)	(pH 5.0-7.0, 37-60 ºC)	Bosar Biotechnology	China

Table 3. 2 Chemicals for sugar determination

Name	Company	Country
A sugar syrup with specified degree of polymerization (DP) Glucose (monosaccharide; DP1) (HPLC grade)	Merck	Germany
Mannose (monosaccharide; DP1) (HPLC grade)	Sigma-Aldrich	St Louis, USA
Maltose (disaccharide; DP2) (HPLC grade)	Sigma-Aldrich	St Louis, USA
Maltotriose (trisaccharide; DP3) (HPLC grade)	Sigma-Aldrich	St Louis, USA
Haltotetraose (tetrasaccharide DP4) (HPLC grade)	Hayashibra biochemica	Japan
Maltopentaose (pentasaccharide DP5) (HPLC grade)	Wako chemical	Japan
Maltohexaose (hexasaccharide DP6) (HPLC grade)	Hayashibra biochemica	Japan
Maltotheptaose (heptasaccharide DP7) (HPLC grade)	Hayashibra biochemica	Japan
Acetonitrile (HPLC grade)	Merck	Germany

Table 3. 3 Chemicals for spray drying process of andrographolide microcapsule

Name	Company	Country
Andrographolide (99%) (HPLC grade)	Sigma-Aldrich	USA
Acetonitrile (HPLC grade)	Merck	Germany
Hydrochloric acid (A.R. grade)	Sigma-Aldrich	USA
Phosphate buffer (A.R. grade)	Sigma-Aldrich	USA

Table 3. 4 Apparatus

Instruments	**Model**	**Company, Country**
Spray dryer	SD-01, Food Processing	Thailand
pH meter	Cyberscan 1000	Eutech, Singapore
Centrifuge	Universal 32R	DJB Labcare, Germany
High Performance Liquid Chromatography (HPLC)	Alltech Associates, Inc. 2051	USA
Columns	Prevail^{TM} carbohydrate ES 250	USA
Rheometer	BOHLIN: C-VOR	England
HPLC	Shimadzu, LC-10A	Japan
Column Ultra	C18 microns 250 m × 4.6 mm	USA
FT-IR spectroscopy	Spectrum One	Perkin-Elmer, USA
Water activity meter	-	Aqua Lab, USA
Magnetic stirrer	Steroma G	Steroglass, Italy
SEM	JSM-7610F	JEOL, Japan
Micrometer	Dial Thickness Gauge 7301	Mitutoyo, Japan
Hot air oven	FD 240	Binder, Germany

3.2 Methods

3.2.1 Optimization of enzymatic hydrolysis of KGM solution

For the study of enzyme type, the 0.05% (w/w) of each enzyme was prepared in distilled water. After that, 9% (w/w) of KGM powder was added and the hydrolysis reaction was performed as mentioned.

For the study of incubation temperature, the selected type of enzyme with suitable from pervious section was prepared. After that, 9% (w/w) of KGM powder was added and the hydrolysis reaction was performed as mentioned above at determined incubation temperatures (40, 50 and 60 ºC).

For the study of enzyme concentration, the selected incubation temperature from pervious section was prepared at desired concentration (375, 750 and 1500 unit per 9 g of KGM. After that, 9% (w/w) of KGM powder was added and the hydrolysis reaction was performed as mentioned.

For the study of speed of stirrer, the selected enzyme with suitable concentration from pervious sections was prepared. After that, 9% (w/w) of KGM powder was added and the hydrolysis reaction was performed as mentioned above at suitable incubation temperatures and stirring speed (0, 100 and 200 rpm).

For the study of KGM solution concentration, the selected speed of stirrer condition from pervious sections was set up. After that, KGM solution at tested concentrations (9%, 12%, 15% and 18% (w/w)) was added and the hydrolysis reaction was performed as mentioned above at optimal incubation temperature.

The suitability of each factor for KGM hydrolysis was selected based on the viscosity reduction of KGMH to less than 100 mPa·s. The percentage of

oligosaccharides was determined by using HPLC and standard curve of each sugar (Appendix A1)

3.2.2 Selection a suitable coating material for andrographolide

The combination of KGMH with either β or γ cyclodextrin (CD) was studied as a coating material for andrographolide. The KGMH was prepared using the optimized enzymatic hydrolysis condition from previous section. Total solid concentration of the coating material combination (12% w/w) KGMH, 10% (w/w) KGMH + 2% (w/w) γ-CD, 10% (w/w) KGMH + 2% (w/w) β-CD) was fixed at 12% (w/w). The 2% (w/w) andrographolide was selected as a core material. The coating material and andrographolide were mixed and left at room temperature for 45 min prior to spray drying. After that, it was fed the spray dryer using a co-current system and rotary atomizer (SD-01, Food Processing Co., Thailand). (Figure 3.1) to perform the encapsulation process. The inlet and outlet air temperatures were set at 170 ºC and 85 ºC, respectively. The encapsulated andrographolide obtained in this process was collected and its physical and chemical properties were analyzed as described below. The encapsulation efficiency (%) and product yield (%) were calculated as shown in equation 1:

$$\text{Encapsulation efficiency (\%)} = \frac{A2}{A1} \times 100 \qquad (1)$$

Where:

A2 is Amount of andrographolide in microcapsules (g) (Appendix A.2)

A1 is Amount of andrographolide before spray drying process (8 g)

Product yield (%) was calculated as shown in equation 2:

$$\text{Product yield (\% w/w)} = \frac{\text{Weight of microcapsules in dry basis (g)}}{\text{Weight of total solid (g)}} \times 100 \quad (2)$$

Where:

Total of solid are konjac glucomannan (g) + mananase (g) + andrographolide (g)

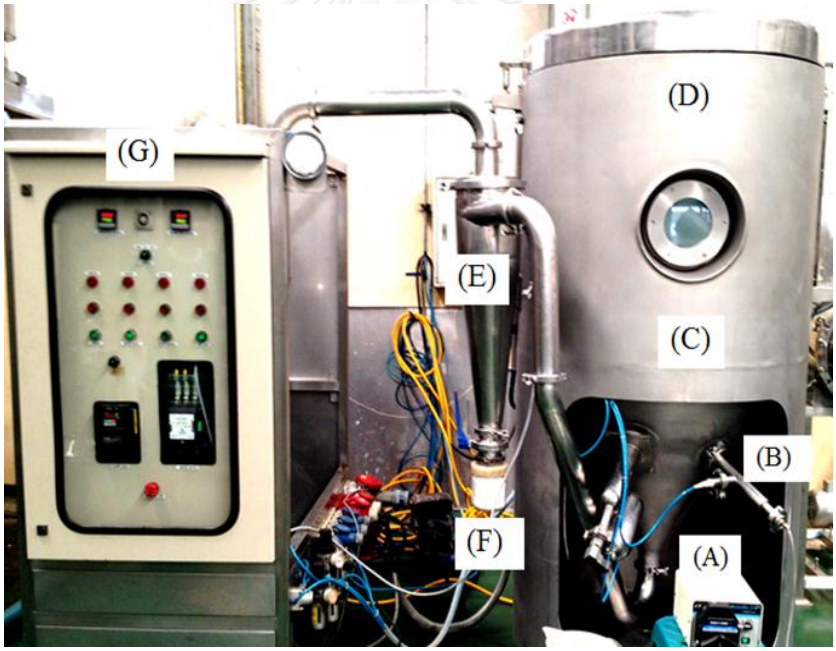

Figure 3.1 Spray dryer used in the experiment; Peristaltic pump (A), Feed tank (B) Drying chamber (C), Atomizer (D), Cyclone (E). Powder collection (F) and Control panel (G)

The flow-chart of the experiments is show in Figure 3.2

3.2.3 Optimization of the encapsulation process for andrographolide via spray drying

The effect of outlet, inlet air temperature and KGMH concentration during spray drying on andrographolide encapsulation efficiency were studied. The KGMH was prepared using the optimized enzymatic hydrolysis condition from previous section. The mixture of 12% (w/w) KGMH and 2% (w/w) andrographolide were applied. A co-current spray dryer (SD-01, Food Processing Co., Thailand) was used to perform the encapsulation process as describe above. The outlet air temperature varied between 75±3 and 85±3 ºC, whereas the inlet air temperature was set to 150, 170 or 190 ºC. KGMH concentration was chosen to be 9, 12, 15, 18 and 21% (w/w). The obtained encapsulated andrographolide was collected and its physical and chemical properties were analyzed as described below. The encapsulation efficiency and product yield were calculated.

3.2.4 Investigation of the releasing behavior of andrographolide from microcapsules during *in vitro* study

Release of andrographolide from the microcapsules was carried out *in vitro* by imitating piglet's digestive system by using 0.05 M hydrochloric acid (HCl) (pH 2.5), phosphate buffer (pH 6.8) and distilled water (pH 6.2). The microcapsules (1.0 g) were soaked at each pH at 30, 60 and 120 min, respectively. Then each sample was centrifuged at the speed 7500 × G for 15 min. The supernatant was collected, filtered through 0.2 μm nylon membrane filter prior to inject into HPLC to analyse concentration of released andrographolide. For the sediment from

the centrifuge, it was dissolved in methanol (10 mL) for 15 min and the remaining andrographolide was analysed by HPLC (see in Appendix A.3).

3.2.5 Analysis

3.2.5.1 Analysis of KGMH properties

The viscosity of KGMH was determined using a rheometer (BoHLIN: C-VOR, England). The cone-plate geometry (40 mm diameter, 4 º cone angle, 150 µm gap) was used with a fixed shear rate of 100 s^{-1} at temperature 21 ºC. The mono-, di-, tri- and oligosaccharides in KGMH were quantified using high performance liguid chromatography (HPLC: Alltech Associates, Inc., USA) (see Appendix A.1).

3.2.5.2 Analysis of encapsulated andrographolide

The obtained encapsulated andrographolide was analyzed using HPLC (see Appendix A2). Its moisture content was measured according to AOAC 2000. The water activity, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR) were analyzed using appropriate scientific equipment following the manufacturers' instructions (see Appendix A.4.1-A.4.4 respectively)

3.2.5.3 Statistical analysis

All data were acquired in 3 replications. Data was shown as mean values with standard deviation. Differences between mean values were established using Duncan's Multiple Range Test with a level of significance of $p \leq 0.05$ (SPSS 17.0 software; IBM SPSS, Chicago, IL, USA).

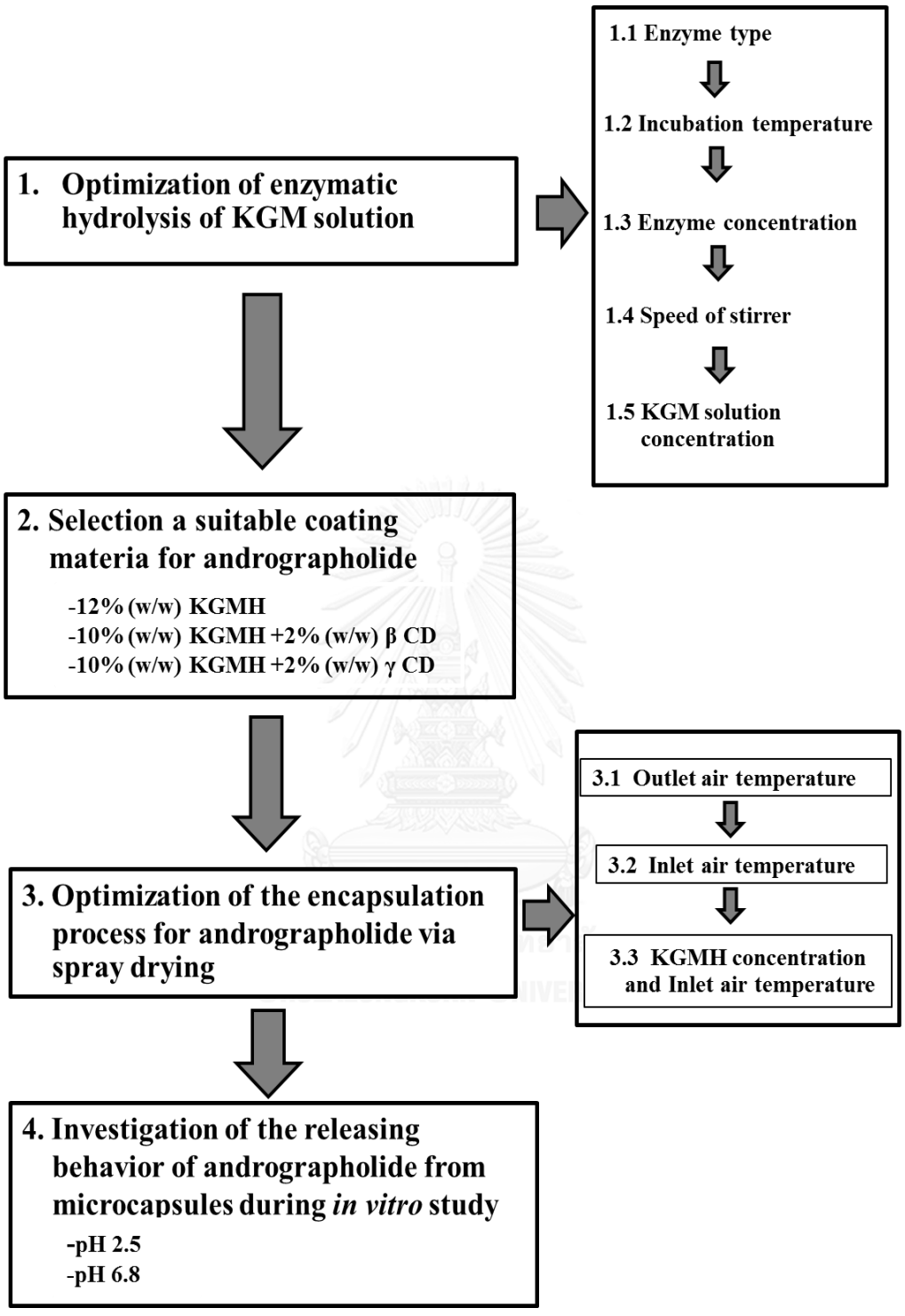

Figure 3.2 Experimental flow chart of the study

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Optimization of enzymatic hydrolysis of KGM solution

4.1.1 Type of enzyme

Changes in the viscosity of 9% (w/w) KGM solution as it was hydrolysed by enzymes, cellulase, mannanase, pectinase as well as combined enzymes (pectinase and mannanase) are shown in Figure 4.1. It can be observed that there is a steady decrease in viscosity of these solutions at 50 ºC, 200 rpm after 6 hours. The decrease in the viscosity was particularly obvious in a solution of enzyme mannanase followed by combined enzyme (pectinase and mannanase), pectinase and cellulase (9.04±0.02, 164.64±3.97, 272.67±4.38 and 454.15±3.42 mPa·s) respectively. Li *et al*. (2004) explained that mannanase can hydrolyse KGM better than cellulase because mannanase's target is β-mannosidic linkage and β-mannosyl-glucosidic linkage. The enzyme can break the KGM structure better than the other enzymes. He *et al*. (2001) reported that enzyme mannanase activity is provided by endo-mannosidase that could hydrolyse 1, 4-β-D-mannopyranosyl linkages within the main chain of glucomannan to produce oligosaccharides and a little chain of monosaccharide. The viscosity limit of solution for feeding to spray drying process is around 100 mPa·s (Iida *et al*., 2008). Therefore, mannanase was selected as it is based on viscosity reduction of KGM solution that was reduced to less than 100 mPa·s within 8 hours.

