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FORMULATION DEVELOPMENT OF FILM COATED TABLETS CONTAINING *MALVASTRUM COROMANDELIANUM* EXTRACT

Miss Pornsri Prasertwaree

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy

Department of Manufacturing Pharmacy
Faculty of Pharmaceutical Sciences
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Thesis Title	FORMULATION DEVELOPMENT OF FILM COATED TABLETS CONTAINING *Malvastrum coromandelianum* EXTRACT
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พรศรี ประเสริฐวารี : การพัฒนาสูตรตำรับยาเม็ดเคลือบฟิล์มที่ประกอบด้วยสารสกัดดายขัด. (FORMULATION DEVELOPMENT OF FILM COATED TABLETS CONTAINING *MALVASTRUM COROMANDELIANUM* EXTRACT) อ. ที่ปรึกษา: ศ.ดร. กาญจน์พิมล ฤทธิเดช, อ.ที่ปรึกษาร่วม : รศ.ดร. สุกัญญา เจษฎานนท์ , 104 หน้า.

สารสกัดพ่นแห้งดายขัด มีฤทธิ์ในการลดระดับน้ำตาลในเลือดของกระต่ายที่ถูกเหนี่ยวนำให้เป็นเบาหวาน และนำมาใช้เป็นสมุนไพรสำหรับรักษาผู้ป่วยเบาหวาน อย่างไรก็ตามเมื่อนำผงยามาทำการบรรจุในแคปซูลชนิดแข็ง 2-3 วัน พบว่าผงยาสีน้ำตาล กลายเป็นสีน้ำตาลดำ และมีการจับตัวรวมกันเป็นก้อน ดังนั้นวัตถุประสงค์ในการศึกษาครั้งนี้เพื่อพัฒนายาเม็ดเคลือบฟิล์มสารสกัดดายขัด โดยจะมีการประเมินผงสารสกัดดายขัด เช่น การกระจายขนาดอนุภาค การไหล และความหนาแน่น ยาเม็ดสารสกัดดายขัดเตรียมโดยวิธีการตอกโดยตรง และมีการเติมสารช่วย และสารช่วยหล่อลื่น เพื่อให้ได้สูตรยาเม็ดแกนที่เหมาะสม กระบวนการในการเคลือบมีการใช้สารก่อฟิล์มดังนี้ ไฮดรอกซีโพรพิลเมธิลเซลลูโลส ไคโตซาน และ โพลีเมทาไครเลท โดยใช้เครื่องเคลือบเม็ดยา โดยยาเม็ดแกนจะมีการประเมิน ความกร่อน ความแข็ง การแตกกระจายตัวของเม็ดยา และ เปอร์เซนต์ค่าการละลายที่ 60 นาที ความสม่ำเสมอของปริมาณตัวยาและปริมาณตัวยาสำคัญของยาเม็ด จะมีการวิเคราะห์โดยใช้ High Performance Liquid Chromatography (HPLC) โดยชนิดของสารก่อฟิล์มได้แก่ ไฮดรอกซีโพรพิลเมธิลเซลลูโลส ไคโตซาน และ โพลีเมทาไครเลท มีอิทธิพลต่อความแข็งแรงในการยึดเกาะและคุณสมบัติในการป้องกันความชื้น ด้วยปริมาณการเคลือบต่างๆกัน โดยจะมีการประเมินทั้งในสภาวะเริ่มต้นและสภาวะเร่ง (45 ± 2 °C และความชื้น 75 ± 5 % RH) เป็นระยะเวลา 4 เดือน ผลการประเมินพบว่าสารสกัดพ่นแห้งดายขัด มีคุณสมบัติการไหลที่ไม่ดี ทั้งๆที่การกระจายขนาดอนุภาคค่อนข้างแคบ และลักษณะของอนุภาคเป็นทรงกลมพื้นผิวไม่เรียบ มีการเกาะกลุ่มกัน จากการศึกษาพบว่าสารสกัดดายขัด ยาเม็ดแกน มีสีน้ำตาลอ่อน ผิวเรียบ เงา และเป็นมัน ยาเม็ดเคลือบฟิล์มไฮดรอกซีโพรพิลเมธิลเซลลูโลส และยาเม็ดเคลือบฟิล์มโพลีเมทาไครเลท ที่เตรียมได้นั้นมีลักษณะทางกายภาพที่ดีและมีการดูดความชื้นต่ำ ส่วนยาเม็ดเคลือบฟิล์มไคโตซานจะมีลักษณะทางกายภาพที่เปลี่ยนแปลงไปอย่างชัดเจน เม็ดยามีการดูดความชื้นสูง สีของเม็ดยาเข้มขึ้น ปริมาณความเข้มข้นของสารก่อฟิล์ม มีผลต่อความชื้น และรูปแบบการละลายอย่างมีนัยสำคัญ ซึ่งชนิดของสารก่อฟิล์มส่งผลเล็กน้อยต่อค่าการกระกระจายตัวของเม็ดยา เมื่อศึกษาความคงตัวของเม็ดยาเหล่านี้ที่สภาวะเร่ง เป็นเวลานาน 4 เดือน พบว่าปริมาณสารสำคัญของยาเม็ดเคลือบฟิล์มที่เคลือบด้วยไฮดรอกซีโพรพิลเมธิลเซลลูโลส และ โพลีเมทาไครเลท มีปริมาณอยู่ในช่วงที่กำหนดตามมาตรฐานเภสัชตำรับ และมีลักษณะที่ดี โดยปริมาณสารสำคัญที่เหลือ คือ 90.41% และ 90.65% ของยาเม็ดเคลือบฟิล์มที่เคลือบด้วย 5% ไฮดรอกซีโพรพิลเมธิลเซลลูโลส และ 2% โพลีเมทาไครเลท ตามลำดับ อย่างไรก็ตาม เมื่อเก็บยาไว้ที่ 6 เดือน ยาเม็ดเคลือบฟิล์มที่เคลือบด้วยไฮดรอกซีโพรพิลเมธิลเซลลูโลส จะแสดงลักษณะปรากฏที่ไม่ดี คือเม็ดยามีการบวม โดยสรุปสูตรยาเม็ดที่เคลือบด้วย โพลีเมทาไครเลท จะแสดงลักษณะปรากฏที่ดี มีความคงตัวดีที่สุด

ภาควิชา.....เภสัชอุตสาหกรรม..... ลายมือชื่อนิสิต.....

สาขาวิชา.....เภสัชอุตสาหกรรม..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2550 .... ลายมือชื่ออาจารย์ทีปรึกษาร่วม.....

4876590333 : MAJOR INDUSTRIAL PHARMACY
KEY WORD: MALVASTRUM COROMANDELIANUM / FILM COATED / TABLET

PORNSRI PRASERTWAREE: FORMULATION DEVELOPMENT OF FILM COATED TABLETS CONTAINING *MALVASTRUM COROMANDELIANUM* EXTRACT. THESIS ADVISOR: PROF. GARNPIMOL C. RITTHIDEJ. Ph.D., THESIS COADVISOR : SUKANYA JESADANONT, Ph.D., 104 pp.

The spray-dried powder of *Malvastrum coromandelianum* water extract has been reported to be able to lower blood sugar for diabetic rabbits. It was also utilized for treating diabetic patients. However, the dried powder turned from pale brown into damp dark brown mass after storage for a few days, even filled into hard gelatin capsule. Therefore, the purpose of this study was to develop film coated tablets of high dose of *Malvastrum coromandelianum* powder. The spray-dried powder was evaluated for particle size, flow, and density. The core tablets were produced on a single-punch tablet press by direct compression method and using various fillers and lubricants to obtain optimal core tablet formulation. The coating process was performed using hydroxypropylmethylcellulose (HPMC), chitosan and Eudragit E100 as film former by Thai coater. These tablets were evaluated for friability, hardness, disintegration and % drug dissolved at 60 minutes. The content was analyzed by high performance liquid chromatography. The influences of type of polymeric films HPMC, chitosan and Eudragit E100 on adhesive strength and moisture protection were evaluated both initially and accelerated conditioned (45 ± 2 °C /75 ± 5 % RH for 4 months). The results found *Malvastrum coromandelianum* spray-dried powder exhibited poor flow property in spite of narrow sizes and spherical shape particles with rough surface and aggregation in cluster. The selected core tablets showed satisfactory properties, having light brown color with smooth and shiny surface. HPMC and Eudragit E100 film coated tablets showed good appearance and physical properties of high mechanical resistance with low moisture sorption. The chitosan coated tablets exhibited the highest moisture sorption and color change. The concentration film coated significantly influenced both moisture and dissolution pattern while the type of polymer had slight effect on disintegration time. After storage at accelerated condition, the content of both HPMC and Eudragit E100 coated tablets was still within the standard of pharmacopoeia and good appearance. The percent remaining of the active compounds was 90.41% and 90.65% of the initial content as HPMC5 and PMC2, respectively. However, HPMC coated tablets showed poor appearance of swelling after storage for 6 months. In conclusion, Eudragit E100 film coated tablets of high dose of *Malvastrum coromandelianum* extract could be prepared with satisfactory properties and stability.

Department.....Manufacturing.Pharmacy...Student's.signature.....

Field of study ....Industrial Pharmacy.....Advisor's signature.....

Academic year ..2007.....Co-advisor's signature.....

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LIST OF ABBREVIATIONS

^{o}C	=	degree Celsius
cm	=	centimeter
cm^2	=	square centimeter
CS3	=	Tablet coated with chitosan at 3% coating level
CS5	=	Tablet coated with chitosan at 5% coating level
CV	=	coefficient variation
DSC	=	Differential Scanning Calorimetric
e.g.	=	*exemplit gratia*, "for example"
et al.	=	*et alii*, "and others"
FDA	=	Food and Drog Administration
g	=	gram
HPLC	=	High performance liquid chromatography
HPMC	=	hydroxypropyl methylcellulose
HPMC3	=	Tablet coated with HPMC at 3% coating level
HPMC3	=	Tablet coated with HPMC at 5% coating level
Kg	=	kilogram
kN	=	kilonewton
kp	=	kilopound
BW.	=	body weight
mg	=	milligram
min	=	minutes
ml	=	milliliter
mm	=	millimeter
mPa	=	millipascal
M.W.	=	molecular weight
N	=	normal (concentration)
pH	=	the negative logarithm of the hydrogen ion concentration
PMC1	=	Tablet coated with polymethacrylate at 1% coating level

PMC2	=	Tablet coated with polymethacrylate at 2% coating level
r^2	=	coefficient of determination
RH	=	relative humidity
rpm	=	revolutions per minute
S.D.	=	standard deviation
sec	=	second
T_g	=	glass transition temperature
µl	=	microliter
µm	=	micrometer
USP	=	The United States Pharmacopoeia National Formulary
g /kg	=	gram per kilogram
w/w	=	weight by weight
v/v	=	volume by volume
WVP	=	water vapour pressure

CHAPTER I
INTRODUCTION

Malvastrum coromandelianum (Linn.) is a Thai medicinal plant that has been claimed to treat diabetes mellitus. The therapeutic use of this herbal remedy with its wide range of applications has been well known in Philippines, tropical America, The plant is widely distributed in the tropics (Merrill, 1912). This plant continues to be used within the framework of folk medicine as effective remedy (Reddy et al., 2000). Different uses are claimed for the plant. The more common usages are as a anti-inflammatory, analgesic, antidysenteric and in the treatment of jaundice and ulcers. The phytochemical compounds of *Malvastrum coromandelianum* have been determined to be alkaloids, flavanoids, lactone glycosides (non-volatile) and saponin (จิรดา และคณะ., 2007).

Plant medicine is normally used as crude or extract. Normally, the ethanolic or aqueous extracts are dried by lyophilization or spray drying which latter technique is more popular. However, the problem of spray dried powder is poor flow ability and instability, due to the amorphous form.

Various dosage forms can be employed. The most commonly used is as tablets. However, the tableting of formulation containing high dose extract is dominated by the poor compression properties of the extract. Moreover, herbal dry extracts are subject to natural variations influencing the formulation and production of the extract in the solid dosage forms. Eggelkraut-Gottanka et al., (2002) have addressed techniques to overcome these problems: wet granulation using nonaqueous solvents, dry granulation by roller compaction and direct compression after loading the extracts onto fumed silica.

Direct compression can provide technical as well as economic benefits. Stability of certain drugs can be improved, and the elimination of a wetting and drying process can be beneficial when formulating drugs that are thermolabile or moisture sensitive (Davies, 2001). After dry mixing with appropriate excipients it is

possible to obtain, by direct compression without manipulation, tablets that are technologically satisfactory in terms of hardness, disintegration and friability (Bonati, 1991). The dissolution rate can be improved by utilizing direct compression. In the section on disintegration, it is stated that for optimal dissolution, the tablets have to disintegrate into its primary particles as quickly as possible (Davies, 2001).

Tablets of plant dry extracts appear heterogenous and their taste and smell are often unacceptable. The unpleasant flavor or odor of certain drugs and the difficulties related to swallowing bitter tasting dosage forms have been reported as the primary reasons for incompliance with drug therapy. Although the presence of artificial flavors and sweeteners can improve the palatability of a dosage form, the application of a coating around the drug particles of around the final dosage form has been demonstrated to provide a superior result by preventing the molecules from reaching the taste sensors (Cerea et al., 2004). This usually requires a film coating using a non modified polymer like hydroxypropylmethylcellulose (HPMC) (Kleinebudde, 2004).

Film coated tablet is a pharmaceutical dosage form which surface is deposited with a thin plastic-like material consisting of polymer. Polymeric film coating have been applied to pharmaceutical dosage form for a variety of reasons including masking unpleasant taste, odor and color of the drug, imparting a more glossy and elegant appearance, protecting the active ingredients against surrounding environment, increasing mechanical stability and preventing dust formation during subsequent packing and shipment, separating incompatible active ingredients, and ensuring the controlled or modified release of drug (Porter, 1990; Radebaugh, 1992; Pourkavoos and Peck, 1993; Bauer et al., 1998; Davies, 2001). The polymeric matters that are widely used in film coating are the cellulose derivatives. The most common material in this class is hydroxypropylmethylcellulose (HPMC). Besides these cellulose ethers, another chemical namely methacrylic acid-methacrylic acid ester copolymers are also possibly used. In addition, chitosan, a natural biopolymer, has a close chemical relative of cellulose and also has the ability to dissolve in aqueous medium, thus this material could be used as film former with aqueous base system for tablet film coating approaching to both fast or extended drug release depending on characteristics of selected films that are in soluble or insoluble form (Phaechamud,

1999). These film formers play a protective role in drug stability and mask the unpleasant taste (Bauer et al., 1998).

The spray dried powder of MC aqueous water extract filled in hard gelatin capsules showed good clinical treatment for diabetic patients. However, the dry powder in capsule turned from pale brown into damp dark brown mass after storage only for a few days. Therefore, the development of film coated tablet of *Malvastrum coromandelianum* powder was of interest. In this investigation, it was aimed to develop the formulation of *Malvastrum coromandelianum* extract film coated tablets for prevention and providing the good stability. Their physicochemical properties were evaluated. The products stored under the recommended conditions following the Thai FDA guidance were also tested.

The objective of the present study were:

1. To evaluate the physicochemical properties of the spray dried powder of *Malvastrum coromandelianum* extract.
2. To develop and evaluate the formulation of *Malvastrum coromandelianum* extract film coated tablets using HPMC, chitosan and polymethacrylate as film former.
3. To study the stability of *Malvastrum coromandelianum* extract film coated tablets under ambient condition (30 ± 2 °C and 75 ± 5 % RH) compared with the accelerated condition (45 ± 2 °C and 75 ± 5 % RH) for 4 months.

CHAPTER II
LITERATURE REVIEW

I. *Malvastrum coromandelianum* (Linn.) Garcke

Malvastrum coromandelianum (L.) Garcke, belonging to the Malvaceae family, an erect, somewhat hairy, branched, suffrutescent, perennial plant 1 metre high or less. Leaves oblong to ovate-lanceolate, obtuse, base usually rounded, irreugularly toothed, 2 to 5 cm long. Fruit consisting of from 8 to 12 carpels, reniform, compressed, hirsute carpels 2 to 3 mm long, each carpel with 8 short straight awns. Abundant in west laces, throughout the Philippines. A native of tropical America, now widely distributed in the tropics (E.D. Merrill., 1912).

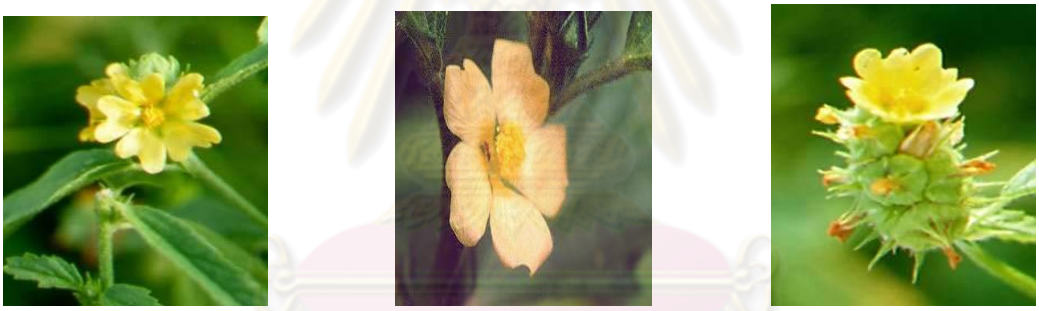


Figure 1. *Malvastrum coromandelianum* (Linn.) Garcke (www.malvaceae.info/General/Malvastrum/gallery.html.)

Malvastrum coromandelianum (L.) Garcke or "Dai-Kat" in Thai was one of medicinal plants scientifically investigated by the Medicinal Plant Research institute, Department of Medical Sciences. We previously showed that water extract of this medicinal plant exhibited hypoglycemic effect in diabetic rabbits. This paper reported chronic toxicity study of this plant in Wistar rats. Water extract of *M. coromandelianum* at the doses equivalent to crude drug 0.2, 2 and 20 g/kg/BW/day was given and hematological parameters of all extract-treated groups were not significantly different from those of female rats, but not male rats, treated with the extract was significantly lower than that of the control group. Biochemical study of

serum samples showed that cholesterol level of male rats receiving the extract the 20 g/kg BW was significantly lower than that of the control. In addition, potassium levels of male rats receiving that extract at the doses of 2 and 20 g/kg BW were also significantly lower than that of the control, while female rats treated with the extract 20 g/kg BW had significantly lower albumin level than their control.

A botanical products are contrast to single chemical entities, there are difficulties inherent in trying to apply pharmaceutical current Good Manufacturing Practices to botanical products. There is variability within the same plant materials this variability may be from grower to grower and crop to crop, and also depends on harvest and postharvest handling. Variability also occurs between different plant parts. Herbals have multiple constituents including active compounds, inactive, unknowns, and elements which are dietary rather than therapeutic. Stability is more difficult to monitor and reference standards are more difficult to establish and obtain than for chemically synthesized drugs (Natalie J., 1998).

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents observed by chromatography. Chromatographic techniques such as HPLC, gas chromatography (GC) have been used for identity tests. Spectroscopic methods such as nuclear magnetic resonance (NMR) and ultraviolet-visible (UV-vis) may also be used for fingerprinting. A marker compounds and chromatographic profiles (fingerprints) are used to help in identification of herbals and in assessment of their potency and stability (Natalie J., 1998).

When embarking on development of assays, it must first be decided which compounds to quantitative. If a principle active component is known, it is most logical to quantitative this compound. Where active ingredients contributing to therapeutic efficacy are known, botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known, a marker substance which should be specific for the botanical could be chosen for analytical purposes, although it should only serve for internal batch control. Single or multiple markers can

be used to ensure that the concentration and ratio of components in and herbal mixture are present in reproducible levels in raw materials, manufacturing intermediates and in the final dosage forms (Natalie J., 1998).

Chromatographic testing of botanicals can be carried out for two purposes: identification and potency determination. Ideally, an active principle can be quantitative. In botanicals, however, this is not always the case. If it is not possible to identify the active principle with certainty, then the second best approach is to identify and develop markers that allow fingerprinting, to ensure batch-to-batch consistency. Identification may be achieved by confirming the presence of active principles, looking for single or multiple markers and by the chromatographic fingerprints (Natalie J., 1998).

Marker compounds may be monitored for stability to determine shelf life of a formulation and packaging system, if real-time and accelerated studies are carried out at different temperature (humidity) conditions. Stability may be investigated in a manner similar to chemical pharmaceuticals; an Arrhenius treatment may be used to predict stability at temperatures other than those studies (Lachman L et al., 1976).