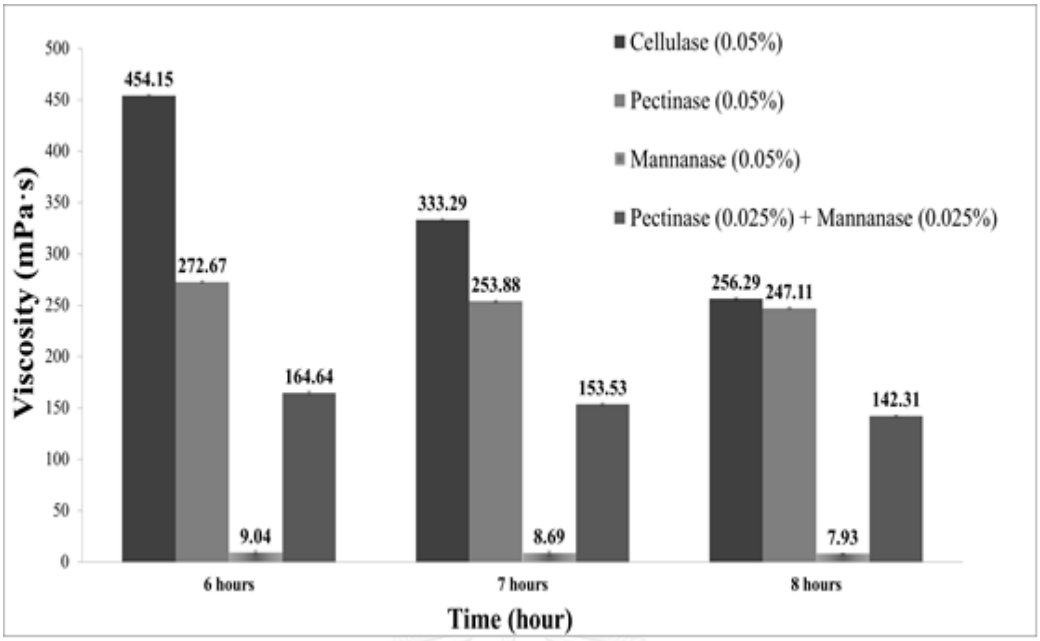

Figure 4.1 Effect of enzyme type on viscosity of 9% (w/w) konjac glucomannan hydrolysate (KGMH) after hydrolysis for 6, 7 and 8 hours

4.1.2 Incubation temperature

Figure 4.2 shows the effects of incubation temperature on viscosity of 9% (w/w) KGM solution. It appears clearly that the difference in viscosity is small when hydrolysis temperature is changed. The viscosity of hydrolysed solution decreased in the following order; 60 ^{o}C > 50 ^{o}C > 40 ^{o}C. The viscosity of the KGM solution after 1 hour was 179.42±0.07, 240.89±0.63 and 1251.42±0.54 mPa·s at 60 ^{o}C, 50 ^{o}C and 40 ^{o}C, respectively.

After hydrolysis for 4 hours, the viscosity of KGM solution decreased to 40.00±0.66, 36.58±0.10 and 32.24±0.25 mPa·s, at 60 ^{o}C, 50 ^{o}C and 40 ^{o}C respectively, that temperature affected the catalytic activity. If the temperature increases, the enzyme catalyzes the binding substrate better, but temperatures higher than 90 °C, will cause a reduction in reaction due to protein denaturation and affect

the structure of the enzyme, especially at the active site of the enzyme binding to the substrate. Thus, appropriate reaction temperature for this reaction is 40 ^{o}C for 4 hours. Moreover, after 4 hours, the viscosity of KGM solution showed no difference at different temperatures, Therefore mannanase 1500 unit at 40 ^{o}C was selected on the basis reduction of viscosity to less than 100 mPa·s within 4 hours.

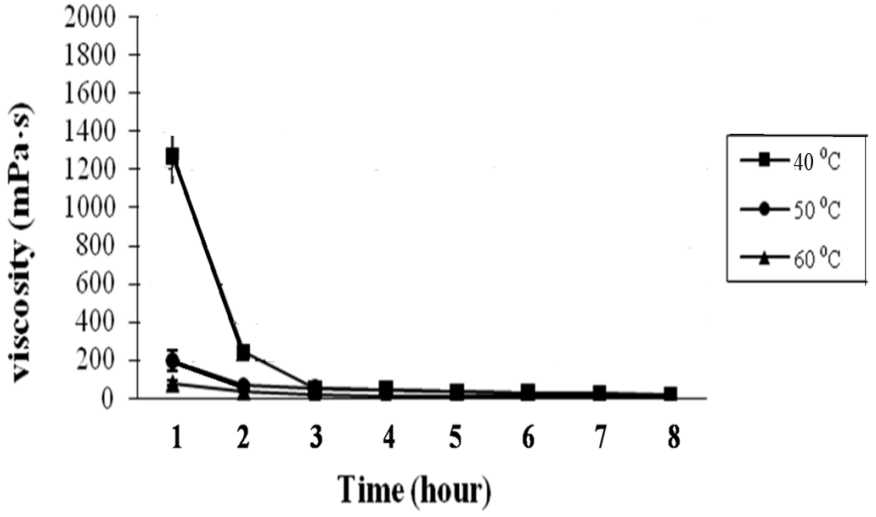

Figure 4.2 Efect of incubation temperature 40 ^{o}C (■), 50 ^{o}C (●) and 60 ^{o}C (▲) on viscosity of 9% (w/w) KGMH after mannanase hydrolysis for 8 hours with a stirring speed of 200 rpm

4.1.3 Enzyme concentration

From the previous experiment, mannanase was proven to be a suitable enzyme to use for hydrolysis of KGM solution. In this step, effects of enzyme mannanase concentration on the viscosity of 9% KGM solution were studied and the results are shown in Figure 4.3. It appears that 1500 unit mannanase can lower viscosity of KGM solution better than a concentration of 750 units and 375 units. The reaction rate is directly proportional to concentration of the enzyme mannanase.

It appears that 750 units and 375 units could not reduce the viscosity of KGM solution to less than 100 mPa·s within 4 hours. This may due to the fact that the amount of enzyme was not sufficient for hydrolysis.

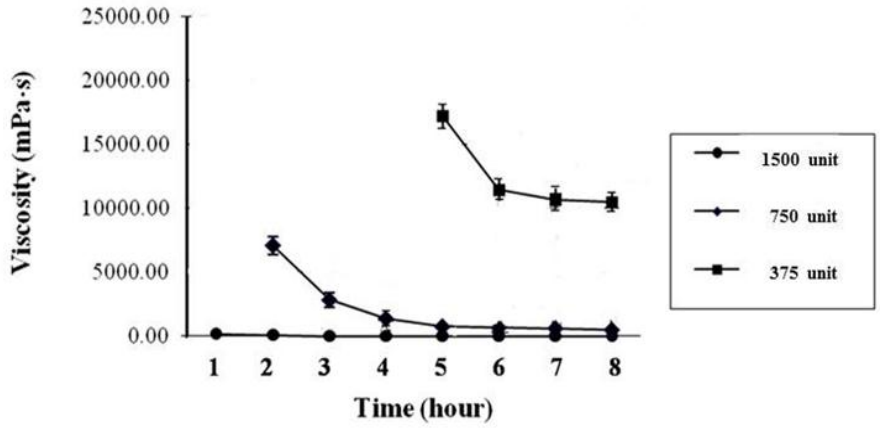

Figure 4.3 Effect of mannanase concentration at 375 (■), 750 (♦) and 1500 (●) unit on viscosity of 9% (w/w) KGMH after mannanase hydrolysis for 8 hours at 40 ^{o}C with a stirring speed of 200 rpm

4.1.4 Speed of stirrer

Different stirring speed has little impact on the viscosity of 9% (w/w) KGM solution during enzyme hydrolysis. After shaking at 200 rpm for 1 hour, see Figure 4.4, the viscosity of KGM solution was lower than at 100 rpm and at 0 rpm at 40 ^{o}C. After 4 hours the viscosity of KGM solution decreased to 33.36±0.16, 41.46±0.18 and 42.39±0.21 mPa·s at 200, 100 and 0 rpm respectively. The viscosity of KGM solution shows no differences related to stirring speeds and the viscosity of KGM solution was less than 100 mPa·s at 40 ^{o}C within 4 hours for all stirring speeds. However, in the next study, aiming at increasing the concentration of KGM,

the stirring process becomes a crucial factor. Therefore, the stirring speed at 200 rpm was selected to ensure uniform mixing of enzyme and substrate solution.

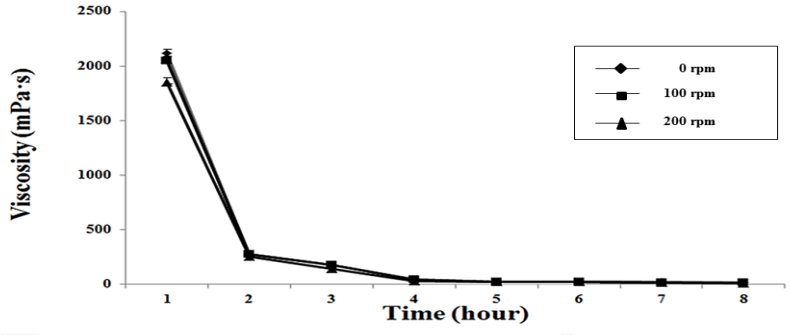

Figure 4.4 Effect of stirring speed 0 rpm (♦), 100 rpm (■) and 200 rpm (▲) on viscosity of 9% (w/w) KGMH after mannanase hydrolysis for 8 hours at 40 °C.

4.1.5 KGM solution concentration

The final viscosity of less than 100 mPa·s is targeted because that viscosity is related to the ability of carrying at the spray drying process. The range of concentration of KGM ranged from 9 to 18% (w/w). It was found that the most suitable concentration was 12% (w/w), as shown in Table 4.1. At KGM concentrations higher than 12% (w/w), the hydrolysate viscosity remained above 100 mPa·s and thus 12% (w/w) was the optimum to be used in the spray drying process. The hydrolysis of the 9% (w/w) KGM gave a lower viscosity than the value required and thus the ability to protect ingredients from heat during drying would have been reduced (Adamiec *et al.*, 2012). The oligosaccharides that were released from the different concentrations of KGM solution following mannanase hydrolysis are

summarized in Table 4.1. There were up to eleven distinct peaks during the elution time of 6 to 13 minutes, which was more than the eight standards designated as DP1 to DP7 (see the methods section Appendix A.1). The comparison of the elution times with those of the standards was performed to identify the possible type of sugar from each peak. This was of interest because oligosaccharides from DP4 up to DP7 have been reported to protect bioactive compounds during microencapsulation in the spray drying process (Inglett *et al*., 1988). Oligosaccharides that were released from the different concentrations of KGM solution by mannanase hydrolysis are shown in Table 4.1. To identify the oligosaccharides, standards were used.

Table 4. 1 Hydrolysed products in the supernatant from a 0.05% (w/w) mannanase hydrolysis

The types of sugar	9% (w/w) KGMH (%)	12% (w/w) KGMH (%)	15% (w/w) KGMH (%)	18% (w/w) KGMH (%)	Retention time sample (min)	Retention time standard (min)
DP1	28.10±0.06	21.03±0.03	28.08±0.05	28.06±0.04	6.50	6.40
DP2	10.00±0.04	12.12±0.04	13.08±0.05	9.09±0.06	7.53	7.40
DP3	11.45±0.03	14.29±0.05	15.02±0.03	14.05±0.02	8.60	8.51
DP4	8.02±0.05	6.10±0.04	4.22±0.03	10.10±0.05	9.70	9.71
DP5	3.06±0.04	6.05±0.03	7.00±0.04	7.08±0.02	10.95	10.96
DP6	10.17±0.05	8.14±0.03	10.20±0.03	8.05±0.05	12.06	12.01
DP7	9.07±0.02	7.08±0.02	9.06±0.04	6.07±0.04	13.00	13.06

The main oligosaccharides from 9%, 12%, 15% and 18% (w/w) solution of KGMH were DP4 to DP7 at 30%, 27%, 30% and 31% respectively, whereas the viscosity of solution were 11.82±0.76, 49.48±6.83, 167.60±7.03 and 285.08±9.20 mPa·s respectively. Furthermore, the type of sugar obtained in the different hydrolysates did not differ between the different initial KGM concentrations. Mannanase could hydrolyze β-mannans with β-1, 4-mannosidic linkages and the release of short β-1-4-manno-oligosaccharides. Accordingly, it has been reported that mannanase hydrolysis was a suitable method to produce manno-oligosaccharides at a large scale (AKINO *et al.*, 1988; Van Zyl *et al.*, 2010). It was found that the maximum amount of KGM that was suitable for mannanase hydrolysis is 12% (w/w) KGM. Beyond that concentration, the viscosity was too high (>150 mPa·s) for solutions undergoing spray drying process. The concentration of KGM and the viscosity of the solution should match the level required for spray drying. If the viscosity is too low, the coating performance will reduce the content of coated ingredients, hence it would not be protected from the heat during the drying process. If the viscosity is too high, the spray drying process could be affected since a high viscosity could prevent adequate atomization of the feed in drying chamber or lead to inadequate drying of the encapsulating material (Rosenberg *et al.*, 1990). Therefore the determination of a suitable concentration of KGMH solution in this study will be used as a process guideline to use KGM solution as a natural coating material for spray drying encapsulation process.

4.2 Selection a suitable coating material for andrographolide

The study was conducted in order to create microcapsule of andrographolide by using the spray drying process. The identification step of the most appropriate

coating materials, which included 12% (w/w) KGMH, 10% (w/w) KGMH + 2% (w/w) β CD and 10% (w/w) KGMH + 2% γ CD is shown in Table 4.2 and Figure 4.5.

Table 4.2 Andrographolide compounds from different types of coating material with andrographolide ratio 12% (w/w): 2% (w/w) from spray drying analyzed by HPLC

Type of coating material	**Encapsulation efficiency (%)**	**Product yield (%)^{ns}**
KGMH	46.63±0.18^{a}	56.05±2.52
KGMH+ γ CD	37.38±0.88^{b}	51.16±3.40
KGMH+ β CD	10.82±0.09^{c}	50.54±2.27

Values in a column followed by different letters are significantly different ($p \leq 0.05$).

^{ns} means non-significant

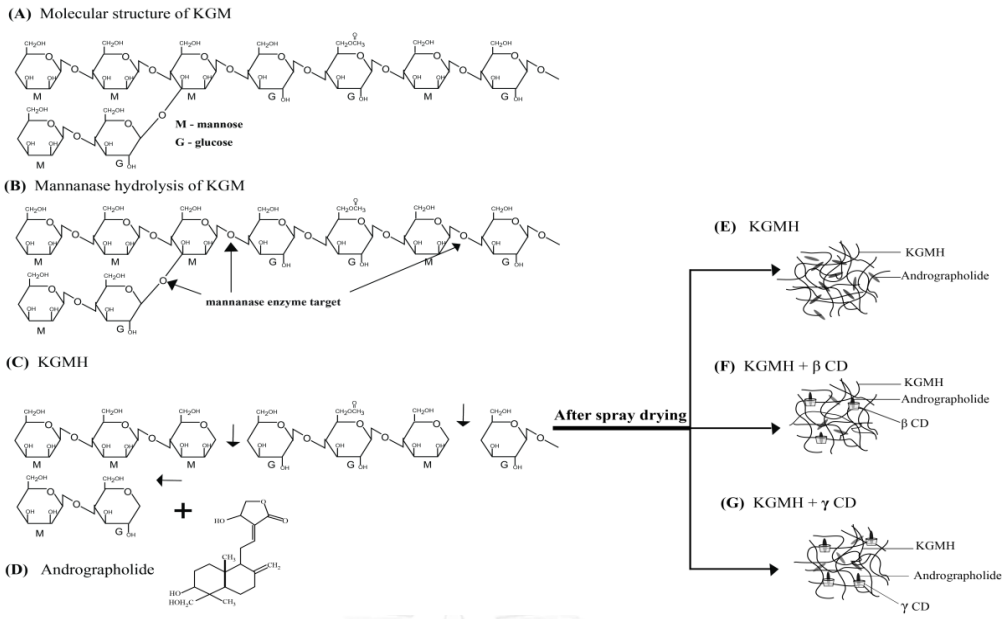

Figure 4.5 The process of enzymatic hydrolysis of KGM (A-C), chemical structure of andrographolide (D) and proposed structure of microcapsule of andrographolide using KGMH (E), KGMH + β CD (F) and KGMH + γ CD (G)

The study was also conducted by maintaining the concentration of the coating material at 12% (w/w) and the concentration of the core material that was used in the experiment to be at 2% (w/w) andrographolide. Furthermore, the study was conducted by using the spray drying process of each material at the inlet air temperature of 170 ºC and the outlet air temperature of 85±3 ºC. Among the coating materials of the product yield (%) of microcapsule, KGMH alone showed no different value of product yield (%) in comparison with KGMH - γ CD and KGMH - β CD that was 56.05±2.52%, 51.16±3.40% and 50.54±2.27% respectively. But, when using the KGMH as a single substance for the experiment, it could be shown that high encapsulation efficiency (%) could be achiever than when using the KGMH - γ CD

and KGMH – β CD as the efficiency was 46.63±0.18% compared to 37.38±0.88% and 10.82±0.09%, respectively. FT-IR spectra of encapsulated andrographolide compared with the control sample are shown in Figure 4.6. A– G, respectively.

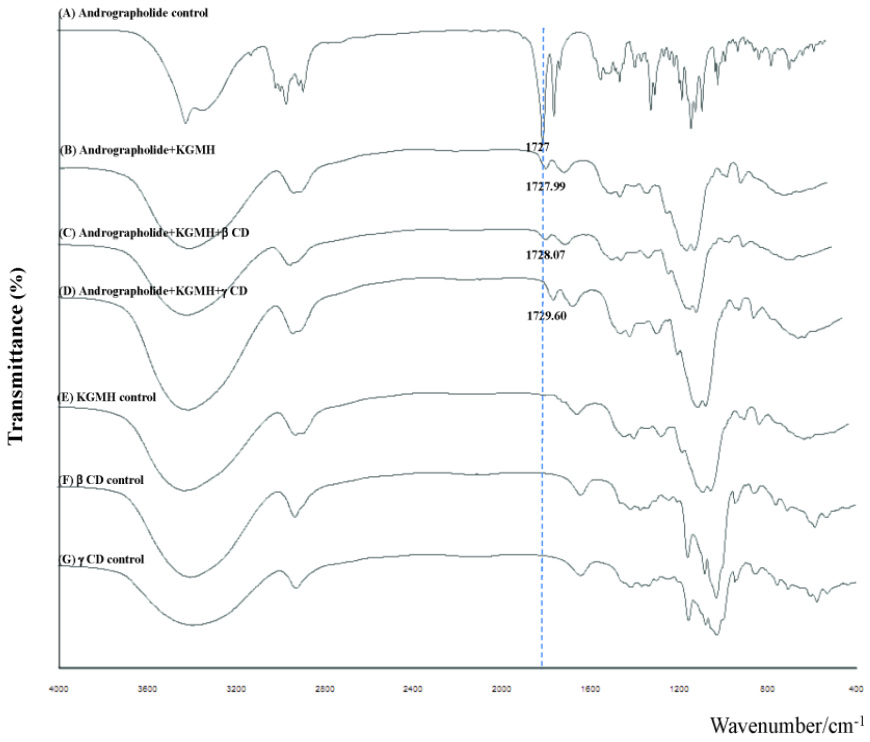

Figure 4.6 The FT-IR spectra of sample preparation andrographolide control (A), Andrographolide +KGMH (B), Andrographolide +KGMH+ β CD (C), Andrographolide+ KGMH+ γ CD (D), KGMH control (E), β CD control (F) and γ CD control (G)

Andrographolide crystals showed the stretching band at 1727 cm^{-1}, which was assigned to C=O (Jug *et al*., 2004). The characteristic stretching bands of andrographolide showed in its physical mixtures with all three samples compared with pure andrographolide. In Figure 4.6 it the presence of C=O in these systems ; KGMH + andrographolide; which KGMH + β CD + andrographolide;

showed the stretching band at KGMH + γ CD + andrographolide showed the stretching band at 1727.99 cm^{-1}, 1728.07 cm^{-1} and 1729.60 cm^{-1} respectively. As for C=O, it was not found in FT-IR spectrum of pure control of three coating materials (Figure 4.6). KGMH alone showed the most effective coating capacity due to the intermolecular force between oligosaccharides that were mixed with the andrographolide, as explained in the Figure 4.5, images E-G. After mixing the KGMH solution with the andrographolide solution, dehydration process took place. The drying process created the binding force between the molecules of the oligosaccharides resulting in enclosing the particles and coating the andrographolide in the particles (see the SEM image in Figure. 4.7, images A and B1), between andrographolide and KGMH (AKINO *et al*., 1988; Wang *et al*., 2012). Moreover, the particle was also found to have a round shape due the continuous phase of the coating oligosaccharides without breaking of the particle. In case of CD (γ CD and β CD), it was made into hydrophobic cavity pocket shape that would coat the andrographolide inside. On the other side of CD that had the open in space, it contains primary hydroxyl groups and secondary hydroxyl groups, which contain numbers of OH groups. Once the CD + andrographolide solution was mixed with the KGMH and went to the process of dehydration, it led to the steric attraction, producing the interruption on the intermolecular forces between oligosaccharides and CD. This phenomenon created the bond angle strain that made the particle into the wrong shape and led to the interruption of the continuous phase of the oligosaccharide (Prankerd *et al*., 1992; Zhou *et al*., 2009) This can be seen under SEM, as shown in Figure 4.7. A2, B2 and A3, B3. The particle came out as an oval shape with the cracks on the surface of the particle.

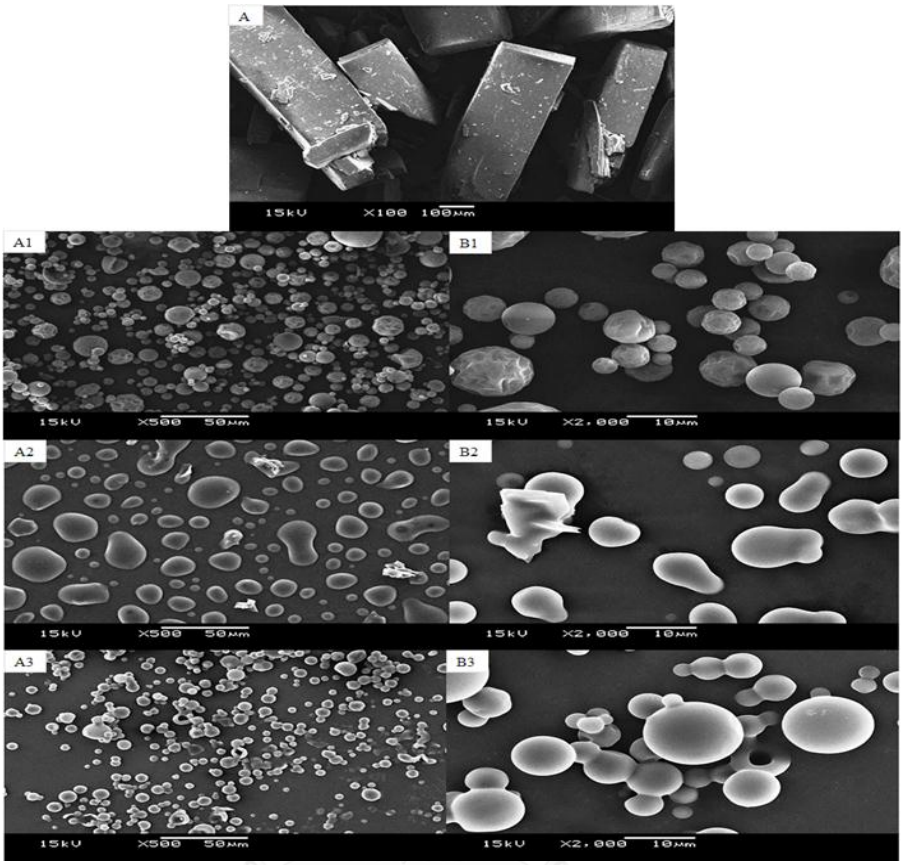

Figure 4.7 SEM of Andrographolide control (100×: A), Andrographolide + KGMH (500×: A1, 2000×: B1), Andrographolide + KGMH + β CD (500×: A2, 2000×: B2), and Andrographolide + KGMH + γ CD (500×: A3, 2000×: B3) spray dried at inlet air temperature of 170 ^{o}C and outlet air-temperature of 85±3 ^{o}C

This phenomenon led to the loss of andrographolide during encapsulation process. Therefore the microcapsules from the KGMH that were developed throughout this study could be used for the andrographolide coating better than using the coating material of KGMH - β CD, whereas the characteristics, the moisture content and the water activity were not statistically different (p>0.05), see Table 4.3.

Table 4. 3 Effect of type of coating materials used in preparation of andrographolide microcapsules from spray drying analyzed for water activity and moisture content

Type of coating material	Water activity (a_w)^{ns}	Moisture content (%) (w.b.)^{ns}
KGMH	0.25±0.00	5.11±0.07
KGMH+ γ CD	0.26±0.00	5.03±0.63
KGMH+ β CD	0.27±0.00	6.06±0.84

^{ns} means non-significant

Furthermore, when using the KGMH alone as a coating material also proved to have lower production costs than the KGMH –γ CD and KGMH – β CD. The development of the KGMH as a coating material, as shown in this study, provides an alternative for the industry to use andrographolide in various applications.

4.3 Optimization of the encapsulation process for andrographolide via spray drying

4.3.1 Outlet air temperature

The results in Table 4.4 show that the outlet air temperature influences the encapsulation efficiency (%) and the product yield (%) of microcapsules significantly. The study also show that the outlet air temperature at 85±3 ^{o}C could achieve a higher the % encapsulation (46.63%±0.18) than using the outlet air temperature at 75±3 ^{o}C (5.32%±0.45), respectively. However, the moisture content (% w.b.) and water activity that were measured in the powder obtained under these two conditions were not different. Spray drying encapsulation of most food ingredients, usually the inlet air temperature may vary from 160-200 ^{o}C and

evaporation occurs immediately. Therefore the dry powders are exposed to moderate outlet air temperature 65-90 ^{o}C which may lead to the thermal degradations of the dry product. However, when high amount of heat is transferred into the droplet the evaporation rate of water from the wall material is high, which results in less heat exposure to the active core material. (Ghandi *et al*., 2012; Ozmen and Langrish, 2003). In addition, outlet air temperature is a very important factor for encapsulation efficiency and product yield. A higher outlet temperature leads to a faster dehydration, a shorter on the drying process and lower density. At a low outlet air temperature, the humidity will be increased and lead to agglomeration and stickiness of product and formation of plastic sticky surface in the dryer. Hence, the outlet air temperature affects the product yield (%) to 56.25%±2.53 (85±3 ^{o}C) and 13.44%±2.43 (75±3 ^{o}C), respectively. Bhandari *et al*. (1997) and Górska *et al*. (2013) investigated the problems associated with spray drying of sugar-rich foods and reported that high outlet air temperature of products could be avoided by spray drying with outlet air condition at least 20 ^{o}C higher than the glass transition temperature of sugar substances. For example, the glass transition temperature of trehalose/maltodextrin, lactose/maltodextrin and sucrose were 57 ^{o}C, 61 ^{o}C and 62 ^{o}C, respectively, and therefore drying for those substances with the less sticky condition, the optimum temperature of the outlet air should be at least 77 ^{o}C, 81 ^{o}C and 82 ^{o}C, respectively.

It is evident from Table 4.1 that the saccharides consisted of DP1-3 from KGMH. Furthermore, % product yield obtained by spray drying under the outlet air temperature 85±3 ^{o}C was higher than that of the outlet air temperature 75±3 ^{o}C, in which at later outlet temperature a significant number of products was found to stick on the surface of spray dryer chamber.

The stickiness of KGMH solution increased with an increase of saccharides (DP 1-3) concentration, which consequently affected the process yield and encapsulation efficiency of spray drying. It is quite possible that with the stickiness and agglomeration of dried particles under the outlet air temperature 75±3 ^{o}C, the saccharides could not be completely dried and sprayed out. Consequently, only small amounts of dried products could come out of the dryer. Thus, the spray drying of KGMH at outlet air temperature of 85±3 ^{o}C seems appropriate to dry the saccharides with high product yield during process, possibly enhancing its encapsulation efficiency.

Based on the results of the experiment described in the present section, the outlet air temperature of 85 ^{o}C was selected to evaluate the effects of inlet air temperature of spray drying on the encapsulation efficiency in the following section.

Table 4. 4 Effect of outlet air temperatures on encapsulation efficiency, product yield and physiochemical properties of andrographolide microcapsules, inlet air temperature at 170 ^{o}C

Outlet air temperature (^{o}C)	**Encapsulation efficiency (%)**	**Product yield (%)**	**Moisture content (% w.b.)^{ns}**	**Water activity (a_w) ^{ns}**
75±3	5.32±0.45^{b}	13.44±2.43^{b}	5.38±0.14	0.28±0.00
85±3	46.63±0.18^{a}	56.25±2.53^{a}	5.11±0.07	0.25±0.00

Values in a column followed by different letters are significantly different ($p \leq 0.05$).
^{ns} means non-significant

4.3.2 Inlet air temperature

In this section, the effects of the inlet air temperature during spray drying at 150 ^{o}C, 170 ^{o}C and 190 ^{o}C, with the outlet air temperature maintained at 85±3 ^{o}C from the previous section was carried out. The result in Table 4.5 show that using the inlet air temperature of 170 ^{o}C (46.63%±0.18), and 190 ^{o}C (42.69%±0.27) resulted in better % encapsulation efficiency than using the inlet air temperature of 150 ^{o}C (12.76%±1.24) but inlet air temperature at 170 ^{o}C and 190 ^{o}C did not show a significant difference in the % encapsulation efficiency (p>0.05). It is obvious from Table 4.5 that, there is no significant difference (p> 0.05) in product yield between the three temperatures, however the uses of higher inlet air temperatures 170 ^{o}C and 190 ^{o}C with shorter drying time result in a decrease in heat/oxygen exposure time of substrates, which show a greater amount of andrographolide retained in microcapsules. Any excess of drying time can potentially degrade andrographolide and has an adverse impact on coating sugar from KGMH. In addition, when comparing between the drying treatments of inlet air temperature 170 ^{o}C and 190 ^{o}C, the andrographolide microcapsule had similar values of the product yield (%), moisture content (% w.b.) and the water activity (p>0.05).