Marker compounds may also be of use in assessing the potency of herbals used for toxicity testing and establishing dosage ranges. Assays for the presence of marker compounds can in this way be useful to validate classical toxicity tests, giving an indication that the constituents of the herb are actually being absorbed and not just passing through the digestive system of the test animals. The concentration of marker compounds can be determined in animal blood and serum, giving and indication of dosage levels (Natalie J., 1998).

II. Diabetes mellitus

The estimated worldwide prevalence of diabetes is 194 million and this is expected to increase to 333 million by 2025 (Diabetes atlas executive summary., 2003). Over 80% of patients have Type 2 diabetes with a significant proportion

remaining undiagnosed. Developing countries, in particular, have a rising incidence of Type 2 diabetes as a consequence of lifestyle factors.

Diabetes affects a patient's quality of life and well-being, with the greatest negative impact occurring in the freedom to eat as desired (Bradley C, 2002).

Diabetes mellitus is a group of metabolic disorders with different underlying etiologies, each characterized by hyperglycemia due to underutilization of glucose. The disease is classified into several major groups.

Type 1 diabetes mellitus, formerly known as insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes mellitus. Type 1 diabetes is a metabolic disorder resulting from absolute insulin deficiency secondary to autoimmune pancreatic β-cell destruction, rendering the pancreas unable to synthesize and secrete insulin (Castano L et al., 1990). Although the peak incidence occurs between the ages of 10 and 14 years, it can occur at any age (Cruickshanks KJ, 1985). The incidence and prevalence of Type 1 diabetes in children < 14 years of age varies globally with a very high incidence (≥ 20/100,000 per year) in the UK, Finland, Sweden, Canada and New Zealand and a much lower incidence (<1/100,000 per year) in China and South America (Karvonen M, 2000).

Type2 diabetes mellitus, formerly known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes mellitus. The pathophysiology of Type 2 diabetes consists of relative insulin deficiency combined with insulin resistance, which increases with obesity. Lifestyle modification and oral glucose-lowering drugs are the initial management tools in Type 2 diabetes; deterioration of glycemic control despite maximal oral therapy is followed by initiation of insulin. However, hypoglycemia, weight gain, suboptimal dose initiation and titration are all barriers to the initiation of insulin therapy in Type 2 diabetes. In contrast to Type 1 diabetes, the insulin resistance associated with Type 2 diabetes requires the administration of higher insulin doses to achieve optimal glycemic control. Type 2 is the most common form, accounting for 90-95% of diabetes in developed countries (Reaven GM, 1988; Sacks DB et al., 1996).

The increasing prevalence of diabetes in most populations, especially Type 2 diabetes, has had a major impact on healthcare systems worldwide (Zimmet P et al., 2001). Factors such as obesity, sedentary lifestyle, ageing population, improved medical care and decreasing mortality rates all contribute to this increase. In 2005, it was estimated that there were 2,103,000 patients with both Types of diabetes in the UK (Diabetes UK, 2005).Overall, 9% of the NHS budget is currently spent on treating diabetes and its complications (Department of health, 2001).

The Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) (Stratton IM et al., 2000) indicated that in Type 1 and Type 2 diabetes, respectively, intensive glucose control delayed or prevented the development of complications. However, intensive glucose control, as defined by maintaining glycosylated haemoglobin (HbA_{1C}) <7%, can be difficult to achieve. In the UK, the National Diabetes Audit predicted that only 56% of patients achieved HbA_{1C} of < 7.5% and 23% achieved that of < 6.5% (Department of health, 2005) The attainment of adequate, yet safe, glycemic control is likely to be further complicated by recent recommendations for HbA_{1C} targets < 6.5% (National institute for health and clinical excellence, 2002)

The epidemic of type 2 diabetes in the latter part of the 20th and in the early 21st century, and the recognition that achieving specific glycemic goals can substantially reduce morbidity, have made the effective treatment of hyperglycemia a top priority (American Diabetes Association, 2005). While the management of hyperglycemia the hallmark metabolic abnormality associated with Type 2 diabetes, has historically had center stage in the treatment of diabetes, therapies directed at other coincident features, such as dyslipidemia, hypertension, hypercoagulability, obesity and insulin resistance have also been a major focus of research and therapy. Maintaining glycemic levels as close to the non-diabetes range as possible has been demonstrated to have a powerful beneficial impact on diabetes-specific complications, including retinopathy, nephropathy and neuropathy in the setting of Type 1 diabetes (Diabetes Control and Complications Trial Research Group, 1993); in Type 2 diabetes, more intensive treatment strategies have likewise been demonstrated to

reduce complications (Ohkubo Y et al., 1995). Intensive glycemic management resulting in lower HbA_{1C} levels has also been shown to have a beneficial effect on cardiovascular disease (CVD) complications in type 1 diabetes (Diabetes Control and Complications Trial., 2005); however, the role of intensive diabetes therapy on CVD in type 2 diabetes remains under active investigation (Bastien A, 2004).

III. Tablets

Tablets are solid dosage forms containing medicinal substances with or without suitable diluents. The vast majority of all tablets manufactured are made by compression and compressed tablets are the most widely used dosage form. Mostly, tablets are used in the oral administration of drugs. Other tablets, as those administered sublingually, buccally or vaginally are prepared to have features most applicable to their particular route of administration. Tablets were traditionally used as dispensing tablets in order to provide a convenient, measured quantity of a potent drug for compounding purposes (Parrott, 1970; Connolly et al., 1990; Davies, 2001; Allen et al., 2005).

Tablets are prepared by three general methods: wet granulation, dry granulation (roll compaction or slugging), and direct compression. The purpose of both wet and dry granulation is to improve flow of the mixture and/or to enhance its compressibility (Allen et al., 2005).

Direct compression

Direct compression is the term used to define the process where powder blends of the drug substance and excipients are compressed on a tablet machine. There is no mechanical treatment of the powder apart from a mixing process. Direct compression avoids many of the problems associated with wet and dry granulations. After compression, tablets may be coated with various materials. However, this method places greater demands on the excipients, particularly the filler. The designed specifically excipients for use in direct compression formulations are introduced, although they trends to be expensive (Davies, 2001).

IV. Tablet dosage form of dry plant extract

The formulation of plant extracts into dosage forms is a complex operation which cannot be regarded only as a problem of pharmaceutical technology. Unlike pure active principles, whether synthetic or natural, extracts are raw materials that always contain, alongside variable but small amounts of the active principles, large quantities of secondary material that can appreciably affect the technology of preparation and the stability of the finished pharmaceutical form (Bonati, 1991). Dry herbal extracts are widely used as solid dosage forms (Eggelkraut-Gottanka et al., 2002). Several problems may arise in the course of processing, especially if the formulation involves large quantities of extracts. Sugars or saponins, frequently present as secondary components of extracts, dissolve in water used for mixing and form granule hard flakes of glassy appearance, making the granules hard to compress (Bonati, 1991 and Kleinebudde, 2004). The problem found in granulation with aqueous solution is the formation of compressed tablets that are difficult to disintegrate. To overcome the problems, wet granulation with organic solvents, dry granulation by roller compaction or use of non-hygroscopic ready-granulated extracts in direct compression method is particularly useful (Bonati, 1991).

V. Film coated tablets

Pharmaceutical coating technologies for solid oral dosage forms are generally based on the use of polymeric materials in solution or dispersed in aqueous or organic vehicles.

Film coated tablets are compressed tablets coated with a thin layer of a polymer capable of forming a skin-like film over the solid substrate. The substrate can be tablets, beads, granules, capsules, pellets, drug powders or particles (Porter and Bruno, 1990). Though new uses of coatings are being continually developed, the following categories cover most current uses (Seitz, 1988 and Radebaugh, 1992) : (a) protection of drugs in the substrate from environmental factors such as light, moisture and air, in order to improve chemical and physical stability, (b) modification of

product appearance to enhance to marketability and product identity or hide undesirable color changes of the substrate, (c) masking of unpleasant taste, texture and odor, (d) enhancement of swallow ability, (e) a mechanical barrier to the interaction of incompatible ingredients by coating one or more of the individual ingredients, (f) improved handling during packaging operations by reducing dust formation, (g) controlled or modified release of drugs (e.g. enteric coating and sustained release).

Gastric-soluble polymers are used to protect ingredients from light, moisture and oxygen, for taste masking and for identification if a colored film is used. Intestine-soluble polymers and permeable polymers which provide drug diffusion are utilized for retardation or local effects (Cole, 1995).

1. Taste masking property of the film

The unpleasant flavor or odor of certain drugs and the difficulties related to swallowing bitter tasting dosage forms have been reported as the primary reasons for incompliance with drug therapy (Aronson and Hardman, 1992). Although the presence of artificial flavors and sweeteners can improve the palatability of a dosage form, the application of a coating around the drug particles or around the final dosage form has been demonstrated to provide a superior result by preventing the molecules from reaching the taste sensors.

The use of pH dependent polymers offers a different approach to taste masking than addition of artificial flavors or the use of rapidly disintegrating films (Cerea et al., 2004). Polymers with basic amino groups are used for flavoring and taste masking. They do not dissolve but swell in saliva (pH 6.8-7.4), and dissolve only in the acid environment of the stomach (Lin et al., 2000; Cerea et al., 2004).

Film coating using HPMC also have become popular because they give a mask the unpleasant taste of the drug substances, superior appearance, act as protection for fragile tablets and stable in the presence of heat, light, air and moisture and its film are flexible, tolerate the presence of colorants and other additives and are resistant to abrasion (Seitz, 1988; Nagai et al., 1997). The main reason for the

extensive use of HPMC as a film-coating polymer is that it is soluble in some organic solvents and also in water over the entire biological pH range. Film coating could therefore be done using an organic solvent system and the film formed will dissolve in the digestive juices, leading to complete release of the active ingredients.

Bajdik et al. (2004) studied the crystal coating of dimenhydrinate by using as film former to promote the tablet masking, increasing the flowability, compressibility, protect from several harmful factors. (light, moisture, and heat transition) and prepared the tasteless solid dosage form.

Tablets prepared using plant dry extracts appear heterogeneous and their taste and smell are often unacceptable. This usually requires a film coating using a non modifying polymer like HPMC (Kleinebudde, 2004).

2. Protective property of the film

Film permeability is usually related to the hydrophobic or hydrophilic nature of the polymer. Permeation is important in coating that enhance stability by protecting the substrate from gases such as oxygen or carbon dioxide or from water vapor (Radebaugh, 1992).

Munden et al. (1964) studied the water-vapor transmission and oxygen permeability of a variety of free films that included cellulose acetate butyrate. The method for water permeability used a modified version of ASTM E96-53T. Films that were very permeable to water vapor were almost impermeable to oxygen and vice versa.

Swarbrick and Amann (1972) studied parameters affecting water-vapor transmission through cast and sprayed cellulose acetate phthalate (hydrophilic) and n-butyl methacrylate (lipophilic) films, using a permeation cell. Significant differences in permeability were observed with the cellulose acetate phthalate films, depending on whether moisture was present on one or both side. These differences were not seen with n-butyl methacrylate films. The authors attributed the observed behavior to

partial hydration of the cellulose acetate phthalate film, a phenomenon to remember when designing realistic testing conditions. The method of film preparation, cast of sprayed, did not affect the permeation characteristics of cellulose acetate phthalate films.

The moisture absorption of HPMC coated pharmaceuticals may occur at very high humidity. The water vapor permeability (WVP) of various viscosity grades of HPMC was studied. The WVP tended to decrease as viscosity decreased. The WVP pf applied films was always higher than that of free films, which might reflect higher porosity (Nagai et al., 1997).

Although chitosan films are highly impermeable to oxygen, they have relatively poor water vapor barrier characteristics. Plasticizers have negative effects on barrier properties and positive effects on mechanical properties. The functional properties of chitosan films are improved when chitosan is combined with other film-forming materials (Xu et al., 2005).

Eudragit ® E has been demonstrated to be an effective moisture protective film coating (Chowhan et al., 1982; Thoennes and McCurdy, 1989; Cerea et al., 2004).

A. Film forming polymer

In this study three types of film-forming polymers which were interested are HPMC, chitosan and polymethacrylate (Eudragit®E 100). There are various in the properties as following:

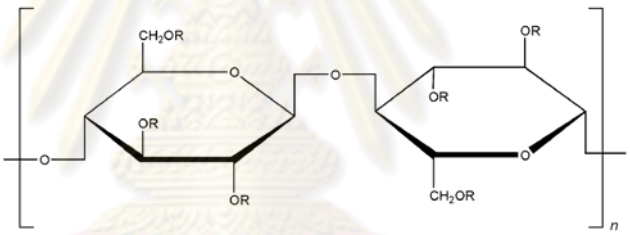
Hydroxypropylmethylcellulose (HPMC)

Hydroxypropylmethylcellulose (HPMC), a cellulose derivative, is widely used as an excipient in oral and topical pharmaceutical formulations. HPMC is primarily used as tablet binder, in film-coating and as a rate-control polymer for sustained release tablet matrix. It is also used as an emulsifier, suspending agent,

stabilizer, thickening agent and protective colloid. HPMC is generally regarded as a nontoxic and nonirritant material (Kibbe, 2000).

HPMC is an odorless and tasteless, white or creamy-white colored fibrous or granular powder. Its chemical structure is shown in figure 2.It is soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol and ether, but soluble in mixtures of methanol or ethanol and dichloromethane. HPMC powder is a stable material although it is hygroscopic after drying.

HPMC absorbs moisture from the atmosphere, the amount of water absorbed depending on the initial moisture content and the temperature and the temperature and the relative humidity of the surrounding (Kibbe, 2000).



R is H or $[CH_2CH(CH_3)O]_mH$

Figure 2. Chemical structure of hydroxypropylmethylcellulose (HPMC)

Chitosan

Chitosan is a polysaccharide, similar in structure to cellulose. Both are made by linear β-(1-4)-linked monosaccharides. However, an important difference to cellulose is that chitosan is composed of 2-amino-2 deoxy-β-D-glucan combined with glycosidic linkages. The primary amine groups render special properties that make chitosan very useful in pharmaceutical applications. Compared to many other natural polymers, chitosan has a positive charge and is mucoadhesive (P.C. Berscht et al., 1994). Therefore, it is used extensively in drug delivery applications.

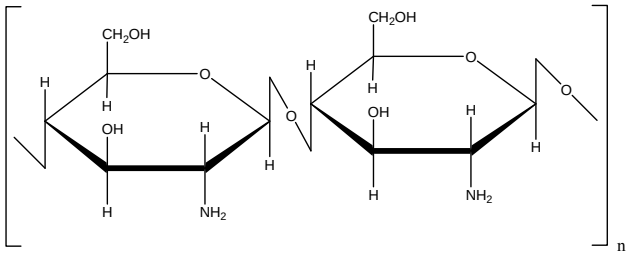


Figure 3. Chemical structure of chitosan

Chitosan, being a cationic polysaccharide in neutral or basic pH conditions, contains free amino groups and hence, is insoluble in water. In acidic pH, amino groups can undergo protonation thus, making it soluble in water. Solubility of chitosan depends upon the distribution of free amino and N-acetyl groups (T. Sannan et al., 1976). Chitosan is biocompatible with living tissue since it does not cause allergic reactions and rejection. It breaks down slowly to harmless products (amino sugars), which are completely absorbed by the human body (S. Nicol et al., 1991).

Chitosan could be used as film former for coated pharmaceuticals. Since it is soluble in diluted acidic medium, attempt to prepare in insoluble from is to expand its use in controlled systems. Dry heat treatment to chitosan acetate film was reported to increase its water resistance which was attributed to the cross-linking of chitosan molecules and/or the formation of anhydrous crystalline in the structure. In addition, chitosan acetate film was reported to be poorly soluble under accelerated conditions and became insoluble after moist heat treatment (Lim and Wan, 1995; Yamada, 1992).

Phaechamud (1999) also found that the hydrolysis of chitosan acetate which was resulted from the interaction between NH_3^+ of chitosan and CH_3COO^- of acetic acid changed the physicochemical properties of the propranolol HCI coated tablets especially the color and solubility of coated films. The drug release was markedly decreased. Chitosan acetate gave appropriate film characteristics for sustained-release coating in all pH range media.

The chitosan citrate film coated onto the core tablet has the satisfactory characteristics, smooth homogenous and well attached. However, the dissolution of the model drug, propranolol hydrochloride, from coated tablets was pH dependent. Moreover, the retardation of disintegration and drug dissolution of these coated tablets was evident after exposure accelerated conditions but not in the case of one year storage at room temperature (Phaechamud et al., 2000).

Ritthidej et al. (2002) reported that the interactions between chitosan and carboxylic acids were associated with electrostatics reaction in aqueous solutions and formed salts in cast films. Moreover, moist heat treatment of 60 °C and 75% RH could change ionic interaction to rather homogeneous amide formation in chitosan film. Therefore, the percentage of water sorption and dissolution of chitosan salt films were depending on the type of carboxylic acid added.

Polymethacrylate

Eudragit ® E is a cationic polymer prepared by copolymerization of buthyl methacrylate, 2-dimethyl aminoethylmethacrylate and methyl methacrylate with a mole ratio of 1:2:1, and is always used as a film-coating former (see Table). The product is commercially available as Eudragit® E 100 (M.W > 150,000), in the form of cylindrical granules approximately 1 to 3 mm long. Eudragit ® E is soluble at a pH below 5.5 (Lehmann, 1968). This polymer can prevent the release of the delivered drug in saliva (pH 6.8-7.4) and dissolves in gastric fluids (pH 1.0-1.5) (Ishikawa et al., 1999). Given a neutral or slightly alkaline environment, the film swells strongly in water and disintegrates (Kibbe, 2000; Cereaet al., 2004). Furthermore, this polymer has been demonstrated to be an effective moisture protective film coating. Eudragit acrylic resins have been widely used in the pharmaceutical industry as a film coating for bitter taste prevention and controlled drug delivery. (Chowhan et al., 1982; Thoennes and McCurdy, 1989). Furthermore, the use of a polymer soluble at gastric pH such as Eudragit® E, would allow rapid release of drug for absorption in the gastrointestinal tract.

For application, Eudragit® E is diluted or dissolved to 4 to 10 %. Addition of plasticizer is not necessary, but talcum or magnesium stearate should be used as a glidant (Bauer et al., 1998). A self-adhesive film for transdermal use, it was found that triacetin is a good first plasticizer for drug free Eudragit E film. On the other hand, for film coating of the tablets, tributyl citrate was indicated that it may be the best choice of plasticizer for Eudragit film, particularly for the Eudragit E film (Lin et al., 2000).

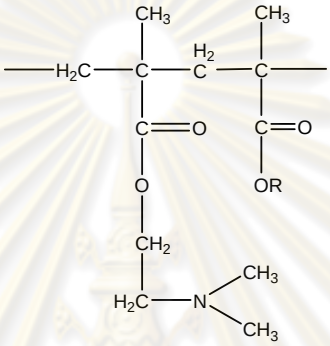


Figure 4. Chemical structure of polymethacrylate

B. Plasticizer

Most of the polymers that are used in pharmaceutical film coatings are amorphous in nature (Porter and Bruno, 1990). Because the glass-transition temperature (Tg) of many of the polymers used in film coating are in excess of the temperature conditions (Porter and Bruno, 1990), they exhibit brittle, tough, hard and stiff properties and require the addition of a plasticizing agent to obtain an effective coating that is free of cracks, edging or splitting. Plasticizers function by weakening the intermolecular attractions between the polymer chains, which generally results in a decrease in the tensile strength, a lowering of the films (Felton and McGinity, 1997). Plasticizer not only enhances flexibility and reduces the brittleness of the film but also may control the drug penetration through the polymeric film (Porter and Bruno, 1990; Bruno et al., 1998). Thus, plasticizer plays an important role in the polymeric film coating (Felton and McGinity, 1997; Lin et al., 2000).

Plasticizers are usually high-boiling liquid, sometimes also polymeric substances of low molecular weight which should disperse as homogeneously as possible in the film formers to be modified. By interacting with the film-forming polymers, they alter certain physical and mechanical properties by enhancing the mobility of the polymer chains. Plasticizers act by penetrating between the chains of the film-forming polymer and interact with functional groups, thereby reducing the interactions among the polymer chains in the film and softening the matrix. The glass temperature of the system decreases as a result of the increased segmental mobility, and the film becomes plastic in the temperature range for processing or use. Crosslinked, polymers become rubber elastic. As far as polymeric film coating are concerned, these effects can be achieved by either “external plasticizing” i.e. adding suitable substances to the coating formulations, or “ internal plasticizing” i.e. copolymerization with softening monomers of greater chain length (Seitz, 1988; Radebaugh, 1992; Bauer et al., 1998).