These results suggest that differences observed in the encapsulation efficiencies could be attributed to different temperatures of inlet air in the spray drying treatment, and a maximum value of 46.63%±0.18 was obtained when the inlet air temperature was 170 ^{o}C. In spray drying process used in microencapsulation, the exposure time is an important factor since extended exposure time decreases the encapsulation efficiency, and also affects the properties of the andrographolide inside particle. It was found that the andrographollide inside microcapsule obtained by the

spray drying under the inlet air temperature of 170 ^{o}C and 190 ^{o}C may exhibit higher encapsulation efficiencies in comparison with the microcapsule powder produced from the spray drying under the inlet air temperature of 150 ^{o}C ($p \leq 0.05$), due to shorter exposure to heat/oxygen during the spray drying treatment. Owing to a small amount of andrographolide inside KGMH from microcapsule and its oxidation susceptibility, the severity of spray drying conditions can affect the possible degradation of the sugar from KGMH leading to changed physicochemical properties in the end product. Thus, the production of andrographolide microencapsules by spray drying under mild conditions seems appropriate to promote encapsulation efficiency and prevent the degradation of andrographolide. Based on the results of the experiments described in the present section, both inlet air temperatures 170 ^{o}C and 190 ^{o}C were chosen to evaluate the effect of coating material concentration on the microencapsulation of andrographolide described in the following section.

Table 4. 5 Effect of inlet air temperatures on encapsulation efficiency, yield and physicochemical properties of andrographolide microcapsules, outlet air temperature at 85±3 ^{o}C

Inlet air temperature (^{o}C)	**Encapsulation efficiency (%)**	**Product yield (%) ^{ns}**	**Moisture content (% w.b.)^{ns}**	**Water activity (a_w) ^{ns}**
150	12.76±1.24 ^{b}	50.67±2.27	4.91±0.38	0.25±0.00
170	46.63±0.18^{a}	56.25±2.53	5.03±0.63	0.26±0.00
190	42.69±0.27^{a}	51.30±3.41	5.04±0.64	0.27±0.00

Values in a column followed by different letters are significantly different ($p \leq 0.05$).
^{ns} means non-significant

4.3.3 KGM concentration and inlet air temperature

Coating material is an important parameter to prevent degradation of andrographolide. It was assumed to tolerate high temperature and convenience to use for spray drying. Table 4.6 show the ability of coating material to maintain quality of the andrographolide from different concentrations of KGM. It shows that the 18% and 21% (w/w) KGMH could maintain a higher % encapsulation in the microcapsules than those of 15%, 12% and 9%. The increase in concentration of KGM increased the ability to maintain the % encapsulation.

The results also demonstrate that using the KGM with concentration of 18% (w/w), with the inlet air temperature at 170 ^{o}C and 190 ^{o}C, led to a high % encapsulation at 78.20%±5.47 and 81.45%±0.97, respectively. However, using the KGMH with concentration of 21% (w/w) with the inlet air temperature of 190 ^{o}C, led to the highest percentage of the % encapsulation of 89.94%±0.27. From the results, it can be implied that a higher amount of KGM and increase in inlet temperature can increase % encapsulation. Therefore, KGMH from KGM with a concentration of 21% (w/w), with the inlet air temperature of 190 ^{o}C and the outlet air temperature at 85±3 ^{o}C was capable to achieve the highest encapsulation efficiency for the microencapsulated product. This may due to KGMH at 21% (w/w) has high a concentration of oligosaccharides such as DP4 to DP7 (Wattanaprasert *et al*., 2012). It lead to the establishment of the stronger intermolecular force between oligosaccharides that were entrapment with the andrographolide. Yang *et al*. (2009) explained that the KGMH could provide adamant retention capability of bioactive compounds very well. Adamiec *et al*. (2012) found that the KGMH can protect and retain of total kaffir lime oil in microcapsules at inlet air temperature of 180 ^{o}C with

the best functional properties in term of retention of oil encapsulated. Therefore, the solution of KGMH could encapsulate andrographolide inside the microcapsules quite well.

Table 4. 6 Effect of concentration of KGM solution before enzymatic treatment and of inlet air temperatures on encapsulation efficiency, yield and physicochemical properties of andrographolide microcapsules produced with outlet air temperature 85±3 ^{o}C

Concentration of KGM (% w/w) and Inlet air temperature (^{o}C)	**Encapsulation efficiency (%)**	**Product yield (%)**	**Moisture content (% w.b.)**	**Water activity (a_w) ^{ns}**
9/170	2.39±0.16^{f}	13.02±0.74^{e}	6.11±0.01^{a}	0.25±0.00
12/170	46.63±0.18^{c}	56.25±2.53^{ab}	5.03±0.36^{c}	0.26±0.00
15/170	36.63±3.89^{d}	56.18±2.42^{ab}	5.18±0.28^{bc}	0.25±0.00
18 /170	78.20±5.47^{b}	59.67±4.01^{a}	4.99±0.18^{c}	0.26±0.00
21/170	81.22±0.31^{b}	45.00±2.12^{bcd}	3.54±0.70^{d}	0.22±0.00
9/190	21.87±5.44^{c}	35.65±8.16^{d}	5.65±0.54^{b}	0.27±0.00
12/190	42.69±0.27^{cd}	51.30±3.41^{bcd}	5.04±0.64^{c}	0.27±0.00
15/190	45.63±4.07^{c}	41.45±5.58^{cd}	5.44±0.04^{bc}	0.29±0.00
18 /190	81.45±0.97^{b}	46.03±11.24^{bcd}	5.00±0.23^{c}	0.26±0.00
21/190	89.94±0.27^{a}	57.50±6.36^{abc}	3.90±0.23^{d}	0.22±0.00

Values in a column followed by different letters are significantly different ($p \leq 0.05$).

^{ns} means non-significant

Figure 4.8 shows morphology of andrographolide microcapsules from the SEM of 2000× and 8000×, showing that the sample has a different appearance.

The concentration of the coating (A-D), the spheres were found in the concentration of 9% (8000×) and of 12% (8000×) or both spherical and dents in a concentration of 15% (8000×) and dents of the more concentrated of 18% (2000×) and of 21% (2000×). At a magnification of 8000× the dents on the surface of the particles appear were clearly. Dents occur in line with the ability to retain andrographolide in the microcapsules.

The morphology of andrographolide microcapsules from the SEM of 2000× and 8000×, on the surface, indicates its importance in appearing which was very important to guarantee the effective protection of the encapsulated material. It was found that the microencapsulated andrographolide using 21% (w/w) KGMH exhibited certain wrinkles on the surface, indicating a better compatibility of KGMH concentration than the other concentrations.

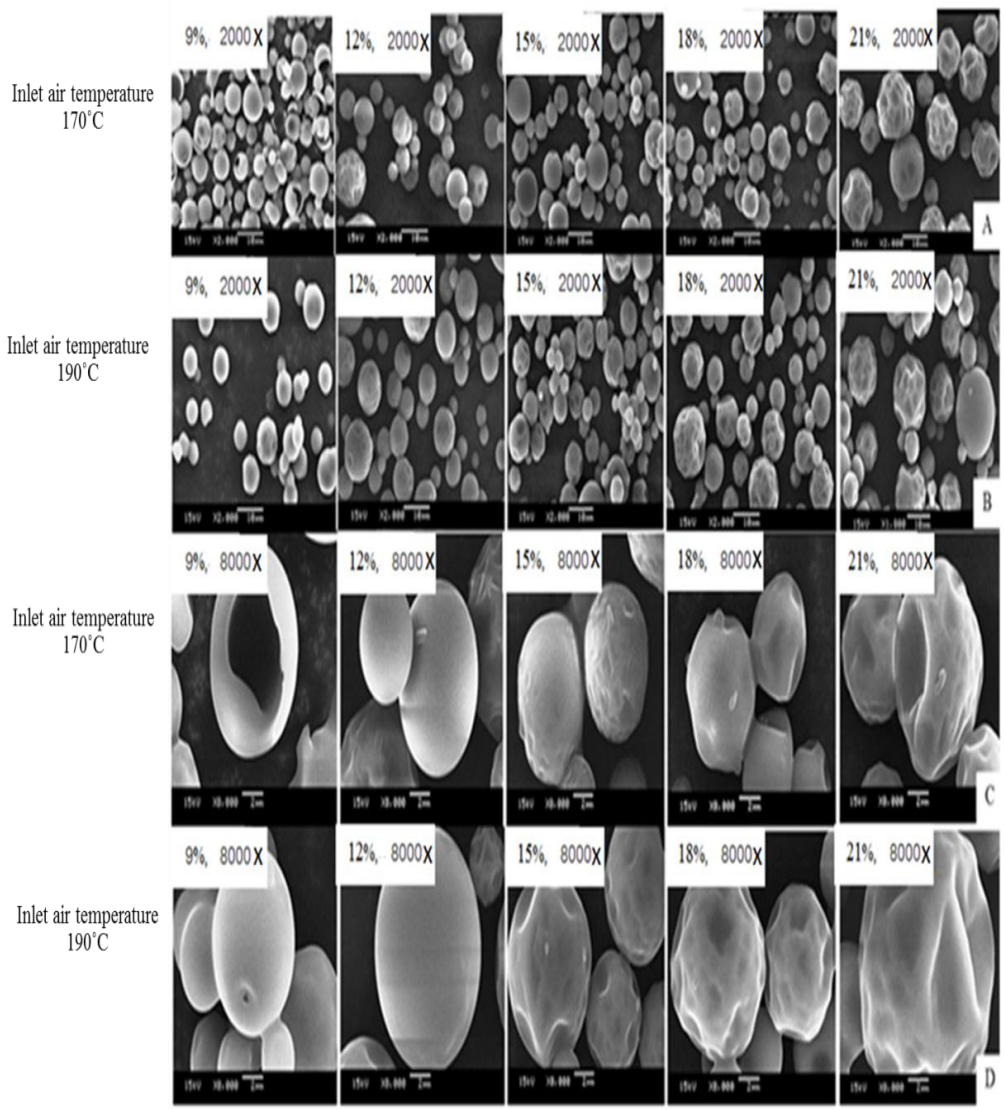

Figure 4.8 Scanning Electron Micrographs of andrographolide microcapsules from drying at inlet air temperature of 170 ^{o}C 2000× (A), inlet air temperature of 190 ^{o}C 2000× (B), inlet air temperature of 170 ^{o}C 8000× (C) and inlet air temperature of 190 ^{o}C 8000× (D)

4.4 Investigation of the releasing behavior of andrographolide from microcapsules during *in vitro* study

The releasing performance of andrographolide from the microcapsules is shown in Table 4.7. The microcapsules that were produced at inlet air temperature of 190 ^{o}C, with the outlet air temperature at 85±3 ^{o}C and using the concentration of the coating material of 21 % (w/w) could tolerate the condition in the simulation model of piglet's digestive system. The digestive system starts of the piglet starts in the stomach at stomach of the piglet at the pH 2.5 and then goes through the gallbladder at pH 6.8 within 60 min. From the experiment, it was shown that the KGMH microcapsules could protect the encapsulated andrographolide until it reached the target. Microcapsules produced from KGM can maintain the andrographolide at pH 2.5 and 6.8 for 30, 60 and 120 min, respectively. The release of the andrographolide was found to be 0.05 – 0.10 % (w/w) which was not significantly different (p>0.05). Furthermore, the remaining andrographolide in microcapsules was at 2.49 – 3.20 % (w/w). The study on the release of the solution shows that the andrographolide microcapsule is good in maintaining its status when it went through the simulation of the pig's digestive system (*in vitro* study) under both acidic conditions before reaching the small intestine. Furthermore, its ability to maintain the concentration a bioactive compound still reached more than 70%. When the microcapsules reach the small intestine of the pig, the enzyme that digesting oligosaccharides (which is known as coating material) will be digested. This will lead to the release of the andrographolide into the target in the colon, which it will create immunity and reduce the diarrhea of the pig, which is known as one of the worst diseases spreading in the pig farming (Kidder and Manners, 1978; Low and Partridge, 1980).

Table 4. 7 Concentration of andrographolide released in *in vitro* system and the remaining andrographolide

pH	Time (minutes)	Released andrographolide (% w/w) [ns]	Andrographolide remained in microcapsule (% w/w) [ns]	% Andrographolide remained in the microcapsules [ns]
2.5	30	0.07 ± 0.01	2.77 ± 0.33	81.00 ± 6.72
2.5	60	0.09 ± 0.01	3.11 ± 0.40	91.09 ± 8.33
2.5	120	0.10 ± 0.01	2.49 ± 0.37	72.81 ± 7.60
6.8	30	0.04 ± 0.02	2.71 ± 0.18	79.10 ± 3.65
6.8	60	0.05 ± 0.00	3.20 ± 0.28	93.57 ± 5.85
6.8	120	0.05 ± 0.01	2.93 ± 0.23	85.53 ± 4.82

* Andrographolide in microcapsules before releasing into the buffer is 3.42± 0.11 (% w/w)

[ns] means non-significant

CHAPTER 5
CONCLUSIONS

From the result of this study prove the hypothesis that konjac glucomannan can be used as an appropriate natural coating material in microencapsulation process of andrographolide by using spray drying technique.

Enzyme mannanase was found to be more effective on decreasing the viscosity of 9% KGM solution than combined enzymes (pectinase and mannanase), pectinase, and cellulase. Enzyme concentration has a major impact on reducing viscosity of KGM solution as well as the temperature. Therefore, the best hydrolysis condition from this study is using1500 unit mannanase with 40 °C and a stirring speed at 200 × G for 4 hours.

Mannanase (125 unit per 1 g KGM) was proved to be suitable for hydrolyzing KGM (up to 12% w/w) solution, to the required viscosity (< 100 mPa·s) within 4 hours. HPLC analysis of 12% (w/w) KGMH solution discovered 27% of oligosaccharides (DP4-DP7) formed. Therefore, using enzymatic hydrolysate of KGM solution, it leads to the production of oligosaccharides that can increase the efficiency of the coating material.

The experimental work indicated the type of wall material prepared by the inlet temperature of 170 °C and outlet air temperature 85±3 °C at 12% (w/w), with KGMH providing the highest % encapsulation in comparison with KGMH- γ CD and KGMH – β CD with a significant differentiation on the result ($p \leq 0.05$). KGMH alone would encapsulate more andrographolide inside the particles than using KGMH- γ CD and KGMH – β CD. However, the chemical properties of the microcapsule were

not significantly different (p>0.05) in terms of water activity and moisture content. As a result, it appears that the process is suitable for producing the useful substance.

The outlet air temperature (85±3 ^{o}C, 75±3 ^{o}C), inlet air temperature (150 – 190 ^{o}C) and concentration of KGM influenced the encapsulation efficiency, the product yield and the physical-chemical properties of andrographolide microcapsules. The increase aimed on the inlet air temperature and concentration of KGM, led to the increase of yield and amount of andrographolide retained in the microcapsules. Also, the concentration of KGM proved to have a stronger effect on encapsulation efficiency and yields than the drying air temperature. Therefore, the optimum condition for producing the andrographolide microcapsules was 21% (w/w) KGMH at the outlet air temperature of 85±3 ^{o}C and the inlet air temperature of 190 ^{o}C, which it proved to have the best condition to produce andrographolide microcapsules. *In vitro* study of piglet's digestive system found that KGMH is able to maintain the andrographolide inside the capsule to the target site by more than 70%. The finding can be an alternative approach for produce natural medicine for feeding industry.