Plasticizers for pharmaceutical purposes must be (a) colorless, (b0 odorless, (c) non-volatile, (d) thermally stable, (e) water-resistant, (f) chemically resistant, (g) compatible with the polymeric film formers, (h) non-migrating in films and (i) physiologically harmless (Bauer et al., 1998; Lin et al., 2000).

Plasticizers commonly used in film coatings can be conveniently divided into three groups: 1. the polyols, 2. the organic esters and 3. the vegetable oils and glycerides. The former are used as plasticizers for the water-soluble polymer and the latter two are used for enteric or sustained release coatings. Drugs may also change the mechanical properties and adhesion strength of the film due to drug-polymer interaction (Lin et al., 2000).

For a good film-forming process, the polymers used should show adequate chain flexibility under conventional film-coating conditions. For practical and economic reasons, a plasticizer selected for pharmaceutical purposes will lower the glass temperature of the film-forming polymers effectively at the smallest possible concentration. Moreover, the effectiveness of plasticizers in the coating formulation

depends on further factors, however, e. g. other excipients, solvent systems, application methods, etc.

Polyethylene glycol (PEG), especially a high molecular weight type such as PEG 6000, is a suitable plasticizer for HPMC. Although a greater effect is expected as the content of plasticizer increase, it should preferably be added at the minimum effective level (usually 20-30 % based on the polymer). Excessive amounts of plasticizer may cause tablet tacking, plasticizer bleeding, color depletion, or interaction with the active ingredients. PEG is also effective as a plasticizer to some extent but tends to volatilize during the coating process (Nagai et al., 1997).

Phaechamud (1999) was studied the effect of plasticizers on the physicochemical properties of chitosan citrate films. It was found that an addition of 25% w/w propylene glycol could produce the satisfactory chitosan citrate film.

Lin et al. (2000) investigated the effect of the organic esters used as plasticizers on water absorption behavior and adhesive property of Eudragit® E films and on the glass transition temperature (Tg) and plasticizer permanence of Eudragit® E film. Eudragit® E film plasticized with triacetin showed a slight water absorption, but when plasticized with diethyl phthalate (DEP), dibutyl phthalate (DBP), or tributyl citrate (TBC) did not. Eudragit® E film exhibited a greater adhesiveness than the Eudragit® RL or RS film, particularly with higher plasticizer concentration. Weight loss of the Eudragit® E film plasticized with triacetin or DEP was more pronounced with aging, but when plasticized with DBP or TBC weight loss was not seen. The results indicated that TBC may be the best choice of plasticizer for Eudragit® E film, particularly for the Eudragit® E film.

C. Solvents

Currently, the solvent will usually be water, although certain types of film coat may require organic solvents to be used. Commonly used solvents include alcohols (methanol, ethanol and isopropranol), esters (ethyl acetate and ethyl lactate), ketone (acetone) and chlorinated hydrocarbon (dichloromethane and

trichloromethane). The polymer should not only be soluble in the chosen solvent but should adopt a conformation in solution that greatest cohesion strength (Radebaugh, 1992).

Tablets are film coated by the application or spraying of the film-coating solution on the tablets in the coating pans. The volatility of the solvent enables the film to adhere quickly to the surface of the tablets.

Due to both the expense of the volatile solvents used in the film coating process and the environmental problem of the release of solvents, pharmaceutical manufacturers generally favor the use of aqueous-based system. The aqueous film coating technology has been widely utilized for the application of polymer film coating to pharmaceutical dosage forms. One of the problems attendants to these, however, is the slow evaporation of the water-base compared to the volatile organic solvent-based system (Porter and Bruno, 1990; Allen, Popovich and Ansel, 2005).

CHAPTER III
EXPERIMENTAL

I. Materials

All materials used in this study were obtained from commercial sources and use as received.

1. **Active ingredient:**

   Malvastrum coromandelianum spray-dried powder (Preserved Food Specialty Co., Ltd., Thailand)

2. **Tablet diluents:**

   - Lactose (Meggle Granulac® 200, Germany)
   - Colloidal silicon dioxide, Aerosil® ®(ZB 55869 Maxway Co., Ltd, Thailand)
   - Magnesium stearate USP (Faci Asia Pacific PTE, Ltd.)
   - Talcum (Haichen Talc Powder, China)

3. **Film formers:**

   - Hydroxypropylmethylcellulose, Methocel® E 5 LV Premium (Batch No. RD 19012404, Colorcon Ltd., England)
   - Hydroxypropylmethylcellulose, Methocel® E 5 LV Premium (Batch No. RD 19012406, Colorcon Ltd., England)
   - Chitosan M.W. 50000 (Seafresh Chitosan Co., Ltd, Thailand)
   - Polymethacrylate, Eudragit® E100, (Lot. No. 8311101177, Rohm Pharma Polymer, Germany)

4. Miscellaneous:

- Polyethylene glycol 6000 (Batch No. 427124/1 33901, Fluka Chemie Gmbh, Switzerland)
- Titanium dioxide (Batch No. UOJ0018, Rama production Co., Ltd, Thailand)
- Simethicone C100F (Batch No. 56180, Basildon Chemical Co., Ltd, England)
- Tartazine lake (Lot. No. 409154, Adinop Co., Ltd, Thailand)
- Brilliant blue lake (Rama production Co., Ltd, Thailand)
- Caffeine (Batch No. 014k0036, Sigma-Aldrich, Inc., USA)
- Citric acid monohydrate BP (Lot. No. 788, Srichand United Dispensary Co., Ltd, Thailand)
- Propylene glycol USP XXII (Lot. No. 2985424084, Srichand United Dispensary Co., Ltd, Thailand)
- Sodium Hydroxide
- Potassium Dihydrogen Orthophosphate, (Batck No, F2H145, Ajax, Finechem, Australia)
- Triacetin (Lot. No. 435795/1 24402107, Fluka)
- Acetonitrile HPLC (Burdick&Jackson, USA)
- Methanol HPLC (Burdick&Jackson, USA)
- Other solvents were AR grade

Equipment:

- Analytical Balance (Model XP 205, Mettler Toledo, Switzerland)
- Analytical Balance (Model PB 303, Mettler Toledo, Switzerland)
- Analytical Balance (Model PB 3002, Mettler Toledo, Switzerland)
- Analytical Balance (Model A 200 S, Sartorius, Germany)
- Scanning Electron microscope (Model S-2500, Hitachi, Japan) for morphology analysis of *Malvastrum coromandelianum*
- Hot air oven (Model BM600, memmert, Germany)

- Moisture Analyzer (Model HR83, Mettler Toledo, Switzerland)
- pH meter (Model 210 A+, Thermo Orion, Germany)
- Frita vibration (Model FT-150M, Fitra, Spain)
- Cubic- shape mixer (Model AR 400, ERWEKA, Germany)
- Single punch tableting machine (Viuheng Engineering, Thailand)
- Thai coater 15″ (L) (Pharmaceutical and Medical Supply, Ltd., Thailand)
- Peristaltic pump, (Model 503503S, watson-Marlow, Ltd, England)
- Modified tapped density tester (Chanchai Engineering, Thailand)
- Powder characteristic tester (PT-R model, Hosokawa Micron, Cheshire, UK)
- Tensile tester (Model LR10K, LLOYRD Instruments Limited, UK)
- Friabilator (ERWEKA TAR 20, Germany)
- Tablet hardness tester (Model 28/205, Dr.K Schleuniger Co., Switzerland)
- Disintegration apparatus (Model ZT 31 , ERWEKA, Germany)
- Dissolution apparatus (Model VK7000, Vankel, USA)
- High performance liquid chromatography (HPLC) (Model SCL-10A VP, Shimadzu, Japan):
  - Degasser (Model DGU-20A_3, shimadzu, Japan)
  - Pump A,B liquid chromatography (Model LC- 20 AD, Shimadzu, Japan)
  - Auto injector (Model SIL-20A, Shimadzu, Japan)
  - Column oven (Model CTO-20A, Shimadzu, Japan)
  - UV-VIS detector (Model SPD-M20A, Shimadzu, Japan)
  - System controller (Model SCL-A, Shimadzu, Japan)
- Differential Scanning Calorimetry (Model DSC822 ^{e} , Mettler Toledo, Switzerland)
- Microscope (Nikon Eclipse E 200 Model C-SHG, Japan)
- UV/VIS Spectrophotometer (Spectrophotometer Jasco V-530, Japan)

II. Methods

1. Characterization of *Malvastrum coromandelianum* spray-dried powder (MCS)

The dried plant was first pulverized. Then it was boiled, filtrated (brix=2 and 1.5 consequently), evaporated and finally spray-dried.

1.1 Morphology study

A small amount of MCS powder was viewed under an optical microscope and a scanning electron microscope (SEM). Before the SEM observation, the particle samples were coated with gold using an ion sputter coater under vacuum conditions. This approach allowed the particles to be observed on size and shape and thus could obtain in–dept morphological information.

1.2 Particle size distribution analysis

Ten grams of MCS powder was weighed and analyzed for particle size distribution using sieve analysis method. The Frita vibration apparatus with standard sieves mesh size number 20 (0.85 mm), 40 (0.425 mm), 60 (0.25 mm) and 80 (0.180 mm) were used. After 10 minutes of vibration, the fractions of each particle size were weighed and calculated in percent of distribution.

1.3 Density and compressibility assay

To determine the bulk density of the sample, a known quantity of the powder (10 g) was gently poured through a 25-ml graduate cylinder. The cylinder was then lightly tapped to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder. The bulk density (ρ_b) was calculated as the ratio between weight (g) and volume (ml).

To determine the ultimate tapped density (ρ_t), the cylinder was

tapped on a tap density tester from a height of 1.5 cm until no measurable change in volume was noticed. The constant volume was read and used to calculate the tapped density. The samples were determined in triplicate. The percent compressibility of the powder was evaluated using the Carr's compressibility index as shown in following equation:

$$\text{Carr's index} = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100 \qquad \text{.....(1)}$$

1.4 Determination of angle repose (α)

The dynamic angle of repose for the powder was determined by the funnel method. The angle of repose was measured from a heap carefully built up by dropping the sample powder through a funnel to the horizontal surface. When the angle of repose came to the desired condition, then the angle measuring arm was moved by fingers to the position at which the angle of repose could be measured in accordance with the display. The angle of repose was averaged from three determinations.

1.5 Determination of flow rate

Thirty gram of the powder, accurately weighed, was filled in a 1.5-cm internal orifice diameter paper funnel that fixed on the clamp. The time was recorded when the powder started to flow until finished. The flow rate averaged from ten determinations was reported in term g/sec.

1.6 Determination of major constituents of *Malvastrum coromandelianum* spray-dried extract

High performance liquid chromatographic method (HPLC)

HPLC method for determination of active constituents from

Malvastrum coromandelianum was modified from Sanya (2004) and validated as the following:

HPLC Analysis

HPLC chromatographic conditions:

Column : Hypersil ® BDS (C18) Column (250x4.6 mm), 5 µm (Phenomenex, USA) equipped with guard column packed with BDS (C18), 5µm set at an ambient temperature

Detector : UV detector at 273 nm

Injector : 20 µl

Mobile phase : Water-acetonitrile linear gradient conditions are described in Table 1

Internal standard : Caffeine 10 µg/ml

Mobile phase were filtered through a membrane filter with a pore size of 0.22 µm and degassed for at least 30 minutes prior to use.

Table 1. Linear gradient condition for HPLC method

Time (min)	Flow-rate (ml/min)	Pump A Water(%)	Pump B Acetonitrile(%)
0	2.2	100	0
30	2.2	85	15
60	2.2	20	80

Validation of HPLC method

The typical analytical parameters to be considered for assay validation are specificity, linearity (R^2), and precision (%CV).

Specificity

The specificity of the internal standard and major constituent peaks was determined by the resolution and tailing factor (symmetry factor). The well resolving peak away from the other peaks and symmetry peaks should be obtained.

The standard solutions of MCS in water at the concentration 5 mg/ml was prepared and evaluated by using the chromatographic conditions as described above.

Linearity

Triplicate injections of solutions containing drug in various concentrations ranging from 2 to 7 mg/ml of each reference standard in ultra-pure water were prepared and analyzed. The linear equation of the curve obtained by plotting the peak area at each level prepared and calculated using the least square method.

Precision

The standard preparation from accuracy section was stepwise diluted with ultra-pure water to obtain the final concentration of 5 mg/ml. Six replicated injections of this standard solution was analyzed. Percentages of coefficient of variation (%CV) were calculated for determination of the precision.

2. Formulation of *Malvastrum coromandelianum* core tablets

The formulations of core tablet are listed in Table 2. Prior to mixing, the excipients (except MCS powder) in the formulations were dried for 2 hours at 60 °C in hot air oven or until constant weight was obtained. MCS, lactose, Avicel® and Explotab® were individually sieved through a 30-mesh screen. On the other hand, Aerosil® , talcum and magnesium stearate were screened through an 80-mesh sieve

prior to mixing. The tablets containing 300 mg of MCS /tablets were prepared by direct compression method.

A batch size of 500 g was produced in a cubic shape mixer. First, MCS was blended in the mixer with lactose, Avicel® and Explotab® by geometric dilution for 10 minutes to obtain a homogeneous powder mixture. Next, Aerosil®, talcum and magnesium stearate were thoroughly blended with the first mixture for 5 minutes each. Finally, the lubricated mixture was compressed into 385 mg tablets using a bi-concave punch of 9.5 mm in diameter on a single punch tableting machine stationed in a controlled humidity room (38-42% RH). The compression force as well as tablet weight were controlled in order to obtain the tablet hardness within the acceptable range of 4-7 kp. The tablets were stored in a desiccator prior to further evaluation.

Table 2. *Malvastrum coromandelianum* core tablets formulation composition

Ingredients (%w/w)	(%) Formulations						
	F1	F2	F3	F4	F5	F6	F7
MCS	78	78	78	78	78	78	78
Lactose	-	-	5	4.5	9.5	9.5	19.5
Avicel®	16.5	11.5	6.5	10	5	-	-
Explotab®	5	10	10	5	5	10	-
Aerosil®	-	-	-	0.5	0.5	0.5	0.5
Talcum	-	-	-	1.5	1.5	1.5	1.5
Magnesium stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5

3. Formulation of *Malvastrum coromandelianum* film coated tablets

3.1 Preparation of *Malvastrum coromandelianum* coated tablets

Three types of film coating polymer hydroxypropylmethylcellulose (HPMC), chitosan and polymethacrylate were applied

on *Malvastrum coromandelianum* core tablets in various coating level as film formers. Titanium dioxide was added in all formulations of the coating solution due to its light-scattering properties that may be exploited as a white pigment and opacifier. The amount added was equivalent to 0.5% w/w of the formulation.

3.1.1 Preparation of hydroxypropylmethylcellulose (HPMC) coating solutions

The 5% w/w HPMC solution containing 20% w/w polyethylene glycol 6000 (PEG 6000) was prepared. Methocel® E5-E15 mixtures in a ratio of 2:3 were previously dispersed and thoroughly hydrated in about 30% of the required amount of water at 70 °C. Cold water was then added to produce the required volume. PEG 6000 was dissolved and stirred for 60 minutes before coating. Sufficient polymers to attain 3 and 5% weight gain film coated tablets were applied.

3.1.2 Preparation of chitosan coating solutions

Chitosan coating solution of 5% w/w was prepared by dissolving chitosan powder in citric acid solutions (mole ratio of glucosamine unit of chitosan: citric acid was 1:1.2) with constant stirring for 14 hours and then filtering through polyester cloth (Phaechamud et al., 2000). The 25% propylene glycol as plasticizer, based on the dry polymer weight, was incorporated into the film coating solution by dissolving for 60 minutes before applying. Pale yellow solution was obtained for the coating on the core tablets. Sufficient polymers to achieve 3 and 5% weight gain were applied.

3.1.3 Preparation of polymethacrylate coating solutions

Eudragit® E100 was dissolved in isopropyl alcohol to obtain 5% w/w concentration and mixed well with the plasticizer triacetin, at the concentration of 20% w/w (based on Eudragit® E100) for 60 minutes before coating. The coating levels were 1 and 2% weight gain based on Eudragit® E100.

3.2 Coating procedure

The most satisfactory formulation of core tablet in section 2 was prepared in large batch (2000g) for film coating. Tablet formulation which gives good appearance tablets with consistent weight and hardness was selected. Moreover, the formulation should exhibit smooth processing and ease on compression. The test tablets (300g) were mixed with 1,700 g placebo tablets which were colored for ease of separation. Coating was undertaken in the perforated coating pan coupled with an air-atomized spray nozzle. Adequate mixing in the pan was achieved by six baffles. The amount of coating applied to each batch was determined from the percentage coating level that had been prior set. Then the coated tablets were dried in the pan with drying air and kept in amber glass containers until evaluation. Table 3 gives details of the coating process parameters used. Samples were removed every hour and then the mean coating weight gain was calculated.

Table 3. Process parameters and levels for the 15-inch perforated coating pan

Parameters	Coating solutions		
	HPMC	chitosan	polymethacrylate
Spray rate (ml/min)	5	5	10
Air pressure (bars)	3.5	3.5	2
Pan rotational speed (rpm)	15	15	10
Inlet air temperature (º C)	60-65	60-65	40-45
Exhaust air temperature (º C)	55	55	45
Tablet bed warming (min)	15	15	15
Total weight gain (%w/w)	3,5	3,5	1,2

3.3 Preparation and Evaluation of cast film

3.3.1 Preparation of polymer solutions

The HPMC solution 5% w/w containing PEG 6000 (20% w/w based on dried polymer) as plasticizer was prepared as described in section 3.1.1

The chitosan solution 5% w/w containing propylene glycol (25% w/w based on dried polymer) as plasticizer was prepared as described in section 3.1.2

The Eudragit® E100 5% w/w solution containing triacetin (20% w/w based on dried polymer) as plasticizer was prepared as described in section 3.1.3

3.3.2 Preparation of cast film

Three types of plasticized polymer film solutions of 5% w/w were prepared as described in section 3.3.1. The film of about 10 μm thickness were obtained by casting technique on the clean smooth surface of Petri dish. In the case of polymethacrylate. The Petri dish was laid with the adhesive plastic tape before casting for ease of peeling. HPMC, chitosan and polymethacrylate cast films were slowly evaporated at 45 ºC in the hot air oven for 12, 12 and 6 hours, respectively. The dried films were kept in a desiccator for further testing.

3.3.3 Evaluation

3.3.3.1 Tensile strength measurement

The film specimens with the size of 1x3 cm^2 were clamped with 2.54 cm pneumatic grips. The rate of strain was 10 mm/min. The stress-strain tester was fitted with a 10 kilonewton load-detecting transducer. Loads and strain data were collected and converted to tensile strength and the percent elongation at break. Five replicates for each film were evaluated.

3.3.3.2 Moisture sorption test

To determine the amount of moisture absorbed, the cast films were carefully cut to size 2 x 2 cm^2 and placed in the desiccator containing saturated solution of potassium sulphate (96% RH) and stored at ambient temperature(30 ± 2

°C). The films were reweighed every 12 hours until saturated with the water. After 48 hours the film were wiped off excess surface water using filter paper and weighed (W_1). The test films were dried at 40 ºC for 24 hours and kept in a desiccator until constant weight for 72 hours prior to reweigh (W_2). The measurement was made in triplicate. The moisture sorption was calculated using the following equation:

$$\% \text{ Moisture sorption} = \frac{(W_1 - W_2)}{W_2} \times 100 \quad \text{.....(2)}$$

4. **Evaluation of *Malvastrum coromandelianum* tablets**

The following physicochemical properties of *Malvastrum coromandelianum* core and film coated tablets were investigated.

4.1 Physical appearances and morphology study

The visual appearance of the tablets was observed every month for four months. At the end of the storage period, tablets stored at both ambient and accelerated conditions were visually observed.

4.2 Friability

The friability of tablets was determined by a tablet friabilator. Twenty tablets were weighed by an analytical balance and averaged " W_0". Filled into the friability tester and rotated at 25 rpm for 4 minutes. The tablets were weighed again after the dust was eliminated, " W ". The percent friability was calculated base on the following equation.

$$\% \text{ Friability} = \frac{(W_0 - W)}{W_0} \times 100 \quad \text{.....(3)}$$

4.3 Hardness

Ten tablets, randomly sampling, was individually subjected to a hardness tester. The tablet hardness was expressed in kilopound (kp) unit. Mean and standard deviation of the tablet hardness were calculated.