Recommendations for further research

In present study, KGMH can be efficiently used as a coating material for andrographolide microencapsulation using spray drying process. However, further research should focus on the following 3 points:

1. The combination of KGMH with other coating material such as gum Arabic should be studied in order to improve the % product yield.

2. The efficiency of KGMH as a coating material could be tried with other core materials such as bioactive compounds for food and natural products.

3. The *In vivo* trial in piglet model could be tested in order to evaluation the actual effect of encapsulated andrographolide.

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APPENDIX

APPENDIX A

Analysis procedures

A.1 Determination of the mono, di, tri saccharides and oligosaccharides produced in the hydrolysate

After the deactivation of enzyme, the hydrolysate was centrifuged at 7,000 × G at 15 °C for 20 min. The supernatant was harvested, diluted ten-fold and filtered through 0.45 μm filter prior to injecting a sample volume of 100 μL into the HPLC column (Prevail carbohydrate ES column 250x4.6 mm). The column was eluted for 20 minutes at a room temperature with a 65:35 (v/v) acetonitrile: water mobile phase at a flow rate of 1 mL/min. Eluates were detected using an ELSD 200 ES (RI detector). Calibration standards for representative oligosaccharides, with an average degree of polymerization (DP) of one (monosaccharide; DP1) to seven (heptosaccharide; DP7) based upon their retention time, were run as above at 2 mg/mL for the monosaccharides (DP1) glucose (Merck, Germany) and mannose (Sigma-Aldrich Chemical, St Louis, USA), the disaccharide (DP2) maltose (Sigma-Aldrich Chemical, St Louis, USA), the trisaccharide (DP3) maltotriose (Sigma-Aldrich Chemical, St Louis, USA), the tetrasaccharide (DP4) maltotetraose (Hayashibra biochemical, Japan), the pentasaccharide (DP5) maltopentaose (Wako chemical, Japan), the hexasaccharide (DP6) maltohexaose (Hayashibra biochemical, Japan), and the heptasaccharide (DP7) maltotheptaose (Hayashibra biochemical, Japan).

```
Injection Date  : 5/1/2013 1:22:22 PM            Seq. Line :   2
Sample Name     : Standard 1                     Location : Vial 1
Acq. Operator   : bussaba                            Inj :   1
                                                 Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 3:59:33 PM by bussaba
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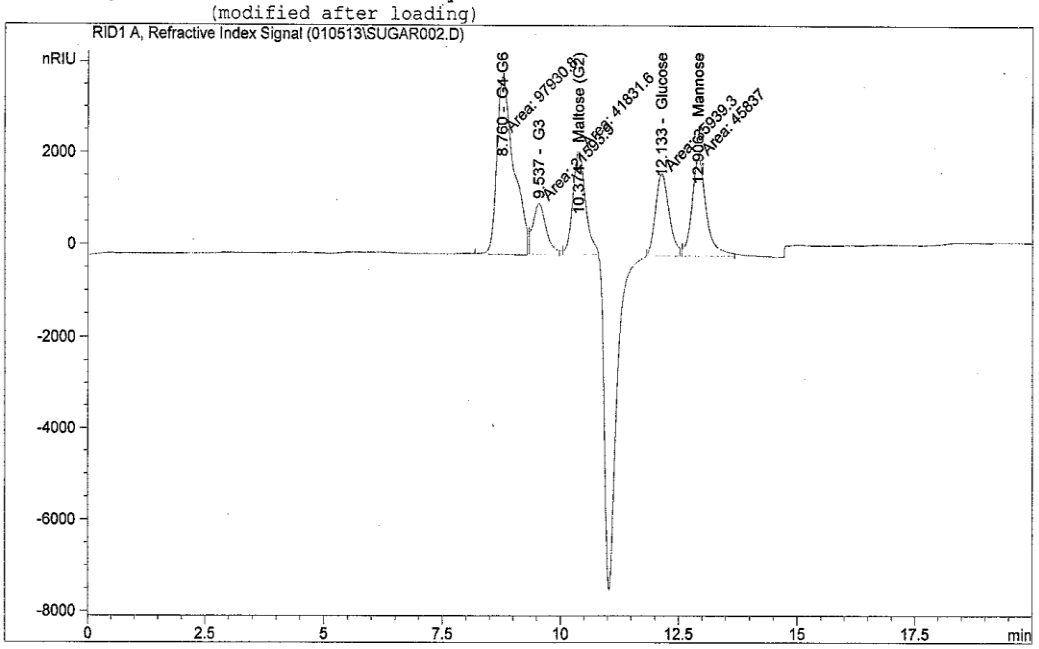


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Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [nRIU*s]                [g/mL]
-------|------|----------|----------|----------|--|------------------
  8.760 MM     9.79308e4  5.38100e-7 5.26965e-2    G4-G6
  9.537 MM     2.15939e4  2.20617e-6 4.76399e-2    G3
 10.374 MM     4.18316e4  1.44492e-6 6.04434e-2     Maltose (G2)
 12.133 MM     3.59393e4  2.56351e-6 9.21307e-2    Glucose
 12.906 MM     4.58370e4  2.21834e-6 1.01682e-1    Mannose

Totals :                              3.54592e-1

 Results obtained with enhanced integrator!

                          *** End of Report ***
```

Figure A1-1.1 Standard calibration of sugar standard

```
Injection Date  : 5/1/2013 1:45:03 PM          Seq. Line :   3
Sample Name     : Standard 2                   Location : Vial 2
Acq. Operator   : bussaba                         Inj :   1
                                               Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 4:10:44 PM by bussaba
                  (modified after loading)
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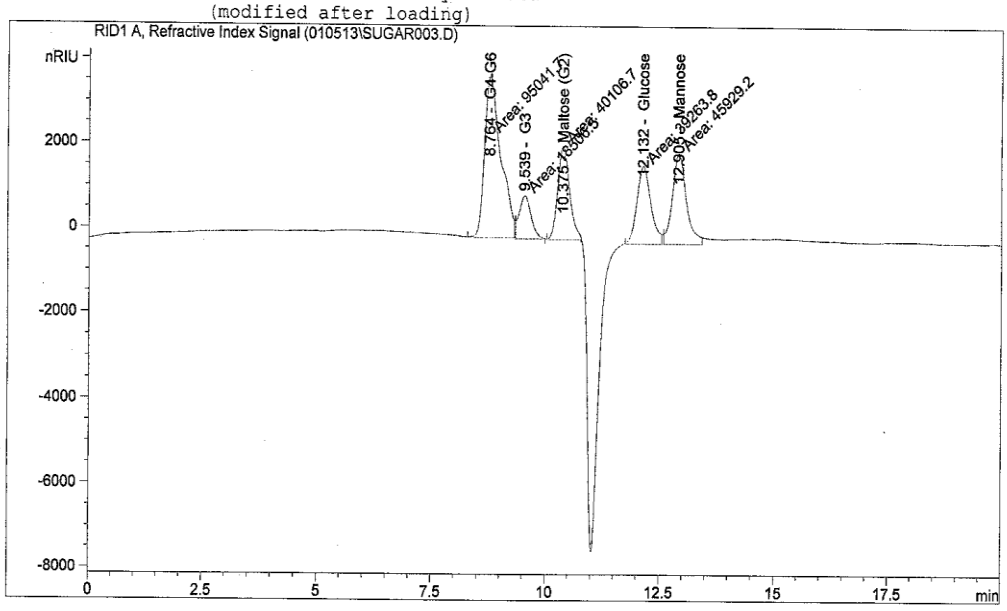

External Standard Report

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Sorted By             :      Signal
Calib. Data Modified  :      5/3/2013 4:10:57 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal
```

RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [g/mL]	Grp	Name
8.764	MM	9.50417e4	5.38100e-7	5.11419e-2		G4-G6
9.539	MM	1.85065e4	2.20617e-6	4.08284e-2		G3
10.375	MM	4.01067e4	1.44492e-6	5.79511e-2		Maltose (G2)
12.132	MM	3.92638e4	2.56351e-6	1.00653e-1		Glucose
12.905	MM	4.59292e4	2.21834e-6	1.01886e-1		Mannose
Totals :				3.52461e-1		

```
Results obtained with enhanced integrator!
```

*** End of Report ***

Figure A1-1.2 Standard calibration of sugar standard (continued)

```
Injection Date  : 5/1/2013 2:07:47 PM            Seq. Line :   4
Sample Name     : Standard 3                     Location : Vial 3
Acq. Operator   : bussaba                            Inj :   1
                                                 Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 4:10:44 PM by bussaba
                  (modified after loading)
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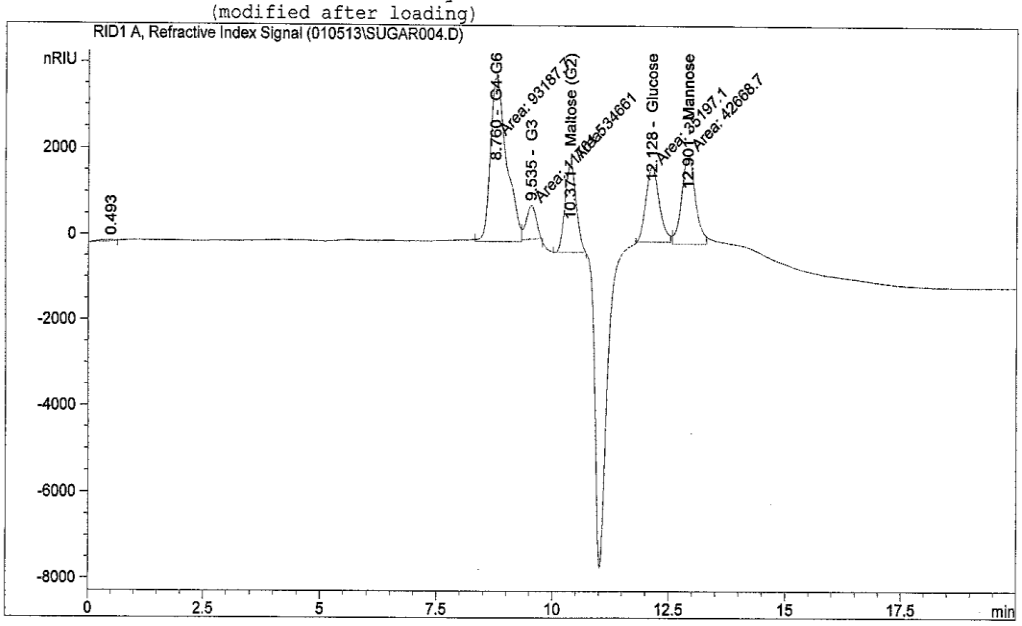


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                        External Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      5/3/2013 4:10:57 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [nRIU*s]                [g/mL]
-------|------|----------|----------|----------|--|------------------
  8.760 MM     9.31877e4  5.38100e-7 5.01443e-2    G4-G6
  9.535 MM     1.17015e4  2.20617e-6 2.58156e-2    G3
 10.371 MM     3.46610e4  1.44492e-6 5.00824e-2     Maltose (G2)
 12.128 MM     3.51971e4  2.56351e-6 9.02283e-2    Glucose
 12.901 MM     4.26687e4  2.21834e-6 9.46535e-2    Mannose

Totals :                              3.10924e-1

 Results obtained with enhanced integrator!

                          *** End of Report ***
```

Figure A1-1.3 Standard calibration of sugar standard (continued)

```
==========================================================================
Injection Date  : 5/1/2013 2:30:36 PM            Seq. Line :   5
Sample Name     : 12% (1)                         Location : Vial 4
Acq. Operator   : bussaba                            Inj :   1
                                                 Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 4:13:24 PM by bussaba
                  (modified after loading)
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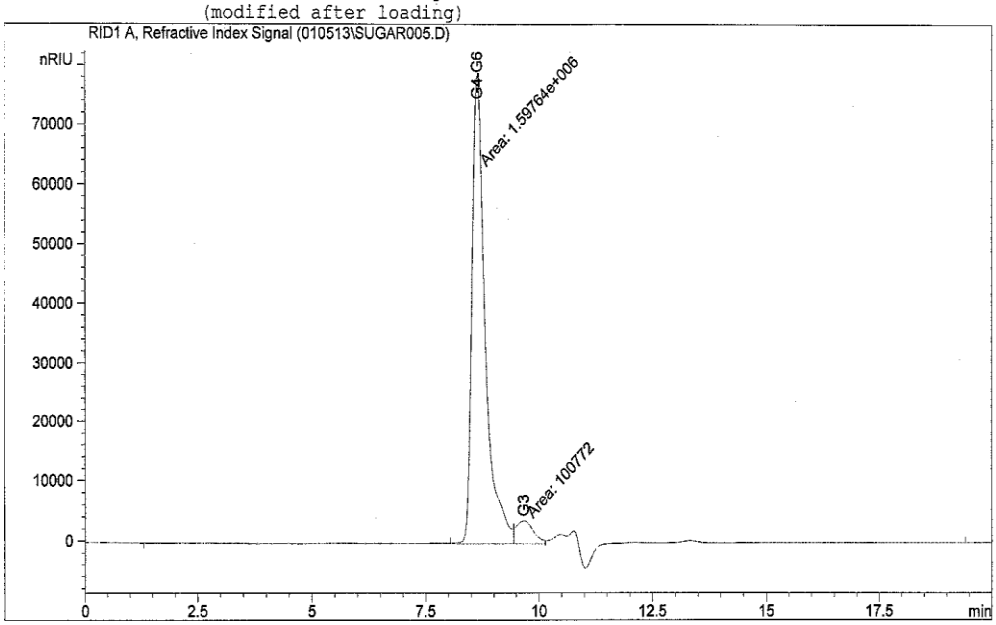


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==========================================================================
                        External Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      5/3/2013 4:10:57 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]                [g/mL]
-------|------|----------|----------|----------|--|------------------
  8.633 MM     1.59764e6  5.38100e-7 8.59688e-1    G4-G6
  9.665 MM     1.00772e5  2.20617e-6 2.22321e-1    G3
 10.374            -          -          -          Maltose (G2)
 12.133            -          -          -         Glucose
 12.906            -          -          -         Mannose

Totals :                                1.08201

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========================================================================
                          *** End of Report ***
```

Figure A1-1.4 Standard calibration of sugar standard (continued)

```
Injection Date  : 5/1/2013 2:53:25 PM            Seq. Line :   6
Sample Name     : 12% (2)                       Location : Vial 5
Acq. Operator   : bussaba                           Inj :   1
                                                Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 4:13:24 PM by bussaba
                  (modified after loading)
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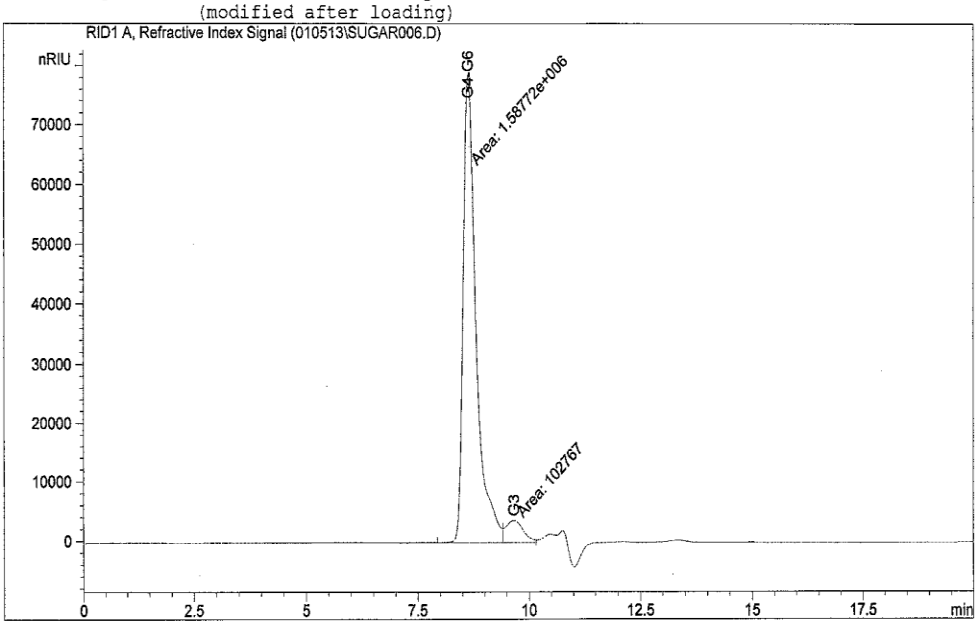