4.4 Average and weight variation

Each of twenty tablets was accurately weighed on an analytical balance. The average weight and standard deviation were calculated.

4.5 Disintegration test

The disintegration test was determined one tablet into each of the six tubes, using distilled water, maintained at temperature 37±1 ºC, as disintegration medium. Analyzes as performed in accordance with standard USP 30 method without disc. Results were reported as the time required for complete disintegration of the tablets.

4.6 Uniformity of dosage unit

Twenty tablets were taken by random sampling. Two tablets were placed into a 50-ml volumetric flask with approximately 30 ml of ultra-pure water and dissolved with sonicator for 15 minutes. Each solution was adjusted to 50 ml with ultra-pure water and mixed well. The sample was filtered through 0.22 µm nylon filter. Filtered solutions were injected onto the HPLC column.

4.7 Assay

The percentage of labeled content was quantitatively calculated by averaging the peak area from HPLC gradient methods as described in section 1.6. The parameters to be considered for validation of HPLC method for the assay of pharmaceutical dosage form were specificity and accuracy (recovery).

Validation of HPLC method for analyzing the pharmaceutical products

Specificity

The specificity of the method was determined by the comparison of standard solutions and test results from analysis of major components in pharmaceutical dosage form. Specificity was established by showing that the major components should have no interference from extraneous components and be well resolved from them.

The standard solutions were prepared and evaluated by using the chromatographic conditions as described in section 1.6.

Accuracy

The accuracy of the proposed method was performed by analyzing placebo spiked with known quantities of active ingredients and evaluated as the percentage of recovery.

Five concentrations (50, 75, 100, 125 and 150% of assay concentration within linearity range) of the standard solutions spiked into the powdered were prepared and analyzed. Three sets of the assay were performed.

Placebo tablets

The placebo tablets (tablets without MCS) were prepared by direct compression methods. The placebo tablets composed of lactose, Aerosil®, talcum and magnesium stearate.

Procedure

Ten placebo tablets, accurately weighed, were ground and mixed. Powder of 85 mg was weighed accurately and transferred to the separated 25-ml volumetric

flask. Aliquot of standard MCS solutions of 2.0, 3.0, 4.0, 5.0 and 6.0 ml MCS were added to each volumetric flask, filled up with ultra-pure water approximately 15 ml and sonicated for 15 minutes, followed by adding ultra-pure water to make up volume. The solutions were filtered through 0.22 μm nylon filter. Filtered solutions were injected into the HPLC column. The percentage of recovery of each reference standard was calculated.

4.7.1 Assay for the pharmaceutical products

4.7.1.1 Standard preparation

Six hundred and twenty five mg of standard MCS was weighed into a 25-ml volumetric flask, dissolved in ultra-pure water and diluted to volume. The solution was quantitatively and stepwise diluted with ultra-pure water. The sample was filtered through 0.22 μm nylon filter. Filtered solutions were injected into the HPLC column.

4.7.1.2 Sample preparation

Twenty tablets were taken by random sampling. Two tablets were placed into a 50-ml volumetric flask with approximately 30 ml of ultra-pure water and dissolved with sonicator for 15 minutes. Each solution was adjusted to 50 ml with ultra-pure water and mixed well. The sample was filtered through 0.22 μm nylon filter. Filtered solutions were injected onto the HPLC column. Each sample was determined in duplicate.

4.8 Dissolution study

A 900 ml of 0.1 N hydrochloric acid as dissolution medium was maintained at temperature 37±0.5°C in a closed system. The large size dissolution apparatus (paddle method) were rotated at a speed of 100 rpm. A portion of

dissolution sample was withdrawn at 15, 30, 45 and 60 minute and assayed by a spectrophotometer. Three tablets of each formulation were determined.

5. Stability study

Stability study of *Malvastrum coromandelianum* tablets was performed according to Thai FDA guideline on stability testing of drug product (จุไรรัตน์, 2547).

The tablets packed in close amber glass container were stored under both accelerated (45±2 °C, 75±5 % RH) and ambient conditions (30 ± 2 °C) for 4 months and randomly sampled at the end of the storage period for analyzing the percent remaining of drug contents by HPLC and studied the solid state transformations of MCS by DSC. The sample preparations were prepared as described in 4.7.1. Moreover, the physical appearance of the tablets was observed every month and the evaluations of the tablets as in section 4 were retested at the end of the storage period.

CHAPTER IV

RESULTS AND DISCUSSION

1. Characterization of *Malvastrum coromandelianum* spray-dried powder (MCS)

1.1 Morphology Study

Figures 5, 6 illustrate the photomicrographs of the MCS powder from optical microscope and scanning electron microscope (SEM), respectively. Particles in spherical-shape with narrow particles size distribution were from optical microscope, while all pictures from SEM demonstrate spherical-shape particles with rough surface and aggregation in cluster.

The pictures from optical microscope of the spray-dried extract from *Phyllanthus niruri* L. indicated the spherical particles (Tatiane et al., 2007) whereas the SEM photomicrographs of soybean dried extract showed that the product was irregular particles and had an agglomerating tendency (Sandra et al., 2007).

A B

Figure 5. Optical microscope photomicrographs of the MCS powder at magnification of (A) 10x and (B) 40x

A B C

Figure 6. SEM photomicrographs of the MCS powder at magnification of (A) 300x, (B) 2000x and (C) 3000x

1.2 Particle size distribution analysis

The particle size distribution of MCS is displayed in Figure 7. The particles were in various sizes. Most of particle sizes were less than 180 μm with highest of distribution of 64.2%.

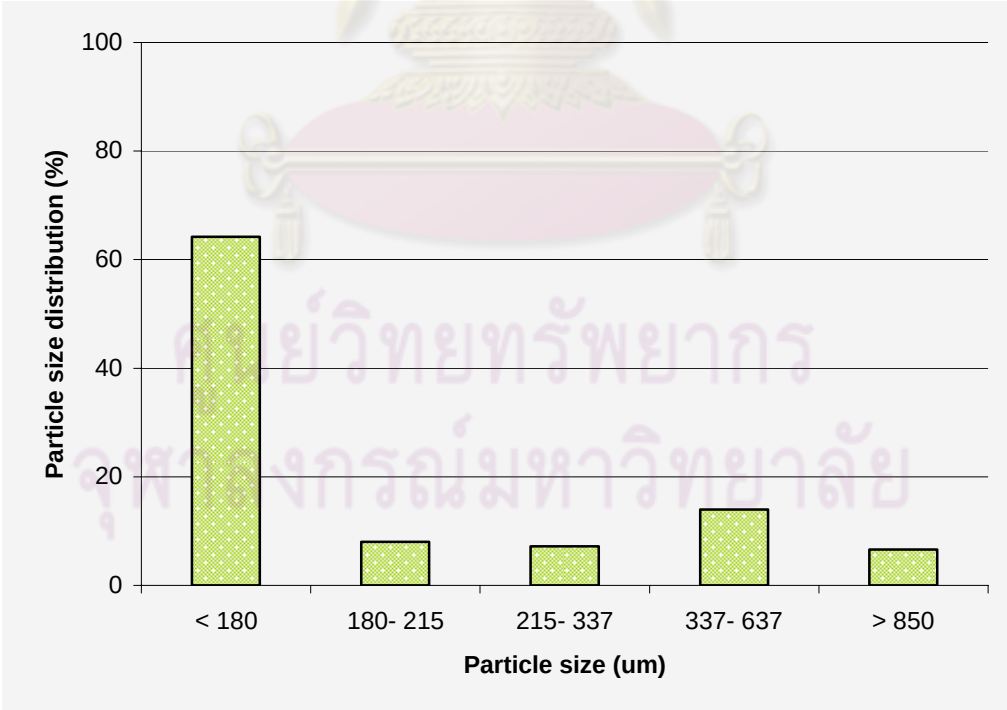


Figure 7. Particle size distributions of MCS

The spray-dried extract from *Phyllanthus niruri* L. classified as a very fine powder with a mean diameter of 10.5 μm (Tatiane et al., 2007) whereas this study found that the particle size from SEM with a mean diameter of 20 μm.

1.3 Density and compressibility assay

The bulk density of a powder depended on the particle size distribution, particle shape and the tendency of the particles to adhere together (Martin et al., 1993). Moreover, the compressibility index was to predict the powder flow characteristics. It is a measure of the tendency for conical formation and a useful measure of flow. The Carr's index classifications are listed in Table 4 (Davies, 2001).

Table 4. Classification of flowability by Carr's indices

Carr's Index (%)	Flow
5-12	Free flowing
12-16	Good
18-21	Fair
23-33	Poor
35-38	Very poor
>40	Extremely poor

The mean of bulk density, tapped density and Carr's compressibility index of MCS were 0.411±0.0025, 0.59±0.0017 and 30.2±0.2268 %, respectively as shown in Table 16, appendix B. For these results, it could not be distinctly concluded about the bulk and tapped density. However, from Carr's compressibility index, the MCS was classified to have poor flow characteristic was confirmed with the photomicrographs from SEM of MCS showed the spherical shape particles with rough surface which aggregate, thus results in poor flow property.

1.4 Determination of angle repose (α)

The angle of repose is a measure of the cohesiveness of the powder, as it represents the point at which the interparticular attraction exceeds the gravitational pull on a particle. A free-flowing powder will form a cone with shallow sides and hence a low angle of repose, while a cohesive powder will form a cone with steeper sides. (Davies, 2001).

The angle of repose of MCS powder measured by the funnel method was 50.27±0.58°. The MCS powder was classified to have poor flowability as shown in Table 5. This was corresponded to the compressibility index.

Table 5. The relationship between the angle of repose and flowability (Nagel and Peck, 2003).

Angle of repose (θ)	Flowability
≤ 38°	Good
38° - 42°	Fair
≥ 42°	Poor

1.5 Determination of flow rate

The flow rate of MCS powder could not be measured by the funnel method both glass and paper funnel, because the extract could not flow pass the funnel orifice. It may be from the static phenomena of spherical shape with rough surface and aggregation of the particles. It was shown that the powder did not have good flow property.

1.6 Determination of major constituents of *Malvastrum coromandelianum* spray-dried powder

High performance liquid chromatographic method (HPLC)

HPLC is the most suitable method to determine the major constituents of this herbal extract both raw material and pharmaceutical dosage form because of its high sensitivity, specificity and convenience for the research.

In preliminary study, HPLC isocratic method was tried to establish but was not successful. It could not attain good separation of the major components from the herbal extract because of the wide range polarity among the compounds. Consequently, the HPLC gradient method was preferred in this investigation. The adjustment of mobile phase composition for linear gradient system was undertaken to obtain the satisfactory single run which showed resolution of internal standard and components. The HPLC chromatogram of internal standard as caffeine peaks at retention time about 19.84 minute and major component of MCS solution peaks at retention time about 30.48 minute as shown in Figure 8.

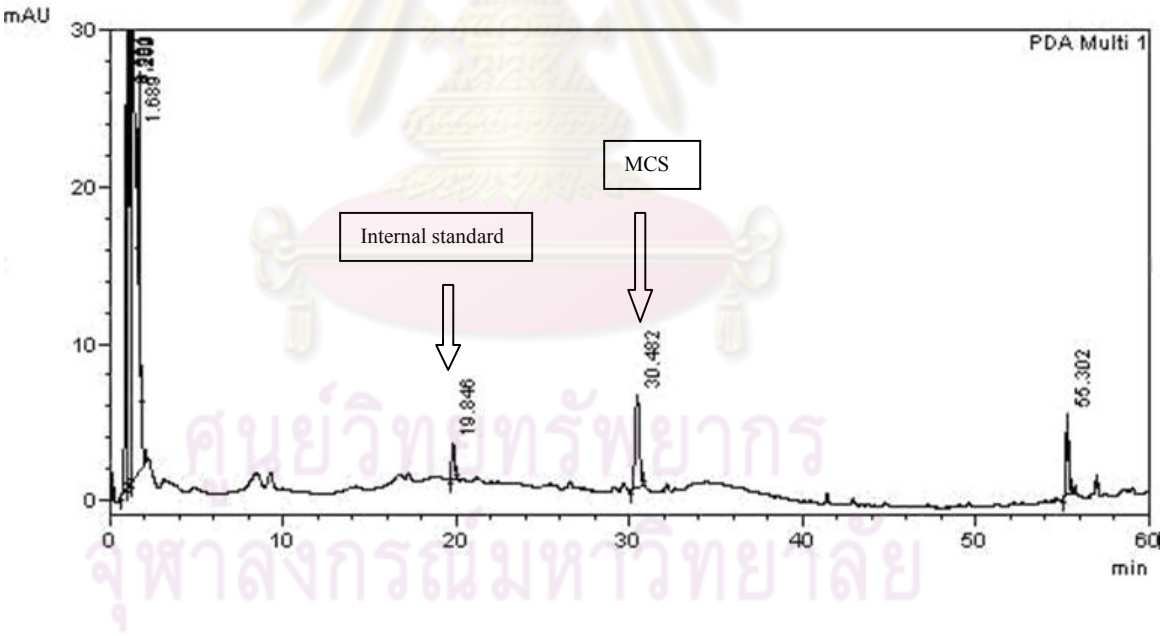


Figure 8.HPLC chromatogram of internal standard and MCS

The goal of the optimization phase is to define improved experimental conditions resulting in a higher separation quality of the fingerprint and if possible in a reduced analysis time. G. Alaerts et al., (2007) can improved experiments had to

performed HPLC instrument, the selected gradients for the optimization results, *Liquorice, Cascara, Curcuma and Artichoke.*

Validation of HPLC method

Analytical method validation is a process to evaluate that the method are suitable and consistent for application. The analytical parameters considered in this validation study were specificity, linearity, accuracy and precision.

Specificity

The specificity of each peak was present as the resolution factor and tailing factor. The retention times of caffeine and major component were about 19.8 and 30.4 respectively. The resolution factors were 49.52 and 26.85 respectively and the tailing factors were 1.129 and 1.081 respectively. They showed the satisfactory resolution, symmetry peak and conformed the USP30 specification.

Linearity

A linearity study was carried out to determine whether this method could measure accurately different concentrations of MCS solution. The linearity curves of the peak area versus the concentrations of MCS solution is shown in Figure 9.

The concentration that gave linear standard curve was in the range of 2 to 7 mg/ml. The regression coefficients (R^2) for standard curve were 0.9996 for compound. This result showed a good linearity of standard concentrations and peak area.

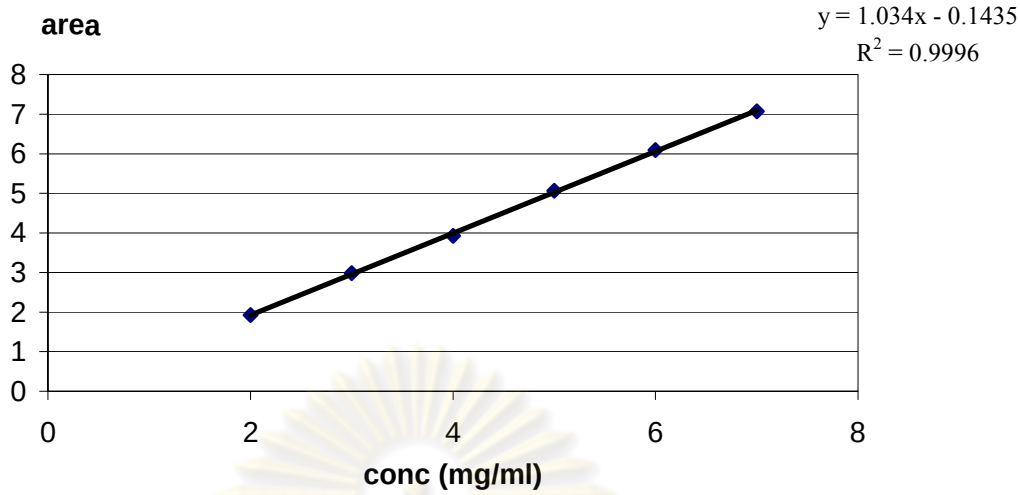


Figure 9. Calibration curve of MCS solution at 273 nm

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from several sampling of the same homogeneous sample. Precision of this method was expressed as the percentage of coefficient of variation (%CV) and the data are shown in Tables 11-13, in Appendix A. The % CV of MCS solutions were 0.8309, 0.9284 and 1.4073 respectively. The low %CV indicated the good precision of this method. The %CV not more than 2 indicated the good precision.

From these satisfactory validation results, this linear gradient HPLC method was employed for the quantitative determination of the major components from *Malvastrum coromandelianum* in this study.

2. Formulation of *Malvastrum coromandelianum* core tablets

The spherical nature of the particles indicates that the material is prepared by spray drying; each particle is almost a perfect minigranule (Ralph, 1990).

In agreement with Palma et al., (2002) this study found that the dry plant

extracts did not currently exhibit the appropriate flowability and compressibility required to be processed by direct compression. Previous reports have addressed techniques to solve this kind of problem, such as wet granulation with non-aqueous solvents, direction compression and selection of suitable excipients for the formulation of dry plant extracts in direct compression tablets (Palma et al., 2002 and Renoux et al., 1996).

For this experiment, the direct compression technology with suitable excipients for the formulation was able to be used for tablet making process. Since there was no stability problem of dry dosage forms. In the dry state there was practically no degradation due to hydrolysis, oxidation or polymerization (Bonati, 1991).

Since the mass powder did not have good flow property. The direct compression excipients could improve the flow property of the drug (Connolly et al., 1990).

2.1 Flow rate

The mean of flow rate powder mixture of each formulation is presented in Figure 10. As a result of static charge, the glass funnel was inappropriate to be used. Thus, it was measured by using paper funnel with an aperture of 1.5 cm.

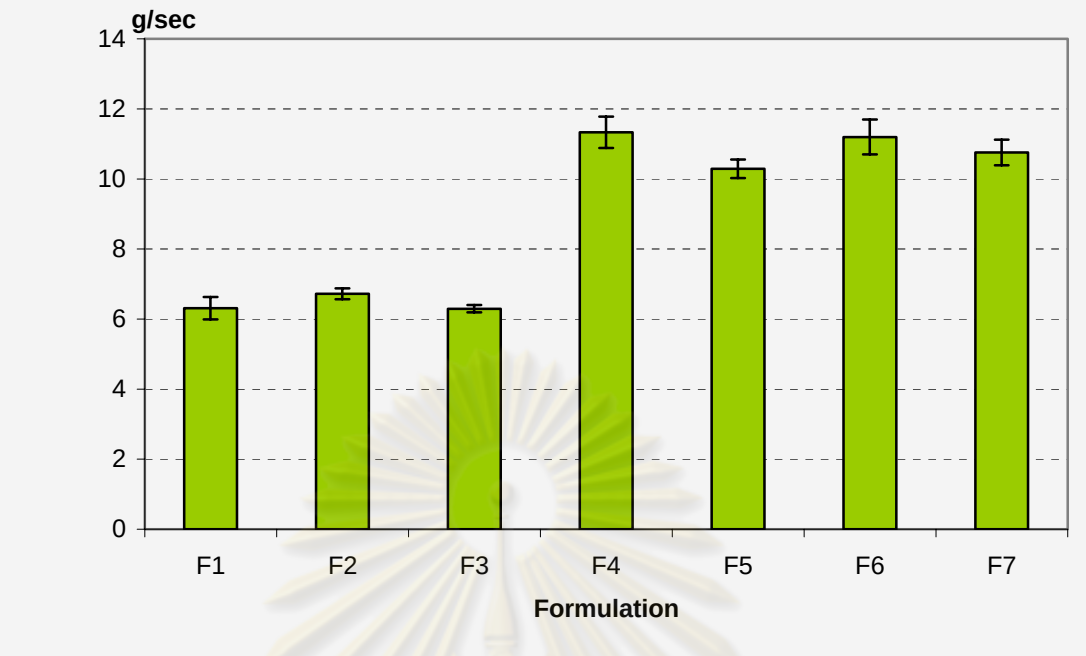


Figure 10. Flow rate of MCS core tablet formulations

Due to the diluents in the formulations, the flowability of MCS was improved. The flow rate of the formulations were in the range of 6.29-11.12 g/sec. It could be ranked as F4> F6> F7 > F5> F2> F1> F3. The formulation F4 contained the diluents : Avicel®, Explotab® and Aerosil® of 10%,5% and 0.5%w/w, respectively. It shows the greatest flow rate (11.12±0.36g/sec). The formulation F7 as the third in the rank composes of the amount of lactose, Aerosil®, talcum and magnesium stearate, 19.5%, 0.5%, 1.5% and 0.5% w/w, respectively while its flow rate was in the third as well (10.75±0.36 g/sec). In case of formulations F6, F5, F2, F1and F3 the flow rates were 11.19±0.19, 10.29±0.26, 6.71±0.15, 6.30±0.32 and 6.29±0.10 g/sec, respectively. On the other hand, formulation F1, F2 and F3 has poor flow rate because they consisted of the adsorbent as Aerosil®. Their flow rates were very similar because their compositions were slightly different.