```
================================================================================
                        External Standard Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      5/3/2013 4:10:57 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [nRIU*s]                [g/mL]
-------|------|----------|----------|----------|--|------------------
  8.633 MM     1.58772e6  5.38100e-7 8.54353e-1    G4-G6
  9.662 MM     1.02767e5  2.20617e-6 2.26721e-1    G3
 10.374            -          -          -          Maltose (G2)
 12.133            -          -          -         Glucose
 12.906            -          -          -         Mannose

Totals :                                 1.08107

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

================================================================================
                           *** End of Report ***
```

Figure A1-1.5 Standard calibration of sugar standard (continued)

```
Injection Date  : 5/1/2013 3:16:08 PM          Seq. Line :   7
Sample Name     : 12% (3)                     Location : Vial 6
Acq. Operator   : bussaba                        Inj :   1
                                              Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 4:13:24 PM by bussaba
                  (modified after loading)
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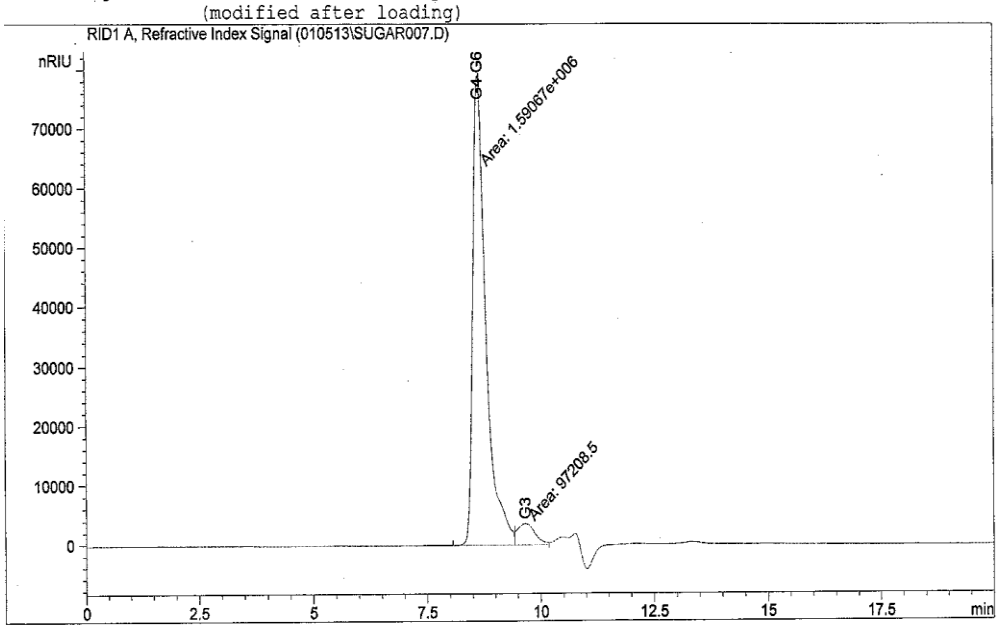


```
================================================================================
                        External Standard Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      5/3/2013 4:10:57 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [nRIU*s]                [g/mL]
-------|------|----------|----------|----------|--|------------------
  8.633 MM     1.59067e6  5.38100e-7 8.55939e-1    G4-G6
  9.664 MM     9.72085e4  2.20617e-6 2.14459e-1    G3
 10.374            -          -          -          Maltose (G2)
 12.133            -          -          -          Glucose
 12.906            -          -          -          Mannose

Totals :                                 1.07040

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

================================================================================
                            *** End of Report ***
```

Figure A1-1.6 Standard calibration of sugar standard (continued)

```
Injection Date  : 5/1/2013 2:07:47 PM          Seq. Line :   4
Sample Name     : Standard 3                   Location : Vial 3
Acq. Operator   : bussaba                         Inj :   1
                                               Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413B.M
Last changed    : 4/30/2013 6:12:03 PM by bussaba
Analysis Method : C:\HPCHEM\1\METHODS\SUGAR(T).M\300413.M
Last changed    : 5/3/2013 4:13:24 PM by bussaba
                  (modified after loading)
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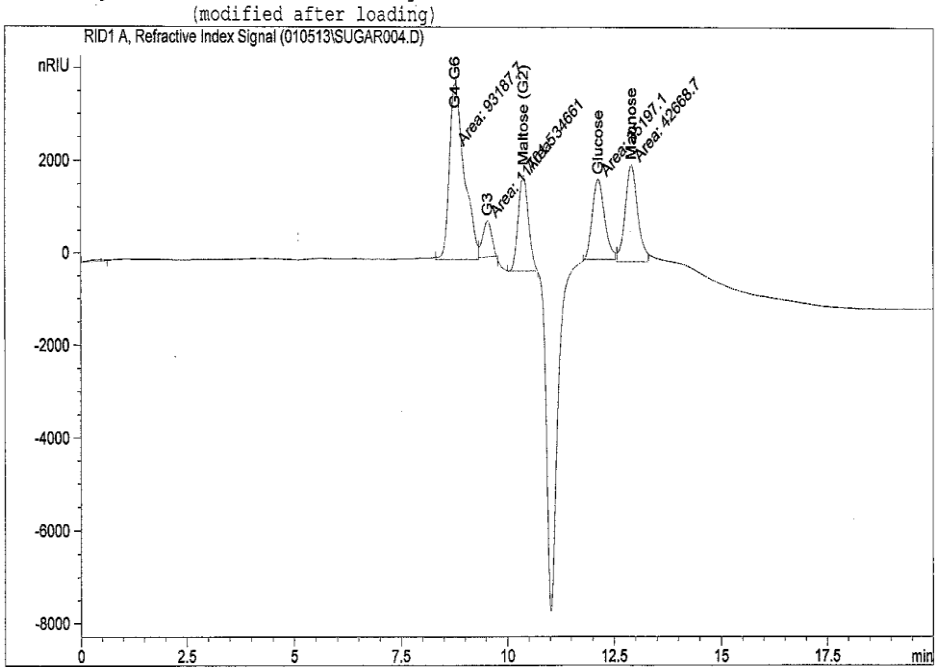



```
                        External Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      5/3/2013 4:10:57 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [nRIU*s]                [g/mL]
-------|------|----------|----------|----------|--|------------------
  8.760 MM     9.31877e4  5.38100e-7 5.01443e-2    G4-G6
  9.535 MM     1.17015e4  2.20617e-6 2.58156e-2    G3
 10.371 MM     3.46610e4  1.44492e-6 5.00824e-2     Maltose (G2)
 12.128 MM     3.51971e4  2.56351e-6 9.02283e-2    Glucose
 12.901 MM     4.26687e4  2.21834e-6 9.46535e-2    Mannose

Totals :                              3.10924e-1

 Results obtained with enhanced integrator!