2.2 Tablet Formulation

Table 2 shows the formulations of MCS core tablets that were produced by direct compression method. In preliminary study, formulation F1 consisted of MCS,

Avicel®, Explotab® and magnesium stearate for 78.0, 16.5, 5.0 and 0.5%, respectively and F2-F3 could not be compressed as tablet because the powder mixture poorly flowed and was unable to fill up the die. Moreover, for formulation F4-F5, adding of Aerosil® and talcum 0.5 and 1.5% w/w, respectively could be made as tablets but the picking problem was found shortly after the operation of compression process. Generally, dry powder extracts did not exhibit the physical-chemical properties required for processing by direct compression (Egglkruat-Gotannaka et al., 2002). To overcome the hygroscopic problem, silicon dioxide, a high porosity excipient, was used (Botani, 1991). Formulation F1 - F6 had a disintegration time problem, which is a longer times than 15 minutes and the data are shown in Figure 11.

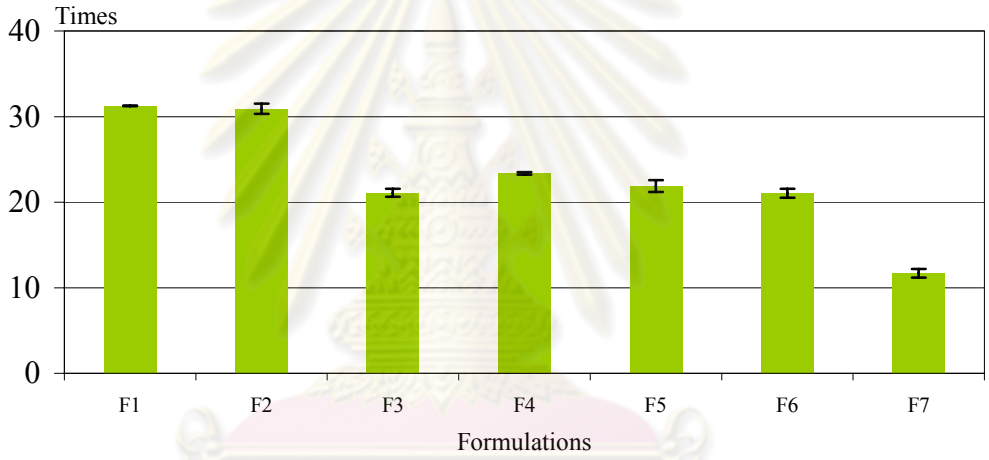


Figure 11. The disintegration time of *Malvastrum coromandelianum* core tablets

Prime particle disintegration in direct-compression tablets depends on the presence of sufficient disintegrating agent and its uniform distribution throughout the tablet matrix. High drug concentrations can lead to cohesive particle bonding during compression with no interjecting layer of binder or disintegrating agent (Ralph, 1990).

The increasing of lactose in formulation F7 could solve this technical problem. Lactose monohydrate had very good flow properties but lack compressibility. Much has been made of the fact that anhydrous lactose contains less moisture than regular lactose and thus was better filler for moisture sensitive drugs. In fact, the surface moisture of the anhydrous and hydrous forms is about the same (0.5%) and the water

of hydration does not play a significant role in the decomposition of active ingredients. Anhydrous lactose possesses excellent dissolution properties, certainly as good as, if not better than, α-lactose monohydrate. However, when the drug makes up higher proportions of the tablet weight, the use of glidants in addition to careful selection of tablet fillers is necessary. The most effective glidants are the micronized silicas (Ralph, 1990).

Not only that this formulation could produce tablets of good appearance but the in-process evaluation results such as friability, hardness, disintegration time and weight variation were also conformed to the specification of the pharmacopoeia. That was the friability of the core tablet was not more than 1.0 %, the hardness of core tablet should be in the range of 5-9 kp and weight variation was in the range of ±5.0% of the average weight. However, smooth tableting process and the satisfactory tablet were still obtained. Accordingly, F7 was the most suitable formulation of *Malvastrum coromandelianum* extract core tablets because it could produce satisfactory tablets by using the lower amount of additives. It was evaluated and used as substrates in the step of film coating development.

3. Formulation of *Malvastrum coromandelianum* film coated tablets

In order to protect the *Malvastrum coromandelianum* core tablets from the environmental factors, the film coating process was utilized. Furthermore, *Malvastrum coromandelianum* extract exhibits a bitter taste, the taste masking is another reason for film coating. In this study, there were 3 types of polymer to be considered; HPMC (Methocel® E5 and E15), chitosan (M.W. 50,000) and polymethacrylate (Eudragit® E100). Similarly, these polymers have good protective and taste masking properties (Bauer et al., 1998; Li et al. 2002). The main reason for the extensive use of HPMC as a film-coating polymer is that it is soluble in some organic solvents and also in water over the entire biological pH range. Additionally, they can dissolve in the gastric juice as well. Chitosan and polymethacrylate are cationic polymer while HPMC is the well known polymer in cellulose derivatives group. Either HPMC or chitosan were prepared in aqueous based system. On the

contrary, polymethacrylate, the representative of acrylic polymer was prepared in organic based system (Hengsawas, 2004).

3.1 Film coating

3.1.1 Film coating process

During coating process, the tackiness problem was found with both chitosan and polymethacrylate film coating formulations, but film-coated tablets rolled easily in coating pan after drying. The problem was attributed to the adhesiveness in nature of the chitosan and polymethacrylate film themselves (Lehmann and Bössler, 1983; Kusonwiriyawong, 1994; Phaechamud, 1999; Lin et al., 2000). In preliminary study, it was found that the rupture of chitosan and polymethacrylate film could occur and this could have a damaging effect on the tablet properties. To overcome such problem, the spraying rate was adjusted by decreasing and the spraying pattern was changed to intermittent, as a consequence, this could lead to much time consuming process. The higher levels of film coating were applied, the more tablet aggregation resulted. In addition, it was found that the film coated tablets of chitosan citrate and polymethacrylate aggregated during the storage as well. On the contrary, the HPMC film coating formulations appeared to have no tackiness problem. If the active ingredient is highly water soluble and its content is very high, the active ingredient may dissolve in the spray mists during the operation, resulting in the active ingredient being included in the film. This is often inconvenient especially if the active ingredient has a bitter taste. Although a method to prevent this phenomenon completely has not yet been found for all cases, a fairly effective method is to keep the particle size of the spray mist small and to use a low spray rate. Satisfactory film coated tablets were obtained.

The tackiness problem was consistent with the study of Eudragit® film former by Lehmann and Bössler (1983). It was recommended that the tablet should not be sprayed too wet, because thicker layer causes slower drying which causes lacquer films remained soft for some time when passed through a tacky phase. Moreover, in this critical period the lacquer layer might be crushed and picked

on coming into contact with other tablets. When the cores become too moist or tacky, spraying should mildly interrupted until the cores were once again dry. Blow drying with warm air should then be implemented for about 5 minutes with the coating pan rotating at a reduce rate. Besides, it was suggested that the film coated tablet of Eudragit® should be spread out on a sheet of filter paper and left to dry overnight in air.

Furthermore, HPMC and chitosan citrate were in water based solutions. The air pressure of the spray nozzle and inlet air temperature should be set higher than that for polymethacrylate which was in organic based solution.

3.2 Evaluation of cast film

3.2.1 Physical appearances

All the plasticized film coating solutions obtained were different in physical appearance. Chitosan film coating solutions were yellowish and clear while both HPMC and polymethacrylate film coating solutions were colorless and clear. The physical appearance of the cast films related to their nature properties of materials, particularly color and solubility. The clarity of the cast films was possibly resulted from the solubility of the polymer. The physical characteristics of a colorless and transparent HPMC solution yielded an off-white opaque and glossy cast film while a chitosan citrate cast film were yellowish transparent and glossy soft film. The polymethacrylate film was colorless transparent and glossy.

3.2.2 Mechanical strength

The tensile strength and percent elongation at break of the cast film are illustrated in Figure12. The tensile strength of cast plasticized films of HPMC, chitosan and polymethacrylate were 0.10, 0.05 and 0.01 Newton/mm^2, respectively. These could be ranked as followed; HPMC > chitosan > polymethacrylate. Furthermore, the percent elongation at break of the plasticized films of HPMC, chitosan and polymethacrylate were 8.64%, 22.71 and 4.75%,

respectively and could be ordered as polymethacrylate < HPMC < chitosan. These data showed that HPMC cast film was stronger and harder than chitosan and polymethacrylate cast film. The chitosan cast film showed the highest elongation at break, which indicated that the polymer yielded the softest film, because chitosan could absorb the highest moisture. The polymethacrylate cast film showed the lowest tensile strength and the lowest elongation at break, which indicated that the polymer yields the brittle film.

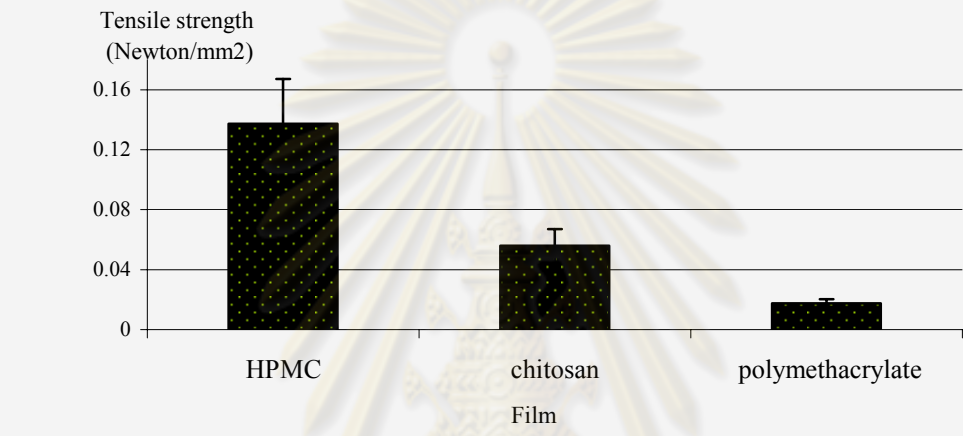


Figure 12. Tensile strength for cast films

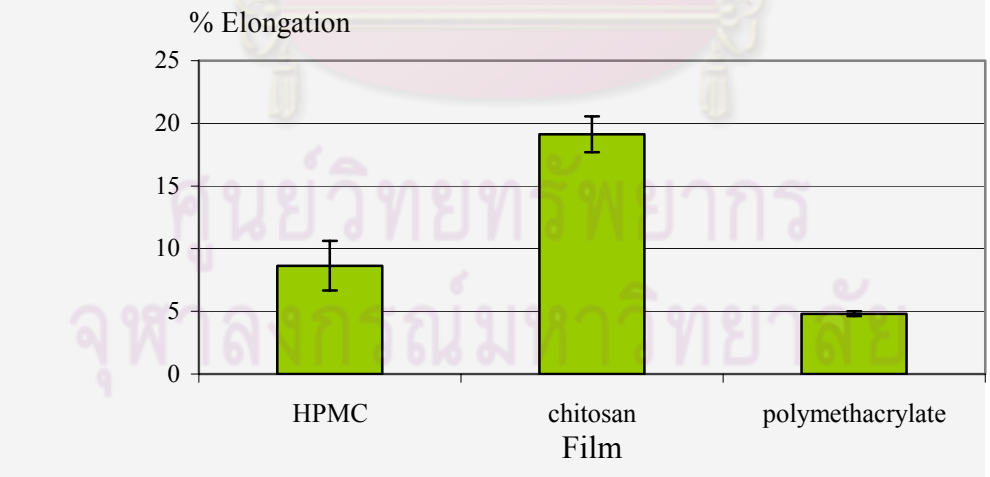


Figure 13. Elongation at break for cast films

In general, HPMC films showed high tensile strength and average percentage of elongation (Li et al., 2002). However, most polymers used alone for film formation are commonly brittle at room temperature and require

plasticizer or another additive to improve their processibility and flexibility (Li et al., 2002). Although HPMC film was also plasticized with PEG 6000, the higher tensile strength of HPMC than that of chitosan and polymethacrylate may be due to the formation of hydrogen bonding when short chain of HPMC E5, inserted between the long chains of HPMC E15. In addition, PEG 6000 molecule was bigger than propylene glycol so the chances of PEG 6000 to interact with HPMC were higher (Sothornvit and Krochta, 2001). Therefore, plasticized HPMC film presented higher intermolecular forces. Moreover, the addition of propylene glycol could shift the fraction behavior of chitosan citrate film from brittle to ductile characteristics. This was due to the alteration of polymeric matrix from a glassy to a rubbery state through plasticizing effect. An introduction of plasticizer promoted ductile fracture owing to a decrease in the intermolecular force along polymeric chain and thereafter the motion of polymeric chain was enhanced as described by Wang et al., (1997). Plasticizers increased film flexibility due to their ability to reduce internal hydrogen bonding between polymer chains while increasing molecular space (Gontard et al., 1993). In addition, the incorporation of the plasticizers also lowered the glass transition temperatures (Tg). The Tg of the triethylcitrate plasticized film is slightly lower than that for the triacetin containing film. This study showed triacetin to be a choice for plasticizing for Eudragit® E film. It not only produced a non-swellable and less adhesive film, but also lowered the Tg of the film to the plasticizer efficacy. Thus, the inclusion of triacetin as plasticizer, was appropriate to reduce the glass transition temperature of the polymethacrylate film so the increasing of the flexibility of film was obtained.

3.2.3 Moisture sorption test

From the data obtained in Figure 14 and in Table 17, in Appendix B, the degree of moisture sorption of the cast films was between 0.50-25.93% and could be ranked as followed: chitosan citrate > HPMC > polymethacrylate were 25.93±0.061%, 11.01±0.063% and 0.50±0.005% respectively. It was found that among three types of film, chitosan citrate film possessed the highest moisture sorption property so it was possible that the increasing of the moisture could soften the film. It may indicate that polymethacrylate film is the best

film to protect the tablets from the moisture, because polymethacrylate could absorb the least moisture.

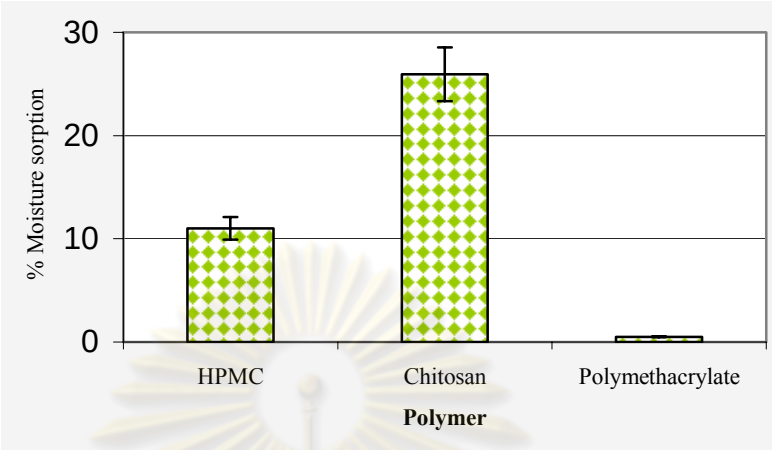


Figure 14. The percentage of moisture sorption of cast films

The two major forces that have been found to affect polymer-tablet adhesion include the strength of the interfacial bond and the internal stresses within the film coating. For pharmaceutical products, hydrogen bond formation is the primary type of interfacial bonding mechanism between the tablet surface and polymer (W.H. Pritchard, 1971.). Dipole-dipole and dipole-induced dipole interactions also occur, however, to a lesser extent. Factors which affect the type or the number of bonds formed between the polymer and the solid surface will influence film adhesion. The second major factor influencing polymer adhesion is the internal stresses within the film. When a polymeric solution or dispersion is applied to a substrate, an internal stress inevitably develops within the film. The total stress within a film is the sum of all the stresses acting on the polymer, including stress due to shrinkage of the film on the evaporation of the solvent, thermal stress due to the difference in thermal expansion of the film and the substrate, and volumetric stress due to the change in volume when a substrate swells during storage (Linda A. et al., 1999).

Polar groups led to water absorption, for instance, from the surrounding air, which led to an increase in the moisture permeation rate by the water plasticizer role into the film matrix (Kim and ustunol, 2001). Interaction between chitosan and carboxylic acids were associated with electrostatic reaction in aqueous

solutions and formed salts in cast films. The presence of hydrophobic and hydrophilic groups especially the carboxyl group in the molecule of acid profoundly affected the water sorption. The more carboxyl and hydroxyl groups and less alkyl group in the molecule of acid would result in the higher water sorption of treated chitosan films. The water was absorbed into chitosan film, thus the protonated amino group and carboxylate ion were equilibrated to the free amine nucleophile and free carboxylic acid (Ritthidej et al., 2002). Citric acid had carboxyl group up to 3 groups and one hydroxyl group so chitosan citrate film could adsorb high water content. Moreover, chitosan was hydrophilic and retained a considerable amount of water, at least in chitosan there existed three predominant absorption sites such as the hydroxyl group, the amino group and the polymer chain end. The polymer chain end was supposed to be composed of a hydroxyl group or an aldehyde group (Gocho et al., 2001).

In this study, chitosan citrate film was plasticized with propylene glycol 25% w/w which was higher amount than the inclusion of PEG 6000 and triacetin 20% w/w as plasticizers in HPMC and polymethacrylate films. The higher amount of hydrophilic plasticizer resulted the plasticized chitosan cast film the highest moisture sorption film. PEG 6000 and propylene glycol could easily dissolve from the film surface and then the water could suddenly penetrate through the pore occurred after plasticizer dissolving. Molecular differences between PEG 6000 and propylene glycol were probably responsible for the different sorption rate of film plasticized with them. PEG 6000 and propylene glycol were polyols with similar straight-chain molecules. However, PEG 6000 (molecular weight of 6000) had two hydroxyl groups while propylene glycol molecule was smaller (molecular weight of 76.1) and had three hydroxyl groups (Kibbe, 2000). PEG 6000 containing films presented higher intermolecular forces and showed a lower capacity to interact with water. Propylene glycol presented more hydroxyl groups to interact with water by hydrogen bonds. Moreover, the chances of PEG 6000 to interact with the polymer chains were higher. This finding was consistent with Sothornvit and Krochta (2001) who studied the effect of two types of plasticizers, glycerol and sorbitol, on water sorption.

At the equal amount of plasticizers, 20% w/w, plasticized HPMC film had the higher percent moisture sorption than polymethacrylate films. This is also true in the case of hydrophobic polymer and plasticizer. Polymethacrylate film with triacetin as plasticizer was unsurprisingly resulted in the film with lower degree of moisture sorption. The chemical structure of Eudragit® E100 had several long chain of hydrophobic group that of ester and aminoalkyl groups. Moreover, triacetin had the hydrophilic property. Thus water absorption of Eudragit® E100 films was dependent on type of Eudragit® E100 polymer and plasticizer used. Triacetin, a plasticizer with high affinity for water, induced slight water absorption for Eudragit® E100 film. Eudragit® E 100 is a copolymer and soluble at pH below 5.5 (Lehmann, 1968). This polymer can prevent the release of the delivered drug in saliva (pH 6.8-7.4) and readily dissolves in gastric fluids (pH 1.0-7.4) (Ishikawa et al., 1999). Furthermore, this polymer has been demonstrated to be an effective moisture protective film coating (Chowhan et al., 1982; Thoennes and McCurdy, 1989)

4. Evaluation of *Malvastrum coromandelianum* tablets

All tablet formulations after freshly prepared and storage at both ambient and accelerated conditions for 4 months were evaluated following this section. The abbreviations of the film coated tablets formulations are shown in Table 6.