                        *** End of Report ***
```

Figure A1-1.7 Standard calibration of sugar standard (continued)

Normalized content of individual oligosaccharide (%) = $\frac{P1}{P2} \times 100$ (A1.1)

Where:

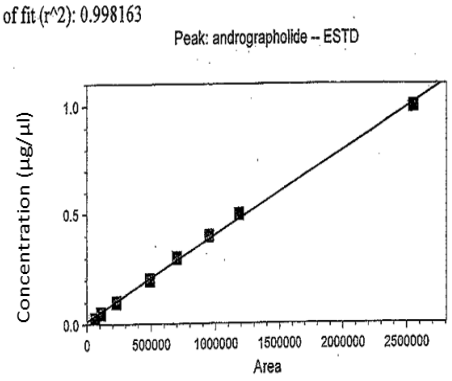
P1= Area of the peak

P2= Total area of all peaks

A.2 Determination of andrographolide microcapsules using HPLC

The analysis of the andrographolide in microcapsules was evaluated using High Performance Liquid Chromatography (HPLC, Shimadzu, LC-10A, Japan), the method followed Kumar *et al*. (2011). Andrographolide microcapsules were extracted by using methanol at the ratio of 0.2 g andrographolide to 10 ml methanol then the solution was sonicated for 15 min until all dissolved. The supernatant was harvested, by filtering through 0.2 μm nylon membrane filter prior to loading at a sample volume of 20 μL injected into the column Ultra (C18 micron 5 m 250 × 4.6 mm). The column was eluted for 10 min at room temperature with a 70:30 (v/v) acetonitrile: water mobile phase at a flow rate of 1.0 mL/min. Elutes were detected using and photodiode array (PDA) (λ 230 nm) detector. The andrographolide standard is 99% andrographolide (Sigma-Aldrich, USA). The calibration curve was potted between andrographolide concentration in 0.025 to 1.0 μg/μl and absorbance at 230 nm (Kumar *et al*., 2011).

y = 3.92833e-007x + 0.0136658
Goodness of fit (r^2): 0.998163



	Level 1	Level 2	Level 3	Level 4	Level 5
Area	65076	108965	228710	490328	699605
Concentration (µg/µl)	0.025	0.05	0.1	0.2	0.3
RF	3.84163e-007	4.58862e-007	4.37235e-007	4.07891e-007	4.28813e-007
Last Area					
Rep StDev					
Rep %RSD					
Rep Area 1	65076	108965	228710	490328	699605

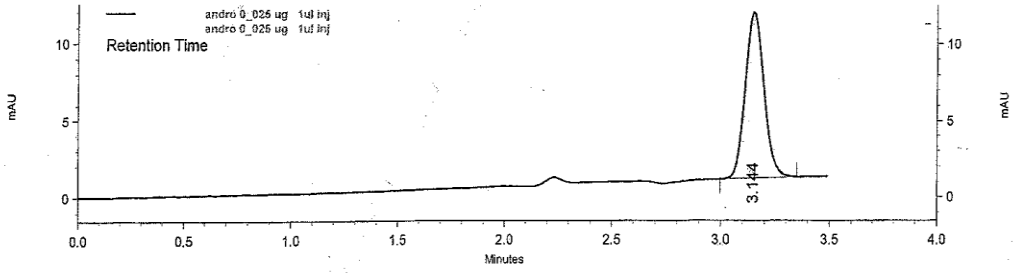
	Level 6	Level 7	Level 8
Area	949548	1182885	2551535
Concentration (µg/µl)	0.4	0.5	1
RF	4.21253e-007	4.22695e-007	3.91921e-007
Last Area			
Rep StDev			
Rep %RSD			
Rep Area 1	949548	1182885	2551535

Figure A2-1 Standard calibration curve of andrographolide standard

Amount of andrographolide in microcapsules was calculated as shown in following step;

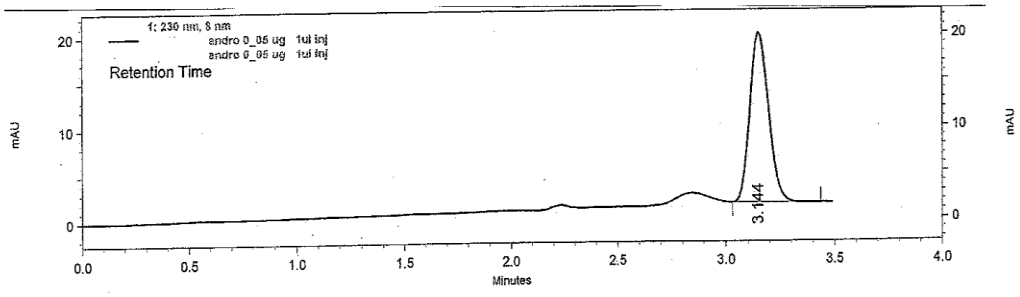
$$\text{Andrographolide (g)} = \frac{\text{Andrographolide concentration from HPLC analysis (µg/µl)} \times \text{Wm (g)}}{\text{Microcapsule concentration before HPLC analysis 20 (µg/µl)}} \quad \text{(A2.1)}$$

Wm is weight of microcapsules (g)



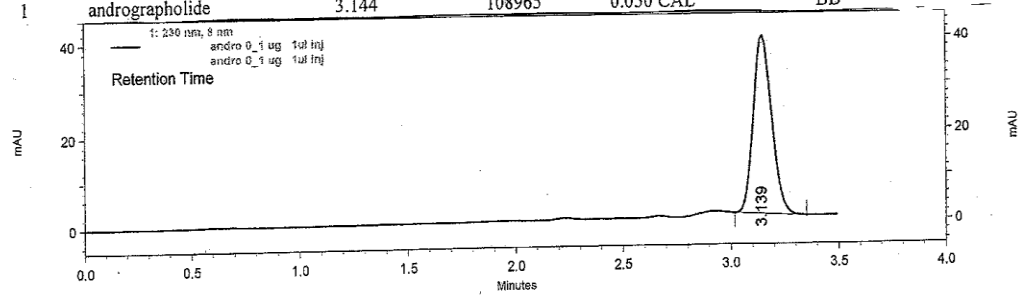
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.144	65076	0.025 CAL	BB



1: 230 nm, 8 nm

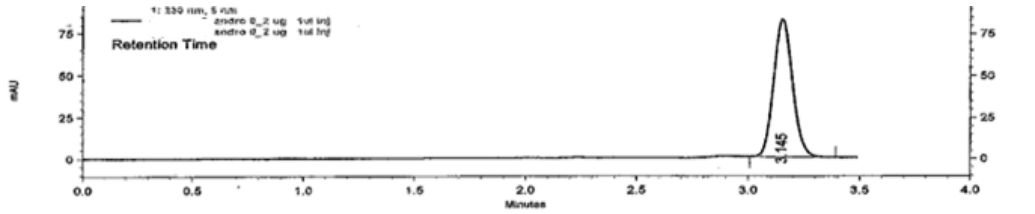
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.144	108965	0.050 CAL	BB



1: 230 nm, 8 nm

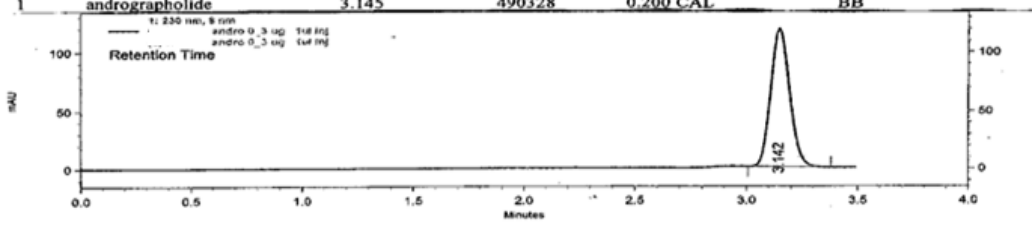
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.139	228710	0.100 CAL	BB

Figure A2-2 Chromatogram of andrographolide standard from each experiment



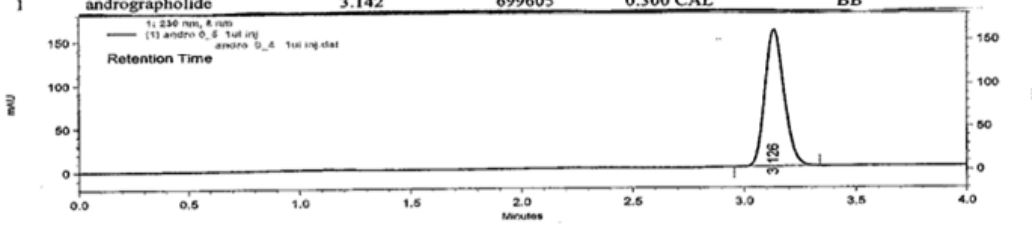
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.145	490328	0.200 CAL	BB



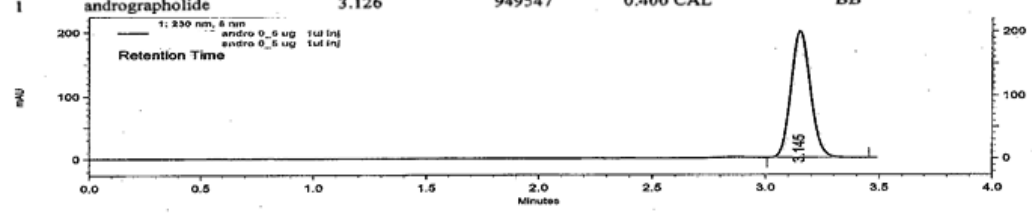
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.142	699605	0.300 CAL	BB



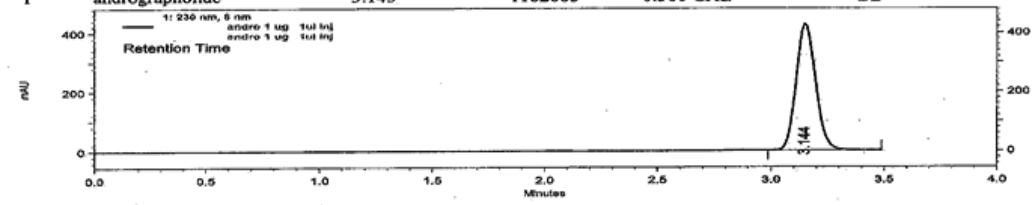
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.126	949547	0.400 CAL	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.145	1182885	0.500 CAL	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.144	2551535	1.016	BB

Figure A2-3 Chromatogram of andrographolide standard (continued)

A.3 Analysis of andrographolide from *in vitro* release by HPLC method

In the dissolution study of andrographolide microcapsules, two dissolution media were performed, simulated pH 2.5 and simulated fluid pH 6.8. The sample from the dissolution study was assayed for the andrographolide dissolved by HPLC at the 230 nm which is the maximum absorption in both media (Table 4.7). The calibration curve was potted between andrographolide concentration in 0.025 to 0.5 µg/µl and absorbance at 230 nm. Both calibration curves of andrographolide using 0.05 M hydrochloric acid (HCl) (pH 2.5), phosphate buffer (pH 6.8) and distilled water (pH 6.2). The microcapsules (1.0 g) were soaked at each pH at 30, 60 and 120 min, respectively. Then each sample was centrifuged at the speed 7500 × G for 15 min. The supernatant was collected, filtered through 0.2 µm nylon membrane filter prior to inject into HPLC to analyses released andrographolide. For the sediment from the centrifuge, it was dissolved in methanol (10 ml) for 15 min and the remained andrographolide was analysed by HPLC (Kumar *et al*., 2011).

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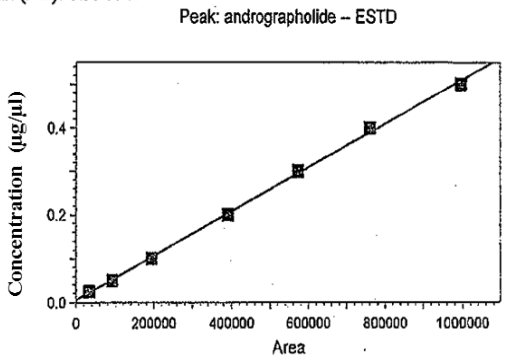
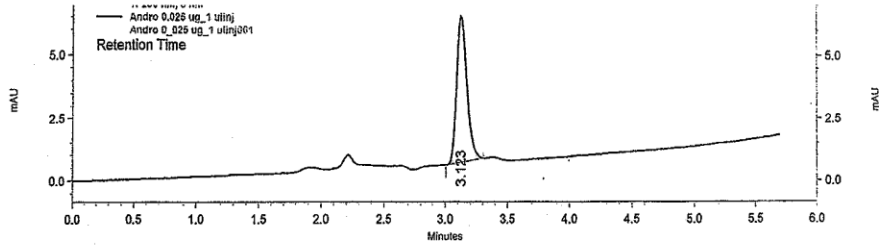
y = 5.04781e-007x + 0.00561417
Goodness of fit (r^2): 0.998743



	Level 1	Level 2	Level 3	Level 4	Level 5
Area	33369	92495	194311	391528	573591
Concentration (µg/µl)	0.025	0.05	0.1	0.2	0.3
RF	7.49199e-007	5.4057e-007	5.1464e-007	5.10819e-007	5.23021e-007
Last Area					
Rep StDev					
Rep %RSD					
Rep Area 1	33369	92495	194311	391528	573591

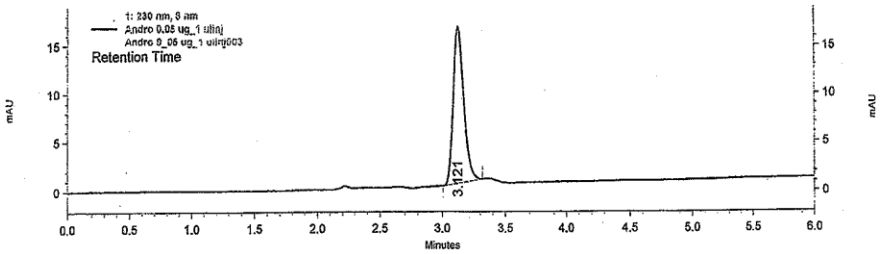
	Level 6	Level 7
Area	760214	996802
Concentration (µg/µl)	0.4	0.5
RF	5.26167e-007	5.01604e-007
Last Area		
Rep StDev		
Rep %RSD		
Rep Area 1	760214	996802

Figure A3-1 Standard calibration curve of andrographolide standard for study *in vitro* release



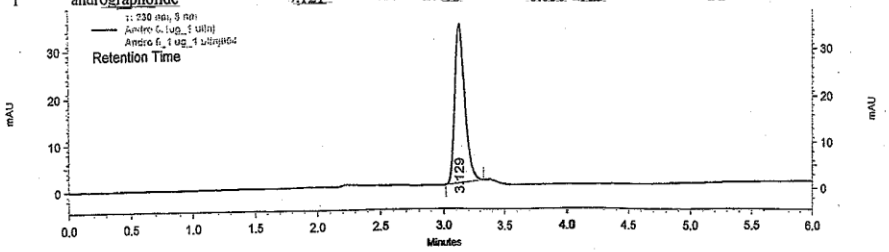
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.123	33369	0.025 CAL	BB



1: 230 nm, 8 nm

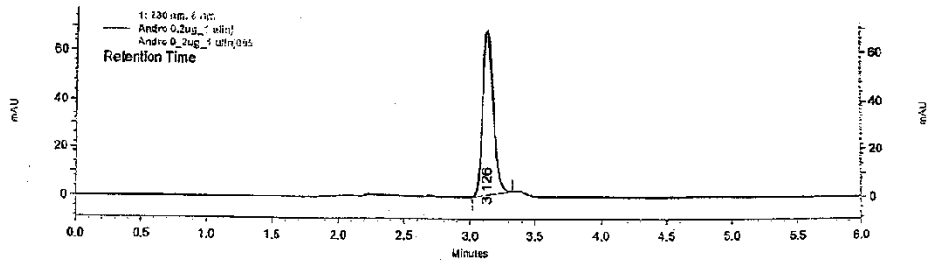
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.121	92495	0.050 CAL	BB



1: 230 nm, 8 nm

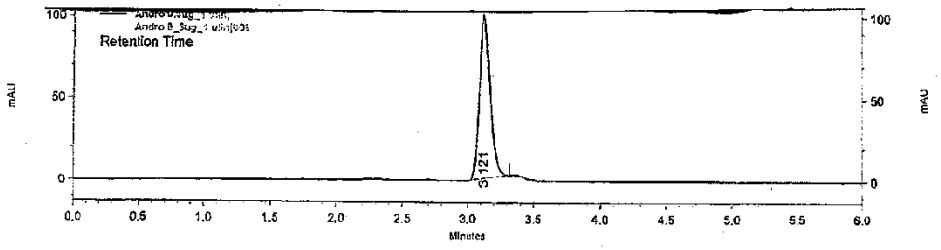
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.129	194311	0.100 CAL	BB

Figure A3-2 Chromatogram of andrographolide standard for study *in vitro* release



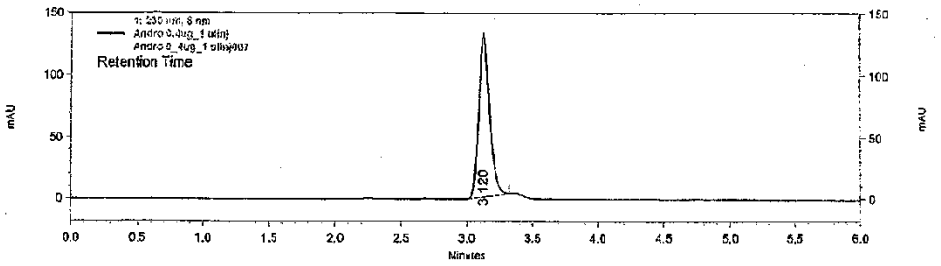
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.126	391528	0.200 CAL	BB



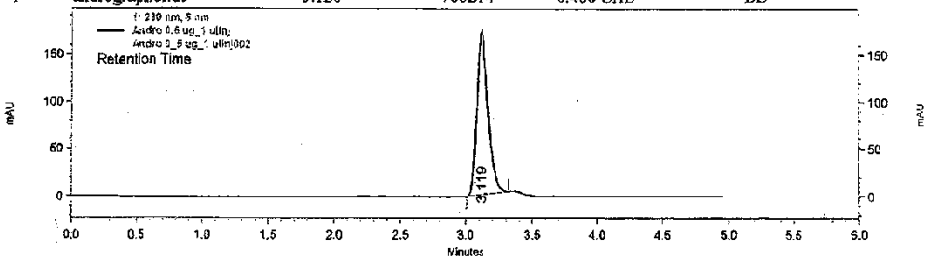
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.121	573591	0.300 CAL	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.120	760214	0.400 CAL	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.119	996802	0.500 CAL	BB

Figure A3-3 Chromatogram of andrographolide standard for study *in vitro* release

A.4 The physical and chemical properties of andrographolide microcapsule

The andrographolide microcapsule was determined the physical and chemical properties as follows;

A.4.1 Moisture content analysis

Dry the empty dish and lid in the oven at 105 ^{o}C for 3 h and transfer to desiccator to cool. Weight the empty dish and lid. Weight about 3 g of sample to the dish, spread the sample to the uniformity. Place the dish with sample in the oven. Dry for 3h at 105 ^{o}C. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample **(AOAC, 2000).**

$$\text{Moisture content (\% w.b.)} = \frac{\text{Weight of sample before drying (g)}}{\text{Weight of sample after drying (g)}} \times 100 \quad \text{(A4.1)}$$

A.4.2 The Water Activity

The water activity of andrographolide microcapsules was determined using water activity analyzer (aw- SPRINT-TH500, NOVASINA, Axair Ltd., Pfäffikon, Switzerland)

A.4.3 Scanning Electron Microscopy (SEM)

The surface morphology of raw materials and the particle microencapsulation was studied by scanning electron microscopy. The samples were fixed on a brass stub using double-sided tape and coated with a thin layer of gold in a vacuum to render them electrically conductive. The photographs were taken at excitation voltage of 10–15 kV.

A.4.4 Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectra were documented using an FTIR spectrometer. The samples were mixed and ground with potassium bromide thoroughly. The sample-KBr pellets were found by condensing the mixture of fine powder under pressure of 15000 pound for 2 min. The pellets were scanned for sixteen times at a resolution of 4 cm^{-1}, from 4000 to 600 cm^{-1} (Jug *et al*., 2004).

APPENDIX B

Raw Data

Table B- 1 Effect of temperature (^{o}C) on viscosity of 9% (w/w) KGM solution at 1 to 8 hours

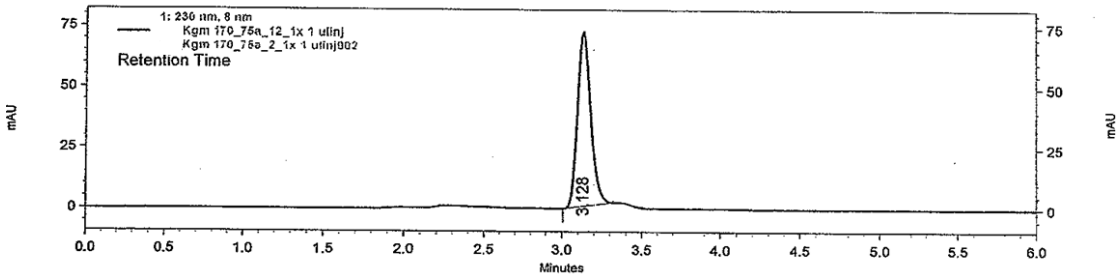
Time (hours)	Temperature (^{o} C)	Viscosity (mPa·s)		
		Sample 1	Sample 2	Sample 3
1	40	1251.09	1252.04	1251.12
1	50	240.50	240.55	241.62
1	60	179.50	179.42	179.35
2	40	242.05	245.42	242.55
2	50	171.71	170.35	171.55
2	60	151.75	151.50	152.39
3	40	167.08	166.50	166.52
3	50	147.25	147.95	148.02
3	60	148.05	148.91	148.99
4	40	32.00	32.22	32.50
4	50	36.52	36.70	36.52
4	60	40.52	39.25	40.22
5	40	11.54	11.13	11.05
5	50	13.04	13.55	13.45
5	60	13.09	13.58	13.52
6	40	10.04	9.97	9.85
6	50	9.55	9.35	9.29
6	60	12.50	11.10	10.80
7	40	9.11	9.15	9.25
7	50	9.00	9.55	9.07
7	60	10.51	9.77	9.85
8	40	9.05	9.10	8.92
8	50	9.02	9.09	8.85
8	60	9.17	9.30	9.94

Table B- 2 Effect of enzyme concentration on viscosity of 9% (w/w) KGM solution at 1 to 8 hours

Time (hours)	Enzyme concentration	Viscosity (mPa·s)		
		Sample 1	Sample 2	Sample 3
1	350	NA	NA	NA
1	750	NA	NA	NA
1	1500	1841.49	1832.94	1841.32
2	350	NA	NA	NA
2	750	7096.25	7097.23	7095.37
2	1500	245.05	255.42	252.55
3	350	NA	NA	NA
3	750	3250.2	3257.35	3255.58
3	1500	145.38	146.55	145.59
4	350	NA	NA	NA
4	750	1894.25	1895.57	1899.32
4	1500	33.05	33.25	33.07
5	350	18730.32	18729.45	18721.55
5	750	743.56	742.36	744.37
5	1500	25.27	25.32	25.45
6	350	11392.15	11384.43	11371.44
6	750	598.48	599.00	599.35
6	1500	20.00	20.58	20.05
7	350	10970.73	10972.43	10970.66
7	750	443.48	443.80	442.75
7	1500	17.55	17.72	17.50
8	350	10082.15	10084.43	10081.44
8	750	403.48	400.80	402.75
8	1500	12.55	12.72	12.50

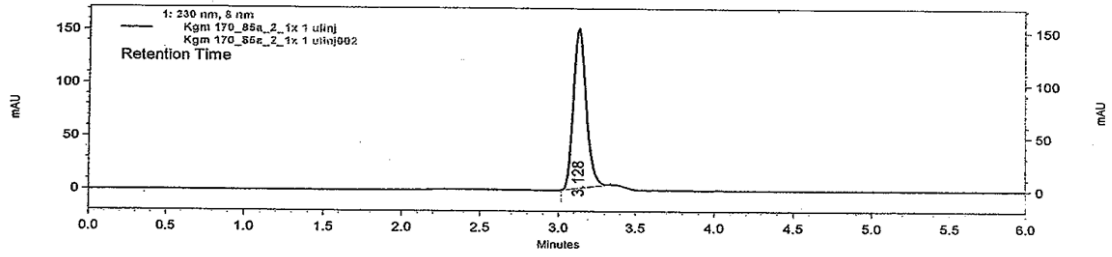
Table B- 3 Effect of speed of stirrer on viscosity of 9% (w/w) KGM solution at 1 to 8 hours

Time (hours)	Speed of stirrer	Viscosity (mPa·s)		
		Sample 1	Sample 2	Sample 3
1	000	2115.20	2117.55	2120.41
1	100	2051.42	20.48.55	2052.55
1	200	1847.42	1846.84	1846.92
2	000	278.23	277.57	277.34
2	100	273.52	273.40	275.30
2	200	255.78	255.88	255.35
3	000	176.25	176.55	176.38
3	100	175.55	175.44	175.39
3	200	145.78	145.88	145.35
4	000	42.15	42.55	42.48
4	100	41.25	41.57	41.55
4	200	33.25	33.55	33.29
5	000	26.37	26.64	26.56
5	100	25.37	25.55	25.21
5	200	25.38	25.46	25.55
6	000	23.35	23.42	23.77
6	100	22.57	22.55	22.32
6	200	20.35	20.67	20.52
7	000	20.21	20.35	20.27
7	100	19.35	19.20	19.45
7	200	17.53	17.27	17.55
8	000	15.32	15.33	15.42
8	100	14.33	14.36	14.55
8	200	12.33	12.32	12.50



1: 230 nm, 8 nm

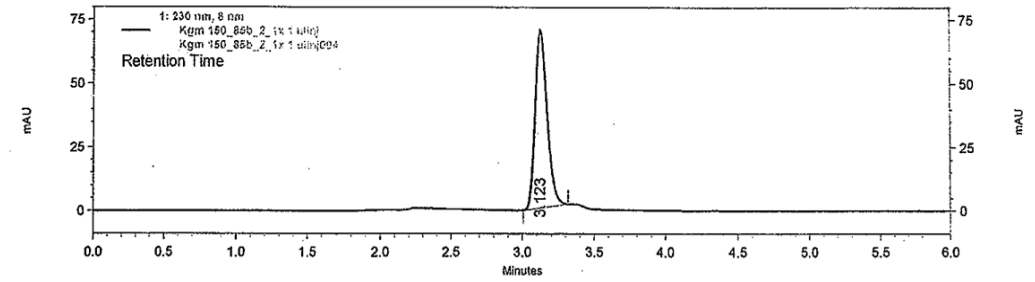
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.128	413553	0.214	BB



1: 230 nm, 8 nm

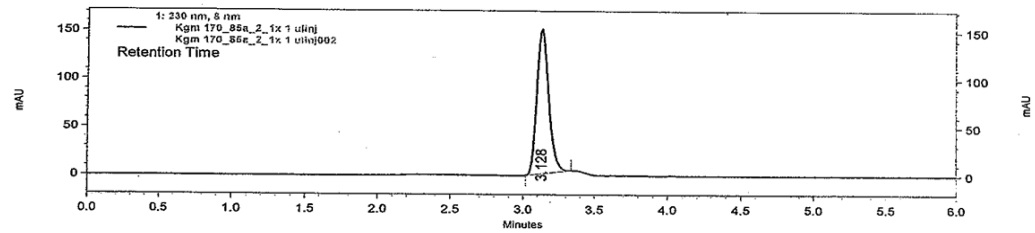
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.128	870859	0.445	BB

Figure B-1 A chromatogram of andrographolide at condition of outlet air temperature 75±3 ºC and 85±3 ºC



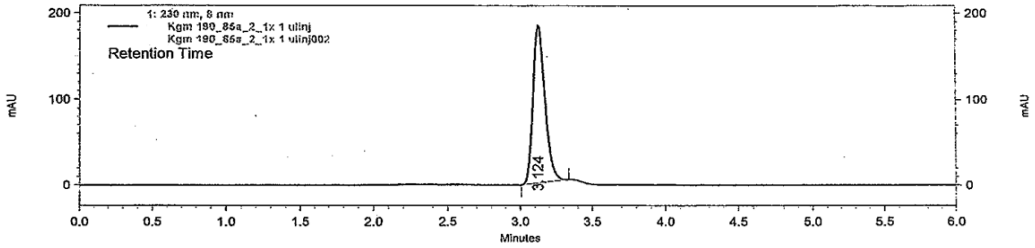
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.123	403241	0.209	BB



1: 230 nm, 8 nm

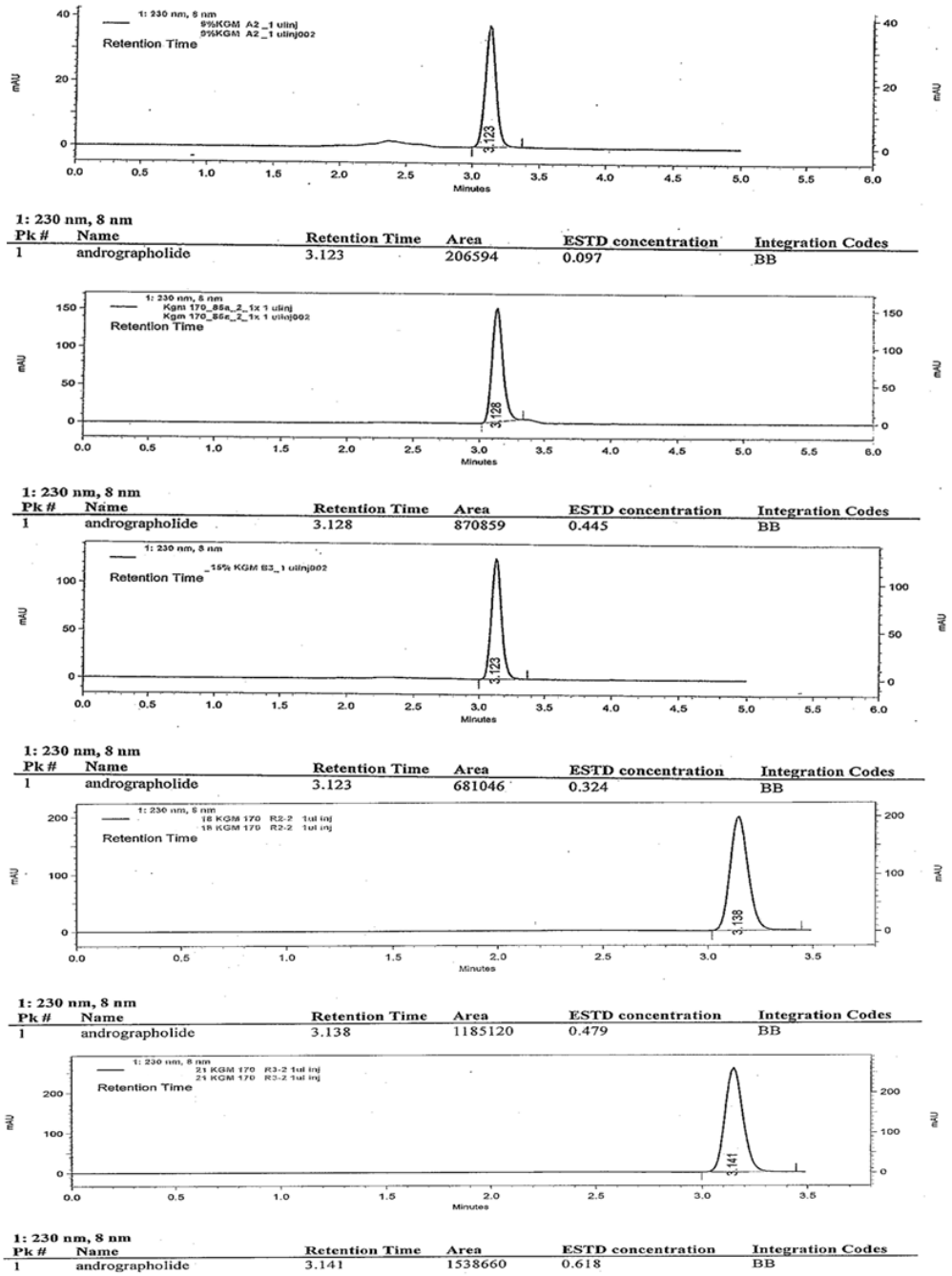
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.128	870859	0.445	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.124	1060243	0.541	BB

Figure B-2 A chromatogram of andrographolide at condition of inlet air temperature 150 ºC, 170 ºC and 190 ºC



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.123	206594	0.097	BB

1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.128	870859	0.445	BB

1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.123	681046	0.324	BB

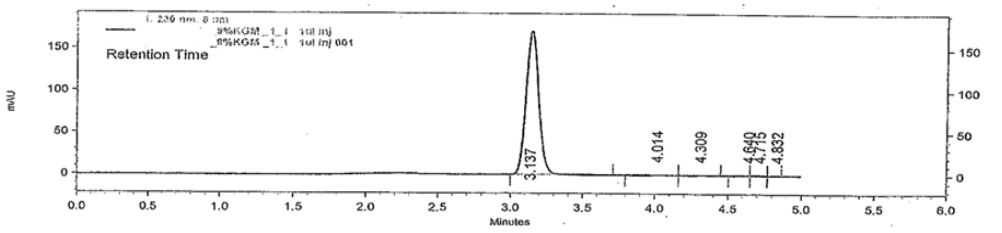
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.138	1185120	0.479	BB

1: 230 nm, 8 nm

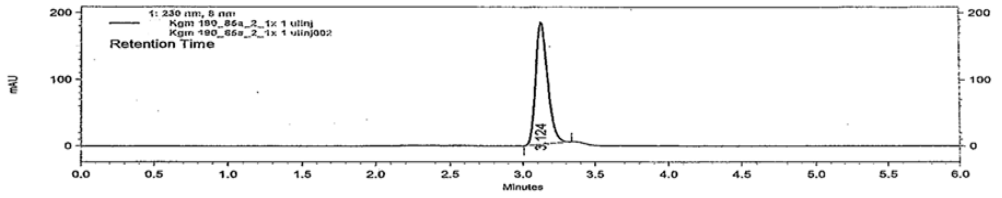
Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.141	1538660	0.618	BB

Figure B-3 A chromatogram of andrographolide in KGMH microcapsule at KGMH concentration (9% to 21%) at inlet air temperature 170 ºC and outlet air temperature 85±3 ºC



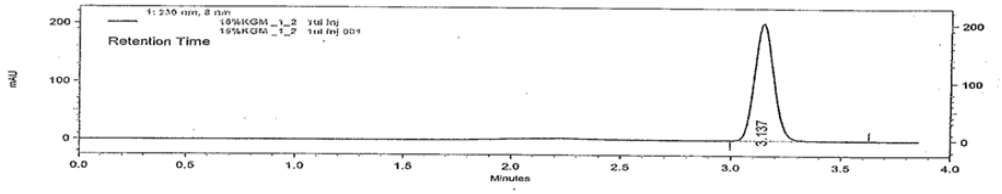
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.137	1040123	0.465	BB



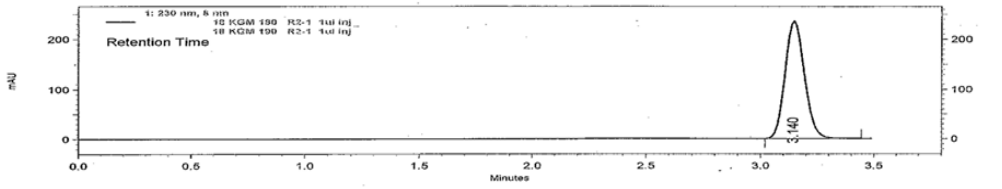
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.124	1060243	0.541	BB



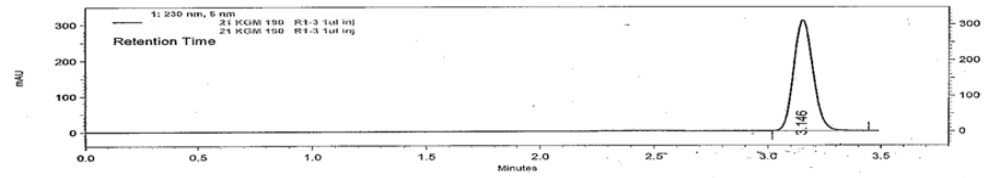
1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.137	1225815	0.548	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.140	1402330	0.565	BB



1: 230 nm, 8 nm

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	andrographolide	3.146	1841170	0.737	BB

Figure B-4 A chromatogram of andrographolide in KGMH microcapsule at KGMH concentration (9% to 21%) at inlet air temperature 190 ºC and outlet air temperature 85±3 ºC

VITA

Miss Supichar Wattanaprasert was born on June 17, 1982 in Prachinburi, Thailand. She got her B.Sc. degree in Biotechnology from King Mongkut's Institute of Technology Ladkrabang in 2004 and M.Sc. in Biotechnology from Chulalongkorn University in 2007.

List of publications

Wattanaprasert, S., Borompichaichartkul, C., Srzednicki, G., & Vaithanomsat, P. (2013). Process Modification of Potential Thai Economical Tuber Crop to be used in Microencapsulation for Nutraceutical Products. The 2nd Asia Pacific Symposium on Postharvest Research Education and Extension (APS2012). Acta Horticulturae, 163-168.

Wattanaprasert, S., Borompichaichartkul, C., Srzednicki, G., & Vaithanomsat, P. (2016). Konjac glucomannan hydrolysate, a potential natural coating material for bioactive compounds in spray drying encapsulation process. Engineering in Life Science. (under review process)

List of conferences

Wattanaprasert, S.; Borompichaichartkul, C.; Srzednicki, G.; Vaithanomsat, P. Process Modification of Potential Thai Economical Tuber Crop to be used in Microencapsulation for Nutraceutical Products. Proceed. 2nd Asia Pacific Symp. on Postharvest Res., Education, and Extension, 18-20 September 2012. Yogyakarta, Indonesia.

Wattanaprasert, S.; Borompichaichartkul, C.; Srzednicki, G.; Vaithanomsat, P. (2013) Preparation of oligosaccharides from konjac glucomannan solution used as natural coating material for spray drying process. The 25th Annual Meeting of the Thai Society for Biotechnology and International Conference, 16-19 October 2013 Bangkok, Thailand.