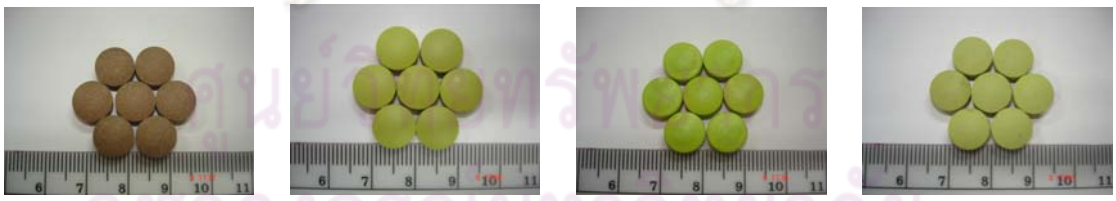
Table 6. The abbreviations of the film coated tablet formulations

Abbreviation	Formulations
Core	Core tablet
HPMC3	Tablet coated with HPMC at 3% coating level
HPMC5	Tablet coated with HPMC at 5% coating level
CS3	Tablet coated with chitosan at 3% coating level
CS5	Tablet coated with chitosan at 5% coating level
PMC1	Tablet coated with polymethacrylate at 1% coating level
PMC2	Tablet coated with polymethacrylate at 2% coating level

4.1 Physical appearances

4.1.1 The color of tablets

The physical characteristics of *Malvastrum coromandelianum* of core tablet were brown and glossy. After freshly prepared, all formulations of coated tablet had green color, smooth and glossy surface, with different shade of green color (Figure 15-16). After storage at ambient condition, the degree of green color of chitosan citrate film coated tablet was slightly increased. Moreover, after exposure to accelerated condition for 4 months the chitosan citrate film coated tablet was pale brown (Figure 18). The degree of color intensity increased as a function of storage time and coating level. That was the longer storage periods was studied and the higher coating level was applied, the more intensity of the color was dominantly detected. Lin et al. (1999) found that the color of chitosan was changed to brown from thermal degradation after storage at high temperature of dry heat treatment. The physicochemical properties of the tablet coated with chitosan citrate especially the color and solubility of the film were changed. In contrast, neither HPMC nor polymethacrylate film coated tablet was changed in color under any conditions (Figure 17, 19).



Core **HPMC** **Chitosan** **Polymethacrylate**

Figure 15. The appearance of core tablets, tablets coated with 5%HPMC, 5%chitosan and 2%polymethacrylate after freshly prepared.

Figure 16. The appearance of core tablets after freshly prepared and storage conditions for 4 months.

Coating level

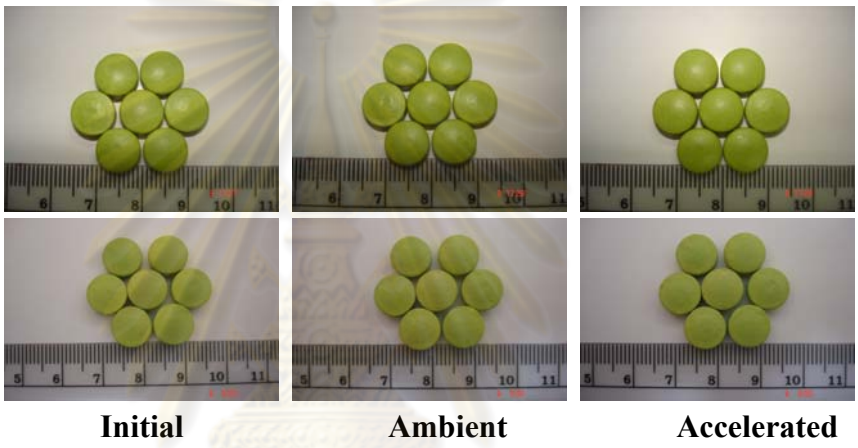


Figure 17. The appearance of tablets coated with 3%HPMC and 5%HPMC coating level and storage conditions for 4 months.

Coating level

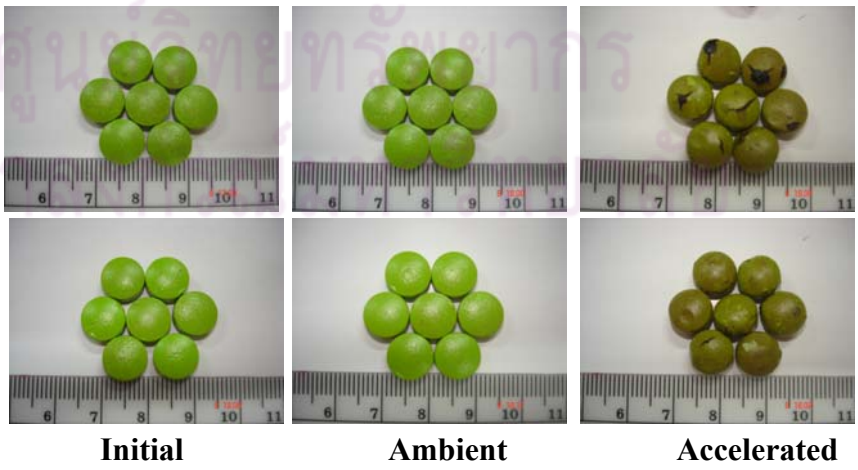


Figure 18. The appearance of tablets coated with 3%chitosan and 5%chitosan coating level and storage conditions for 4 months.

Coating level

Figure 19. The appearance of tablets coated with 1%polymethacrylate and 2%polymethacrylate coating level and storage conditions for 4 months.

4.1.2 Defect of the coated tablets

After preparation and storage at ambient and accelerated conditions, the chitosan and polymetharylate film coated tablets were tacky. The defect regarding picking was slightly found in chitosan film coted tablets whereas HPMC and polymethacrylate film coated tablets could be separated without any defect. Table 7 shows the percentage of defected tablets coated with chitosan citrate by manual coating from 100 tablets. The accelerated condition and the coating level apparently increased tackiness. The chitosan citrate film coated tablets accelerated condition showed more defects than those after preparation and storage at ambient condition. Due to the high humidity of accelerated condition, the tablets coated with chitosan citrate would uptake more moisture from the environment. Therefore they became tacky and had more defect than the tablets stored at ambient condition. The higher percent coating level was applied, the more moisture absorption and defects were increased (Hengsawas, 2004).

Table 7. The percent defect of Malvastrum film coated tablets

	The percent defect of Malvastrum film coated tablets					
	HPMC3	HPMC5	CS3	CS5	PMC1	PMC2
Ambient, at 4th month	0	0	0	0	0	0
Accelerated, at 4th month	0	0	7	12	0	0

4.2 Friability

The percentage of friability of core tablets and coated tablets (after preparation, after storage under accelerated condition for 4 month) are presented in Table18-20 in Appendix B. The friability was omitted in case of tablet storage under ambient condition for 4 months.

From the data obtained, the percentage of friability of core tablets after compression and storage under accelerated condition were 0.246 and –0.0251, respectively. Due to the moisture absorption by the surface of core tablet the percentage of friability after exposure to accelerated condition was less than that after compression. Moreover, the percent friability of core tablets after exposure to accelerated condition had surprising by negative values. This may be attributed to the moisture sorption of surface during accelerated condition. However, the friability of core tablets was rather low, indicating that the composition of the formulation and direct compression method were accepted for producing the core tablets.

The coated tablets picked up moisture more slowly than the core tablets. Most of the formulations of coated tablets were not friable and showed that the weight were unchanged. The coating could improve the friability of the core tablets. In conclusion, the friability of all formulations conformed to the USP 30 specification (less than 1.0%).

4.3 Hardness

The mean and standard deviation of hardness are displayed in Table 21-24, in Appendix B. The hardness of tablet coated with HPMC and chitosan citrate were higher than that of core tablets about 5.97-13.16 kp. An enhancement of coating level enhanced the hardness of tablets coated with HPMC, chitosan citrate and polymethacrylate.

After exposure to the ambient and accelerated conditions for 4 months, the hardness of tablet coated with HPMC, chitosan citrate and polymethacrylate slightly increased. The hardness of tablets coated with polymethacrylate at 1% and 2% coating level were not markedly altered after storage at ambient conditions. This indicated that this film had a satisfactory protective property. Regarding moisture sorption study, it was found that among the three types of film, chitosan citrate film had the highest moisture sorption property so it was possible that the increasing of the moisture could soften the film. While the HPMC and polymethacrylate films absorbed less moisture thus they had greater protection of the tablets from the moisture. However, the mean and standard deviation of the tablet hardness of some formulations; the tablet coated with HPMC at 3% and 5% coating level and chitosan citrate at 3% and 5% coating level after preparation and after storage at ambient and accelerated conditions for 4 month could not be calculated as they are greater than 20 kp, the maximum limit of the apparatus.

4.4 Weight variation

Table 25 in Appendix C, shows the weight variation of all formulations. The weight variations of all formulations were conformed to the specification in official standard USP 30 (average difference of less than 5.0 %). The extremely low of standard deviation of weight variation of core tablets seemed to indicate that the formulation had good flowability. That was agreed with the flow rate of the powder mixture before compression of core tablets. In case of film coated tablet, a narrow range of weight variation could show the thoroughly coating process.

4.5 Disintegration

In this study, the rapidly disintegrating film coated tablets were produced. Therefore, the tablet should disintegrate rapidly in the disintegration medium. HPMC and chitosan citrate are water soluble polymers, while polymethacrylate can not dissolve in pure water (Bauer et al., 1998; Kibbe, 2000; Ritthidej et al., 2002). Lehmann and Bössler (1983) reported that Eudragit lacquer substances did not dissolve in water. Instead, the film coating had to break open, scaled off or became sufficiently permeable for water to penetrate into the core and caused it to burst apart.

The disintegration time of core and coated tablets are presented in Figure 20 and Table 26, in Appendix C. The disintegration time was ranked as: core tablet < HPMC < polymethacrylate < chitosan. The disintegration time of core tablets in deionized water at 37 ºC were within 11 minutes. The disintegration time of all film coated tablets in deionized water were longer than that of core tablets. Increasing of coating level results in longer disintegration time. The thicker the barrier as the coating level was increased, the longer was the disintegration time of the film coated tablet, since the thicker film would require more time in order to be dissolved.

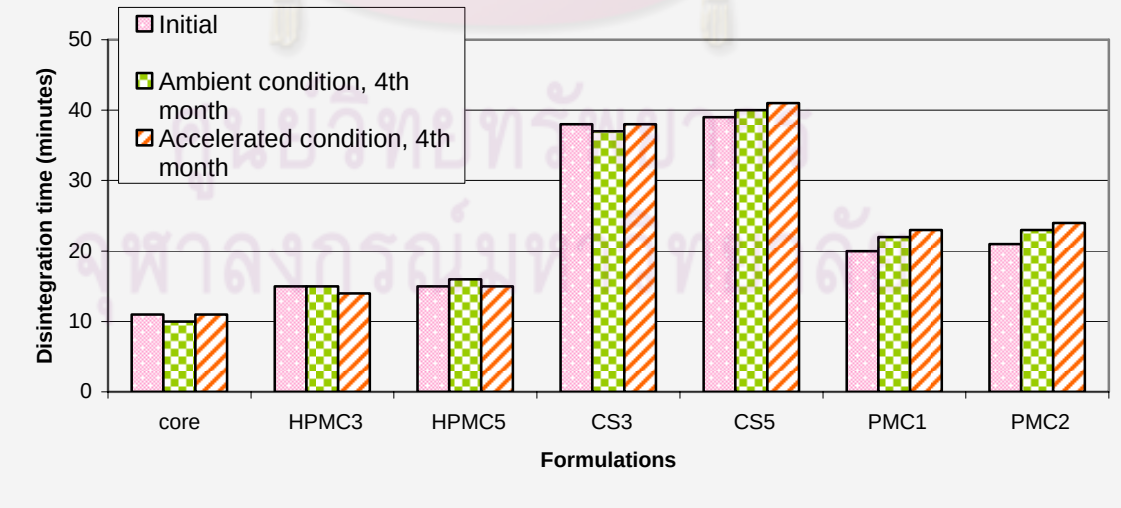


Figure 20. Disintegration time of film coated tablet formulations in de-ionized water at 37 ºC

Moreover, after storage at accelerated conditions for 4 months, the disintegration time of tablets coated with chitosan citrate films were greatly enhanced. The disintegration time of all tablets coated with chitosan citrate films were longer than 30 minutes. The cross-linking between amino groups on chitosan chains and carboxyl groups on citric acid under the accelerated condition could possibly further retard the disintegration of the coated tablet. The more extensive exposure to the accelerated environment possibly enhanced the degree of cross-linking. This finding was consistent with the previous study by Coma et al. (2003). Cross–linking by a polycarboxylic acid seems to be a way to decrease the hydrophilic characteristic and water solubility of cellulosic polymer. Consistant with the formation of a covalent bond between the chains of cellulose, this chemical modification could lead to a decrease in the availability of hydroxyl groups, limiting polysaccharide-water interactions by hydrogen bonding. Cross-linking agents, such as polycarboxylic acids however, partially compensate for the loss of available hydroxyl groups by giving, especially, highly hydrophilic carbonyl groups.

The chitosan citrate dissolved in acidic medium very well. In this study, the use de-ionized water makes it take a long time for disintegration.

4.6 Uniformity of dosage unit

The content uniformity of *Malvastrum coromandelianum* core tablets, freshly prepared, shown in Table 8 was in the range of 101.97-103.73 % of the label amount. The percentage of coefficient variation (%CV) was 0.52.

Table 8. Content uniformity of *Malvastrum coromandelianum* core tablet after freshly prepared

Tablet No.	Constituent (mg)	%label amount
1	49.79	103.73
2	49.23	102.56
3	49.52	103.16
4	48.95	101.97
5	49.72	103.58
6	49.65	103.43
7	49.42	102.95
8	49.51	103.14
9	49.77	103.68
10	49.44	103
Average	49.5	103.125
SD	0.26	0.54
%CV	0.52	0.52

The results passed the specification of general monograph of USP 30, in which the content uniformity of the tablets should be within the range of 85.0-115.0% of the label amount and the percentage of coefficient variation (%CV) should be not more than 2. It also indicated that there was no effect by the method of preparation and the mixing all ingredients by geometric dilution could produce homogeneous mass.

4.7 *Assay*

Validation of HPLC method for analyzing the pharmaceutical products

Specificity

An analytical method is specific if it guarantees that the measured peak

is only related to the substance intended to be analysed, targeted compound, in the presence of the extraneous components. The excipients in the formulation did not interfere with the peak of major components in figure 21.

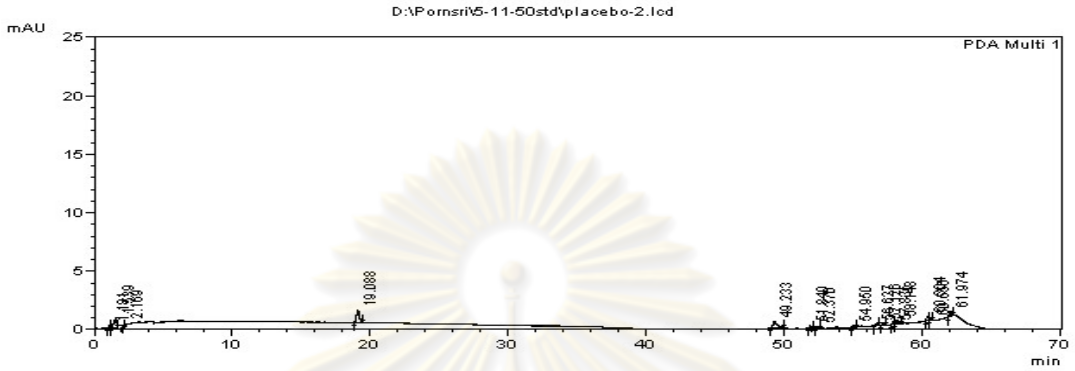


Figure 21. HPLC chromatogram of placebo tablets

Accuracy

The accuracy of the proposed method defined as the percentage of the recovery, is calculated as deviation agreement between the measured value and the true value. The results were shown in Table 13, in Appendix A. The ranges of percentage of recovery were 85.48-98.22% for MCS. Hence this HPLC gradient method is accurate for these compounds assay.

The consequence of the assay is shown in Figure 22. The assay content of the tablet formulations was in the range of 91.05-104.74% labelled amount. The assay amount of the coated formulations could be ranked as HPMC > chitosan ~ polymethacrylate. Although the tackiness problem was found with both chitosan and polymethacrylate, polymethacrylate showed less tacky. Therefore, the period of coating process of polymethacrylate can be done shorter than chitosan. The tablets coated with polymethacrylate were less affected by the environment. Because the polymethacrylate solution was prepared in organic solvent, the low inlet temperature in the coating process was operated. On the contrary, HPMC could expose to high

inlet temperature and aqueous system process was used. It was probably due to failure in the coating and analytical error.

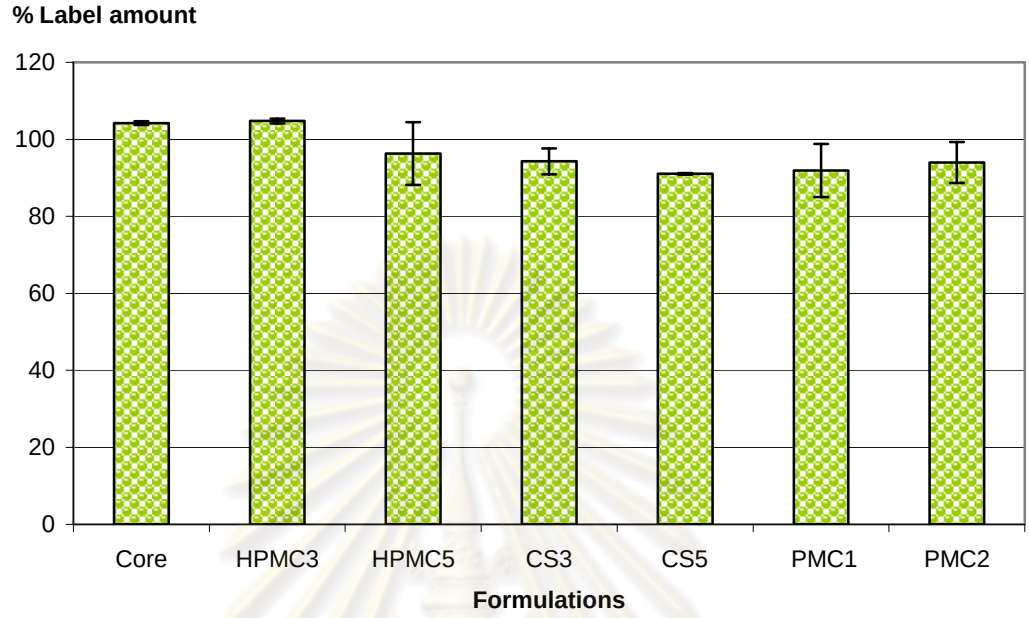


Figure 22. The assay content of the tablets formulations

4.8 Dissolution of the tablets

Figure 23 illustrates the percent dissolution at 60 minutes of the formulation after preparation, storage under ambient condition and accelerated condition at 45±2°C, 75±5%RH for 4 months (mean ±SD). The dissolution of all formulations at the beginning of storage, after storage at ambient condition and accelerated condition for 4 months were in the range of 70.4-80.75%, 76.03-86.10% and 87.62-94.3% labelled amount, respectively.

There were significant differences by using an analysis of variance (ANOVA) and Games-Howell test for post-hoc comparisons of core tablets between the tablets after prepared and accelerated condition and between the tablets after storage at ambient condition and accelerated condition ($p < 0.05$). This was probably due to the decreasing of the amount of major constituents of MCS from core tablets after storage at accelerated condition.

At the same coating level of 3% and 5% coating level, the dissolution of the coated tablets had no significant differences among the polymeric films (p> 0.05).

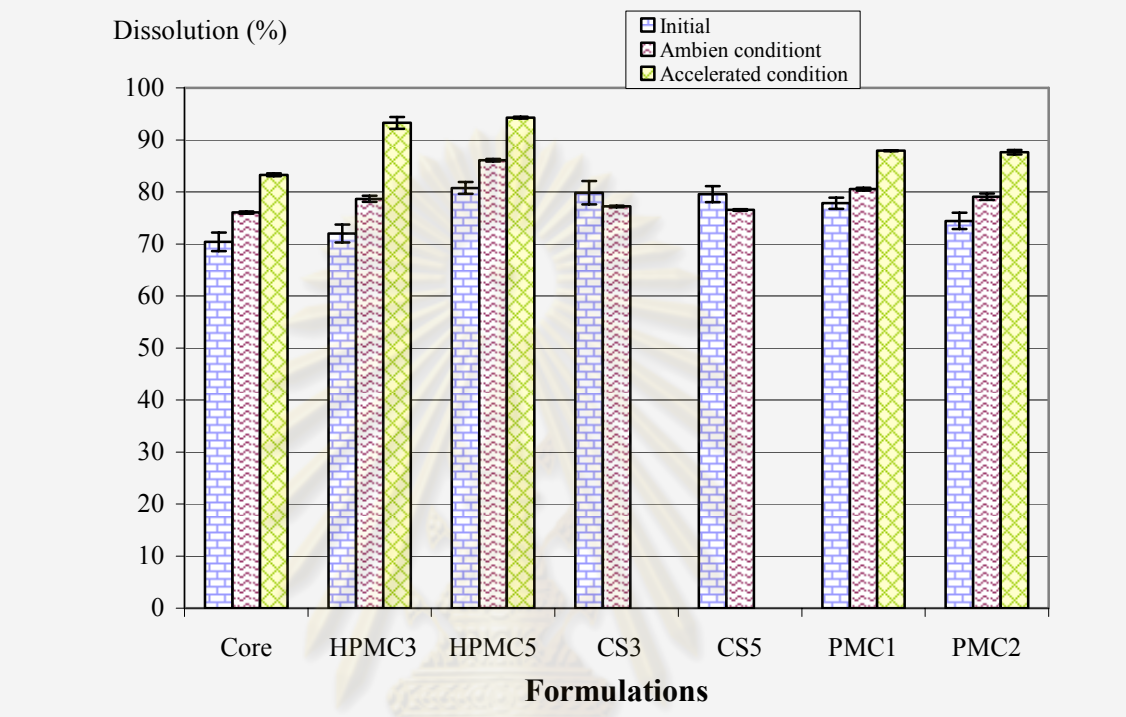


Figure 23. The percentage of dissolution of *Malvastrum coromandelianum* film coated tablets at 60 minutes

The effect of storage conditions on drug dissolution was investigated. From the data obtained, the percent dissolution tended to increase after storage at both conditions. The tablets coated with chitosan citrate after storage at accelerated condition exhibited the lowest percent dissolution. By visual inspection, these coated tablets after dissolution test, their film did not dissolve and remained nearly no change in appearance. There were statistical significant differences in dissolution of the tablets coated with 3% and 5% coating level of chitosan citrate between after freshly prepared or storage at ambient condition for 4 month ($p < 0.05$).

This was probably due to the delay time of the tablet disintegration cause by coating films. This finding agreed with previous studies by Phaechamud et al. (2000)

in that the significantly slower drug dissolution of coated tablets of chitosan citrate film storage in a sealed amber bottle at 45°C, 75%RH, for 1 month was observed. However, the percent dissolution was still passed the specification. Similar to the reason of retardation of disintegration, the cross-linking between amino groups on chitosan chains and carboxyl groups on citric acid under accelerated conditions might be the cause of film property alteration and could possibly retard the drug dissolution of film coated tablet. (Phaechamud et al., 2000).

In case of tablets coated with 3% and 5% coating level of HPMC film, there were significant differences of dissolution by using an analysis of variance (ANOVA) and Games-Howell test for post-hoc comparisons among coated tablets after freshly prepared, storage at ambient condition and accelerated condition ($p < 0.05$).

However, the results from the lower coating level showed no significant differences. It was possible that after exposure to high temperature and moisture for a long period could affect the film properties of HPMC at high coating level. It may be occurred the gel formation of the thick film after storage so it was swelled and delay to dissolve. The increasing of moisture in the coated tablets might from hydrogen bonding between hydroxyl groups on HPMC chain and water molecule caused hydration and swelling of the film prior to the dissolution of the coat. Therefore the higher coating level could form the greater hydrogen bonding and gel forming properties of chitosan in the presence of citric acid in matrix formulations had been reported by Nigalaya et al. (1990).

The cause of tablets dissolution was increased may be result form transformation of compounds. That increased absorbtion at wavelength 273 nm, so percent dissolution was increased. Moreover, analyze percent dissolution by spectrophotometer with make the result was increased.

Film coated tablets chitosan were degrade and had undesirable appearance when stored at accelerated condition, so it is not studied.

Stability study

Chemical stability study

For the stability study of MCS tablets, the storage condition and storage period was performed according to Thai FDA guideline 2004. The percentages of the total remaining contents should be more than 90% and less than 110% of the initial.

The percent content of major constituents of MCS are summarized in Figure 24. The residual contents were calculated by comparing the corresponding values with the initial amount.

From the residual percent of content, the stability of tablet containing MCS extracts could be interpreted that all formulations were stable at either ambient or accelerated condition for 4 months. Thus, it could imply that these tablets had the temporary shelf life for 2 years (จุไรรัตน์, 2547). The residual content was more than 90% of the initial amount. Moreover, after storage at the accelerated condition (45±2°C, 75±5%RH) for 4 months, the percent remaining of the major compounds was 90.41% and 90.65% of the initial content from HPMC5 and PMC2, respectively. It showed a slight decreasing of the major constituents from MCS extracts.

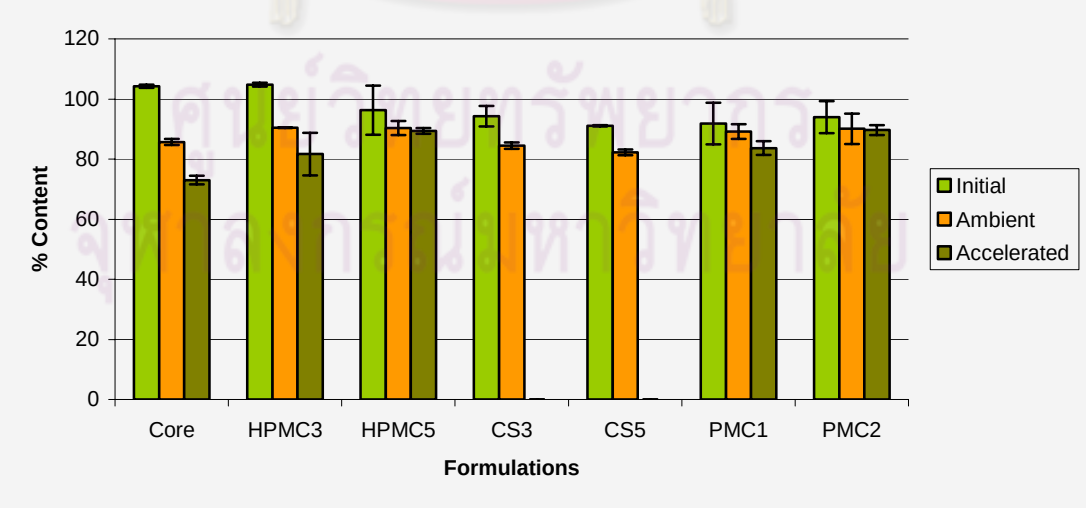


Figure 24. The assay content of the tablets formulations at ambient and accelerated condition

In this study, the coated films could improve the stability of MCS core tablet. The stability of the coated tablets could be ranked: polymethacrylate = HPMC> chitosan. As the coating level was increased, thicker film was obtained. Consequently, more stable was the coated tablets. For chitosan citrate formulations, the contents of the highest coating levels were not clearly different from that of the lower coating level.

The chromatographic profile of the MCS showed 3 major peaks (Figure 25): 1 with a retention time of 20 minutes, was identified as caffeine and used as a internal standard for the MCS. Identification of the next peak may be one of the substances responsible for the therapeutic activity of MCS, which confirmed the absence of any significant chemical interaction among the formulation constituents. No change in the retention time of the major peaks or the appearance of degradation products was observed. The chromatographic profile of the MCS was identical to the chromatographic profile of tablet (Figure 25-31).

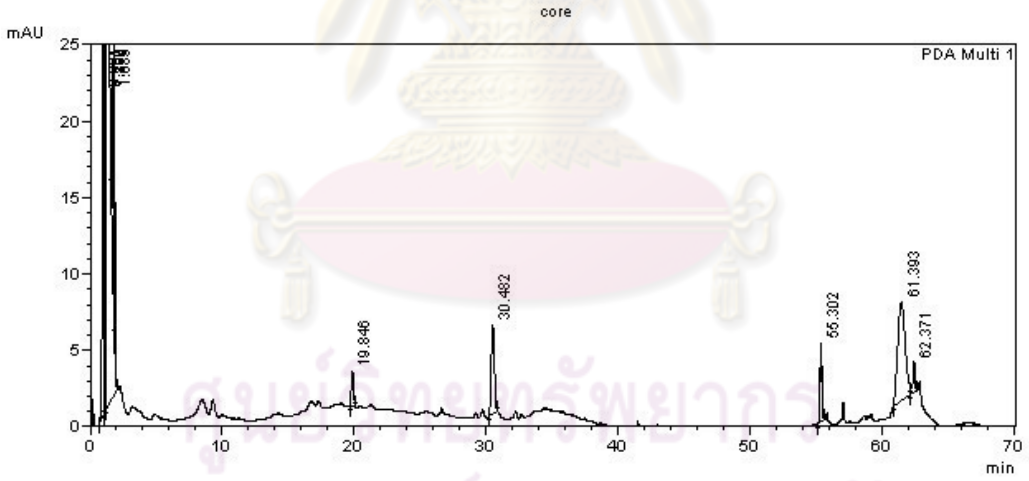


Figure 25. HPLC chromatogram of core tablets

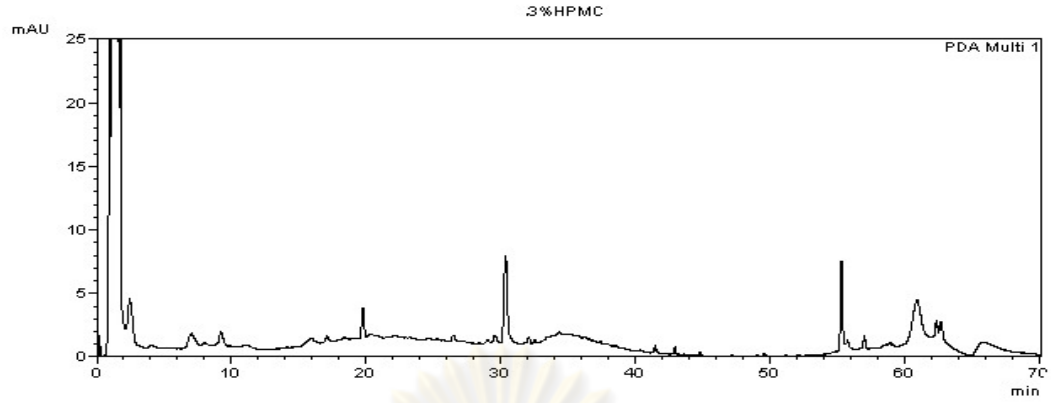


Figure 26. HPLC chromatogram of 3% HPMC film coated tablets

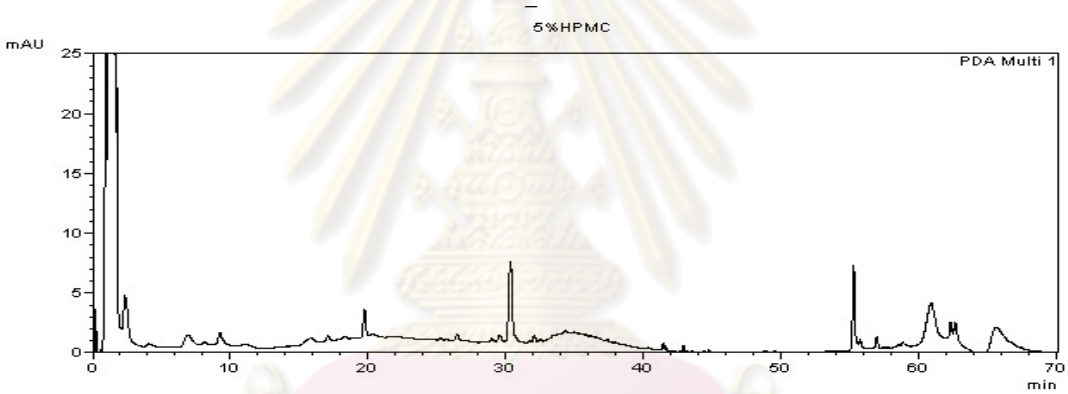


Figure 27. HPLC chromatogram of 5% HPMC film coated tablets

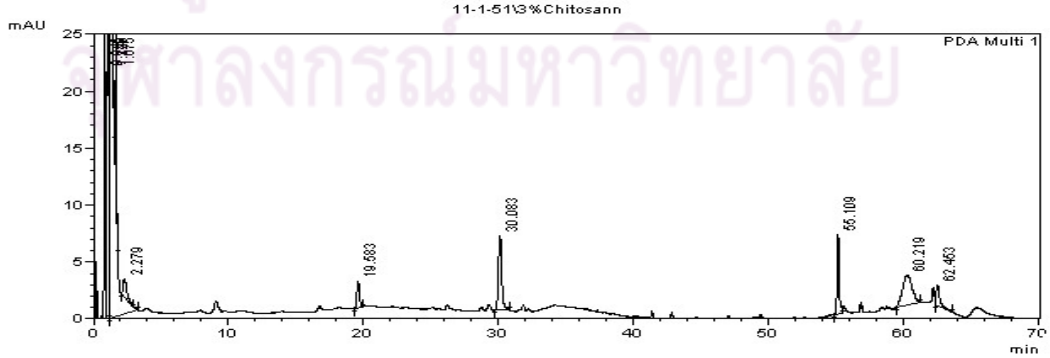


Figure 28. HPLC chromatogram of 3% chitosan film coated tablets

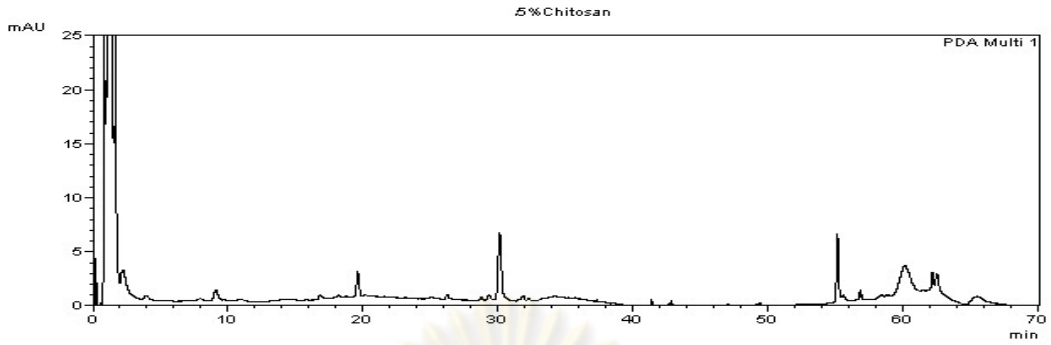


Figure 29. HPLC chromatogram of 5% chitosan film coated tablets

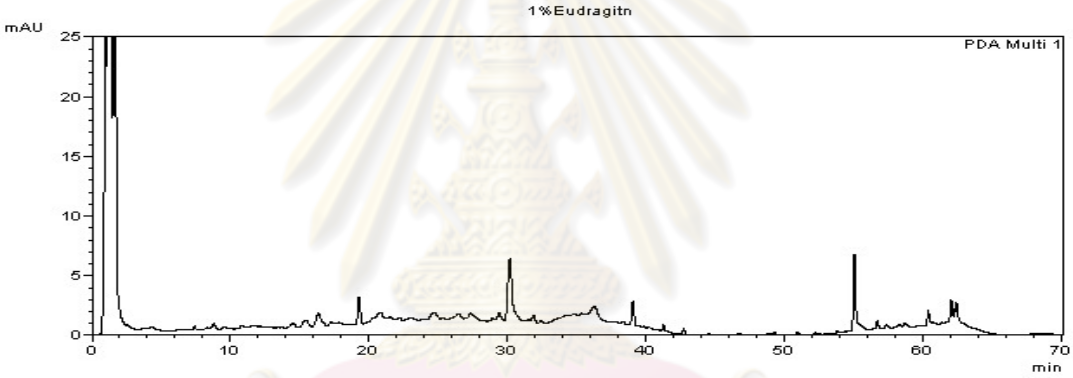


Figure 30. HPLC chromatogram of 1% eudragit film coated tablets

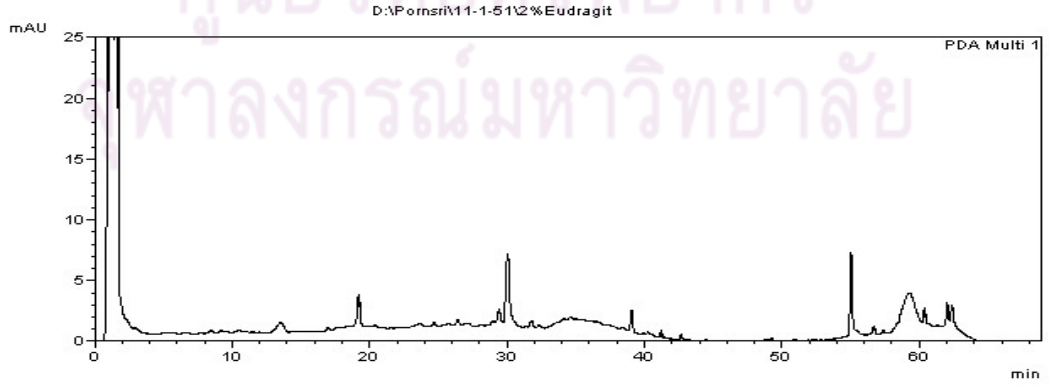


Figure 31. HPLC chromatogram of 2% eudragit film coated tablets

The contents of core tablets and 3% coating level of HPMC film coated tablets were significantly different by using an analysis of variance (ANOVA) and Games-Howell test for post-hoc comparisons both after freshly prepared, storage at ambient condition ($p < 0.05$).

The contents of core tablets with 3% and 5% coating level of HPMC film and 1% and 2% coating level of polymethacrylate there were significantly different by using an analysis of variance (ANOVA) and Games-Howell test for post-hoc comparisons both after freshly prepared, storage at accelerated condition ($p < 0.05$).

From this study it was found that the film coating provided better stability determined from the percent content of MCS. When stored at ambient and accelerated condition, it was found that the percent content of the formulation 5% HPMC and 2% PMC were acceptable according to pharmacopoeia.

The study of MCS powder and film-coated tablet in the accelerated condition at 45 ± 2 °C, 75 ± 5 %RH) for 4 months by thermal analysis with Differential Scanning Calorimetric (DSC) showed that the polymorphism of MCS and film-coated tablet was not different as shown in the Figure 32-33, in Appendix C.

The physical aging of solid state transformations MCS was determined by DSC. The effects of aging on the DSC curve of solid state transformations MCS are shown in Figure 33-40. This figure indicated the partial transformations of solid state transformations form upon storage at 96 %RH for 3 days. The thermograms showed two endothermic peaks at 105 °C and 130 °C when MCS was kept for 3 days. When MCS was stored at temperature and humidity controlled of 30 ± 2 °C in a desiccator, the thermograms showed only one endothermic peak at 100 °C.

The lactose as diluent for core tablet showed 2 endothermic peaks at 150°C and 215°C while other diluents showed no peaks at all.

The core tablets that were kept at 30 ± 2 °C for 4 months showed 2 endothermic peaks at 100 °C and 150 °C the latter peak of 150°C was likely to be the peak of lactose.

Similarly, HPMC3, HPMC5, CS3, CS5, PMC1 and PMC2 after stored at ambient condition at 30 ± 2 °C or 45 ± 2 °C and 75 ± 5 % RH for 4 months also showed 2 endothermic peaks at 100°C (MCS peak) and at 150°C (lactose peak).

CHAPTER V
CONCLUSIONS

The results of this study were concluded as follow:

1. Spray dried powder of *Malvastrum coromandelianum* powder were spherical-shaped particles with rough surface and were aggregated in cluster. Thus, they showed the poor flow property.

2. Core tablets containing *Malvastrum coromandelianum* extract could be manufactured by direct compression method in the controlled humidity environment. The core tablets had good appearance and met the requirement of the compendia.

3. Regarding to the coating process, the tackiness problem was found with chitosan and polymethacrylate film coating formulations. To overcome such problem, the spraying rate was adjusted downward or even if the spraying pattern was changed to be intermittent. As a consequence, this could lead to much consuming process time. Moreover, the higher levels of film coating were applied, the more tablet aggregation was produced. In contrast, the HPMC film coating formulations appeared to have no tackiness problem.

4. The freshly prepared tablets coated with chitosan citrate were greenish and glossy. After storage, the degree of green color of chitosan citrate film coated tablets was increased as a function of time and coating level, especially under the accelerated condition. Meanwhile, both HPMC and polymethacrylate film coated tablets were unchanged in physical appearances under any condition of the storage.

5. HPMC, chitosan and polymethacrylate solutions could be casted into free film. The tensile strength of the plasticized film could be ranked as: HPMC > chitosan > polymethacrylate. On the contrary, the percent elongation at break could be ranked as: polymethacrylate < HPMC < chitosan. The percent moisture sorption of cast films could be ranked as: chitosan citrate > HPMC > polymethacrylate.

6. Evaluation data of core and coated tablets showed that

   6.1 the coated tablets showed improvement in friability compared to core tablets.

   6.2 the hardness of coated tablets with HPMC and chitosan were obviously higher that of core tablets, whereas the hardness of coated tablets with polymethacrylate was slightly higher than core tablets.

   6.3 the weight variation and content uniformity of the tablets were conformed to compendia.

   6.4 the assay contents were in the range of 91.05-104.74% labeled amount.

   6.5 the increasing of coating level and the storage time under the accelerated condition of the film coated tablets showed a tendency to prolong the disintegration time.

6. The film coated tablet of 5% HPMC and 2% PMC formulations were stable under both ambient and accelerated condition for 4 months of storage. However the storage of 5% HPMC for 6 months was physically changed.

7. In conclusion, Eudragit E100 film coated tablets containing high dose of *Malvastrum coromandelianum* spray-dried powder could be prepared with satisfactory properties and stability.

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APPENDICES

APPENDIX A

Table 9. Calibration data of MCS solution at 273 nm.

Concentration (mg/ml)	Area
2	1.922453
3	2.983336
4	3.925411
5	5.067469
6	6.090575
7	7.067717

Table 10. Data of precision of MCS solution at 1st day

Number	Area at 273 nm		
	Caffeine	MCS	Caffeine/MCS
1	30665.1	159953.8	5.216151
2	29892.7	159271.8	5.328117
3	30256.3	159218.3	5.262319
4	30471.2	160722.3	5.274564
5	30258.5	160793.8	5.314004
6	30673.2	160535.8	5.233748
Average			5.271484
%CV			0.830984

Table 11. Data of precision of MCS solution at 2nd day

Number	Area at 273 nm		
	Caffeine	MCS	Caffeine/MCS
1	30110.6	159953.8	5.312209
2	30347.8	163274.5	5.38011
3	30013.1	162210.7	5.404663
4	30333.6	160852.4	5.30278
5	30648.1	161733.2	5.277104
6	30187.4	161793.8	5.359647
Average			5.339419
%CV			0.928447

Table 12. Data of precision of MCS solution at 3rd day

Number	Area at 273 nm		
	Caffeine	MCS	Caffeine/MCS
1	30446.3	161664.5	5.309824
2	30731.7	161814.8	5.265403
3	30485	162006.1	5.314289
4	30562.9	159183.9	5.208403
5	30797.6	158271.8	5.139095
6	30381.6	161884.9	5.328386
Average			5.2609
%CV			1.407399

Table 13. The percentage of recovery of MCS

Analytical concentration (mg/ml)	%Recovery of MCS			Mean	SD
	1	2	3		
2.50	91.0937	85.8505	86.4009	87.8601	1.038491
3.75	85.5617	89.3499	86.3923	87.1074	1.543134
5.00	88.0467	87.0467	85.4835	86.9600	0.996798
6.25	96.0057	88.6748	94.0242	92.8234	2.806771
7.50	96.0342	95.9954	98.2239	96.7592	1.132478

APPENDIX B

Table 14. Particle size distribution

Mesh No.	Aperture (mm)	Particle size (mm)	Particle size distribution (%)
-	-	<0.180	64.2
80	0.180	0.180-0.25	8
60	0.25	0.25-0.425	7.2
40	0.425	0.425-0.85	14
20	0.85	>0.85	6.6

Table 15. Bulk density, tap density and Carr's compressibility index

Sample No.	Measurement values		
	Bulk Density	Tap Density	Carr's Index
1	0.4112	0.5900	30.3050
2	0.4142	0.5920	30.0337
3	0.4091	0.5885	30.4843
Average	0.4115	0.5902	30.2743
SD	0.0025	0.0017	0.2268

Table 16. Tensile strength and elongation at break of cast films

Film formulations	Tensile strength (N/mm^2)[a]	Elongation at break (%)[a]
HPMC	0.10104±0.02952	8.64168±1.97931
Chitosan	0.05513±0.01252	22.7165±6.21524
Polymethacrylate	0.01723±0.00374	4.75053±0.22798

[a] All values were mean±SD of fines sample

Table 17. The percentage of moisture sorption of the cast film

Number of test	%Moisture sorption		
	HPMC	Chitosan	Polymethacrylate
1	11.0852	25.9758	0.5012
2	10.9854	25.9684	0.5046
3	10.9687	25.8651	0.5126
Average	11.0131	25.9364	0.50613
S.D.	0.063	0.06189	0.00585

Table 18. The percentage of friability of core tablet and tablet coated with HPMC with different coating level (A) freshly prepared and (B) after exposure to the accelerated condition for 4 months

Condition	Coating level (%w/w)	Weight (g)		% Friability
		Before	After	
A	Core tablet	7.726	7.707	0.246
	3	7.943	7.943	0.000
	5	8.145	8.145	0.000
B	Core tablet	7.952	7.954	-0.0251
	3	7.982	7.982	0.000
	5	8.723	8.723	0.000

Table 19. The percentage of friability of tablet coated with chitosan with different coating level (A) freshly prepared and (B) after exposure to the accelerated condition for 4 months

Condition	Coating level (%w/w)	Weight (g)		% Friability
		Before	After	
A	3	7.965	7.965	0.000
	5	8.082	8.082	0.000
B	3	7.992	7.992	0.000
	5	8.547	8.547	0.000

Table 20. The percentage of friability of tablet coated with polymethacrylate with different coating level (A) freshly prepared and (B) after exposure to the accelerated condition for 4 months

Condition	Coating level (%w/w)	Weight (g)		% Friability
		Before	After	
A	3	7.741	7.741	0.000
	5	7.789	7.789	0.000
B	3	7.752	7.752	0.000
	5	7.863	7.863	0.000

Table 21. Hardness of core tablet and film coated tablet formulations after preparation, storage under ambient and accelerated condition

Formulations	Hardness (kp)		
	Mean (SD) (n=10)		
	Initial	4^{th} month,RT	4^{th} month, AC
Core	6.84 (1.0)	17.07 (0.42)	>20
HPMC3	>20	>20	>20
HPMC5	>20	>20	>20
CS3	>20	>20	>20
CS	>20	>20	>20
PMC1	16.31 (1.53)	16.46 (2.57)	>20
PMC2	14.03 (2.16)	16.27 (2.08)	>20

Table 22. Hardness of core tablet and tablet coated with HPMC film with different coating level (A) freshly prepared, (B) after exposure to the ambient condition and (C) after exposure to the accelerated condition for 4 months

Tablet No.	Hardness (kp)								
	A			B			C		
	core	3%	5%	core	3%	5%	core	3%	5%
1	7.6	18.6	>20	17.0	>20	19.1	>20	18.7	>20
2	8.2	19.2	18.8	17.4	18.6	>20	>20	>20	19.0
3	7.8	17.5	>20	16.9	19.2	>20	>20	18.9	>20
4	7.2	>20	18.4	17.2	18.7	18.9	>20	>20	>20
5	7.8	17.8	>20	16.8	>20	18.7	18.9	19.2	>20
6	5.2	>20	>20	17.8	18.4	>20	>20	18.7	18.7
7	6	18.4	18.9	17.6	19.1	19.2	19.5	>20	18.9
8	6	18.1	>20	16.8	>20	>20	19.5	>20	>20
9	6.2	18.5	>20	16.5	>20	18.5	19.7	19.1	18.8
10	6.4	17.9	18.7	16.7	18.5	>20	19.4	18.6	>20
Average	6.84	18.25	18.7	17.07	18.75	18.88	19.4	18.6	18.85
SD	1.00	-	-	0.42	-	-	-	-	-
%CV	14.70	-	-	2.45	-	-	-	-	-

Table 23. Hardness of core tablet and tablet coated with chitosan citrate film with different coating level (A) freshly prepared, (B) after exposure to the ambient condition and (C) after exposure to the accelerated condition for 4 months

Tablet No.	Hardness (kp)					
	A		B		C	
	3%	5%	3%	5%	3%	5%
1	18.8	>20	>20	19.0	18.9	>20
2	19.1	19.2	18.9	>20	>20	19.1
3	>20	>20	19.0	>20	18.7	>20
4	>20	18.7	18.9	18.7	>20	>20
5	18.8	>20	>20	18.9	19.0	>20
6	19	>20	18.6	>20	18.8	18.9
7	17.9	18.4	19.2	19.2	>20	18.8
8	17.7	>20	>20	>20	>20	>20
9	>20	>20	>20	18.6	19.2	18.8
10	>20	18.5	18.8	>20	18.8	>20
Average	18.55	18.7	18.9	18.88	18.9	18.9
SD	-	-	-	-	-	-
%CV	-	-	-	-	-	-

Table 24. Hardness of core tablet and tablet coated with polymethacrylate film with different coating level (A) freshly prepared, (B) after exposure to the ambient condition and (C) after exposure to the accelerated condition for 4 months

Tablet No.	Hardness (kp)					
	A		B		C	
	1%	2%	1%	2%	1%	2%
1	18	11.6	17.2	12.6	>20	18.5
2	15	9.6	15.2	15.6	18.7	18.7
3	17	14.2	15.0	16.2	18.5	19.2
4	18	14.	10.4	13.8	18.4	18.9
5	14.6	14.4	18.8	19.8	>20	19.2
6	15	15.2	17.4	16.5	18.4	>20
7	18	14.6	15.6	18.5	>20	18.7
8	14	17.8	18.8	17.2	18.8	>20
9	16.5	14.2	17.5	16.8	18.9	18.9
10	17	14.7	18.7	15.7	17.9	18.2
Average	16.31	14.03	16.46	16.27	18.51	18.78
SD	1.536	2.162	2.574	2.081	-	-
%CV	9.421	15.412	15.64	12.79	-	-

APPENDIX C

Table 25. Weight variation of tablet coated with HPMC film with different coating level (A) freshly prepared, (B) after exposure to the ambient condition and (C) after exposure to the accelerated condition for 4 months

No.	Weight (mg)																			
	A							B							C						
	Core	HPMC 3	HPMC 5	CS3	CS5	PMC1	PMC3	Core	HPMC 3	HPMC 5	CS3	CS5	PMC1	PMC3	Core	HPMC 3	HPMC 5	CS3	CS5	PMC1	PMC3
1	384	397	403	399	405	387	390	388	398	405	397	405	389	390	389	398	399	398	407	388	391
2	386	395	406	400	404	389	389	389	396	406	395	406	388	392	388	399	402	395	409	389	390
3	383	396	410	402	402	388	390	387	402	403	398	408	389	393	389	399	400	397	408	389	390
4	384	395	402	392	406	385	389	385	401	407	397	409	387	392	388	401	401	395	408	388	391
5	379	396	405	401	402	389	388	385	400	409	399	412	385	390	387	400	405	394	405	387	390
6	379	397	407	399	401	389	388	384	399	405	401	411	385	391	388	402	406	398	411	385	390
7	389	398	406	403	401	387	386	379	398	410	400	408	385	390	389	401	407	399	411	385	392
8	385	400	405	404	403	388	392	388	398	412	398	407	389	391	386	399	404	391	410	386	391
9	384	402	405	396	402	384	388	389	397	409	395	409	384	392	385	402	405	392	409	384	392
10	386	397	409	395	400	388	389	396	402	410	402	409	383	389	388	401	404	395	408	384	392
11	385	401	407	404	402	388	388	385	401	408	401	405	385	389	385	399	406	397	408	384	389
12	388	398	410	400	403	388	389	382	399	409	401	406	385	388	389	400	410	395	407	387	388
13	384	392	401	396	403	389	391	386	398	412	400	407	383	389	389	398	410	396	405	387	389
14	386	398	406	403	404	389	390	383	396	412	399	408	385	387	390	397	401	398	403	385	388
15	383	399	408	396	405	388	389	387	398	408	396	402	383	389	390	396	412	399	407	385	389
16	384	402	405	398	408	389	387	386	395	407	398	409	385	389	390	396	412	398	407	385	389
17	379	401	407	397	406	388	390	389	397	409	397	408	382	390	388	397	413	399	408	386	389
18	379	400	409	400	407	388	391	388	398	407	398	409	385	391	385	396	412	392	405	389	389
19	389	399	410	402	409	387	390	389	398	406	397	407	389	390	391	397	411	396	407	389	390
20	387	402	407	399	407	388	392	387	399	407	400	407	390	391	392	399	412	398	409	389	392
Average	384.15	398.25	406.4	399.3	404	387.8	389.3	386.6	398.5	408.05	398.45	407.6	385.8	390.1	388.3	398.8	406.6	396.1	407.6	386.5	390.1
SD	3.16	2.71	2.56	3.26	2.53	1.32	1.55	3.43	1.93	2.45	2.03	2.21	2.41	1.49	1.94	1.95	4.61	2.44	2.03	1.87	1.31
%CV	0.82	0.68	0.63	0.81	0.62	0.34	0.40	0.88	0.48	0.60	0.51	0.5	0.62	0.38	0.50	0.48	1.13	0.61	0.49	0.48	0.33

Table 26. The disintegration time of core tablets and tablet coated with different coating level (A) freshly prepared or as received, (B) after exposure to the ambient condition for 4 months and (C) after exposure to the accelerated condition for 4 months

Formulation	Coating Level (%w/w)	A	B	C
Core tablet	-	11	10	11
HPMC	3	15	15	14
	5	15	16	15
Chitosan	3	38	37	38
	5	39	40	41
polymethacrylate	1	20	22	23
	2	21	23	24

Table 27. Dissolution data of *Malvastrum coromandelianum* core tablets and tablets coated with HPMC, chitosan and polymethacrylate after exposure to the ambient and accelerated conditions (n=3)

Storage condition	Times (min)	% Residual amount						
		Core	HPMC3	HPMC5	CS3	CS5	PMC1	PMC2
Ambient	15	40.98	61.75	72.14	41.72	67.55	40.82	44.75
	30	71.21	77.40	81.62	74.20	77.71	67.43	69.21
	45	76.37	78.63	84.14	79.89	76.63	79.93	79.08
	60	76.03	78.67	86.10	77.21	78.53	80.52	76.63
Accelerated	15	41.84	35.98	50.42	-	-	40.11	55.91
	30	67.96	73.08	88.60	-	-	63.86	78.60
	45	78.85	88.86	90.64	-	-	81.17	82.62
	60	83.29	93.28	94.30	-	-	87.92	88.02

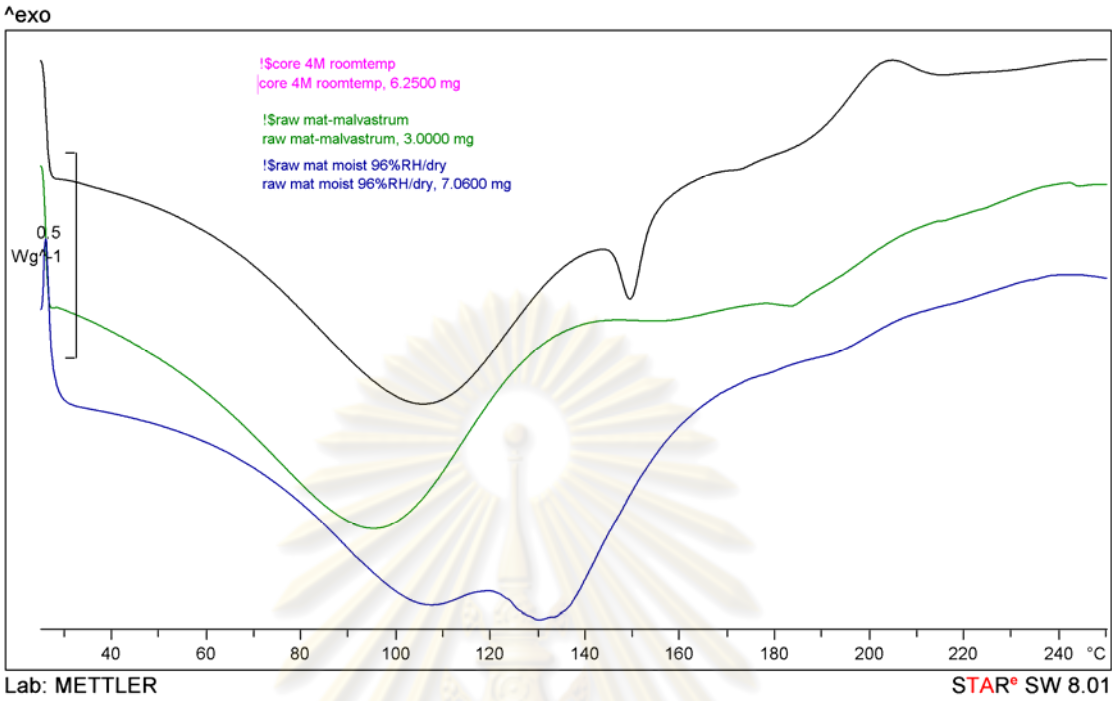

Figure 32. The thermogram of MCS and core tablets at room temperature

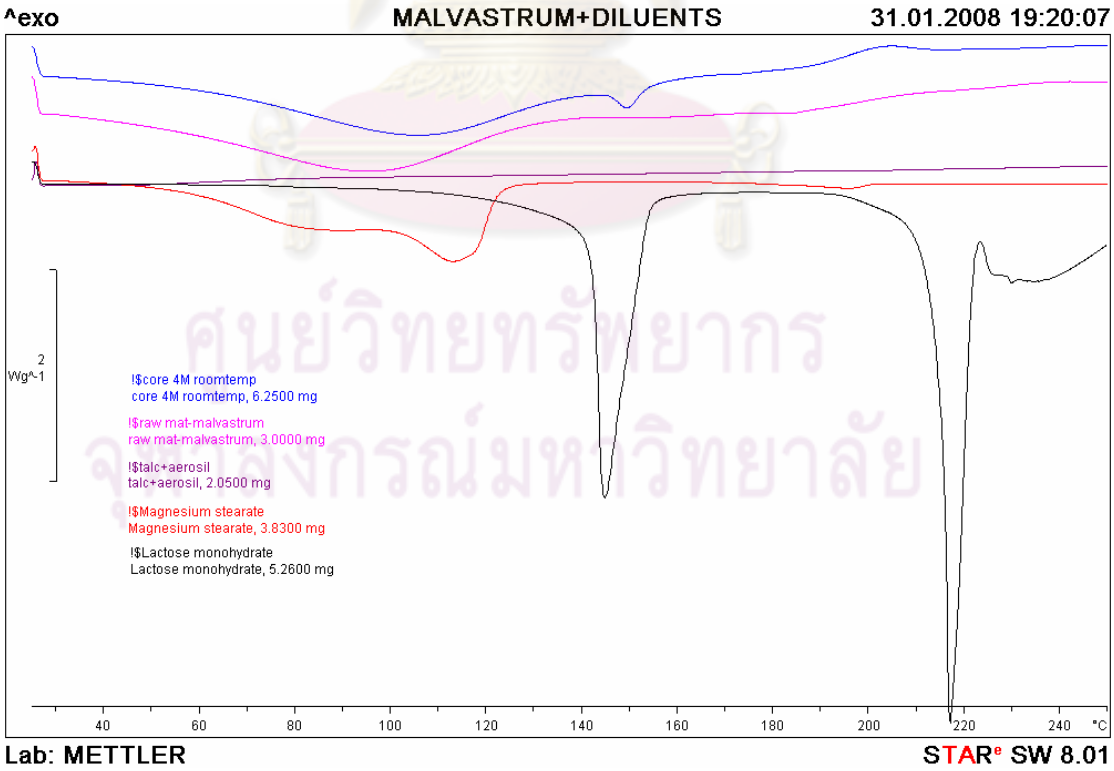

Figure 33. The thermogram of core tablets and diluents in the formulations

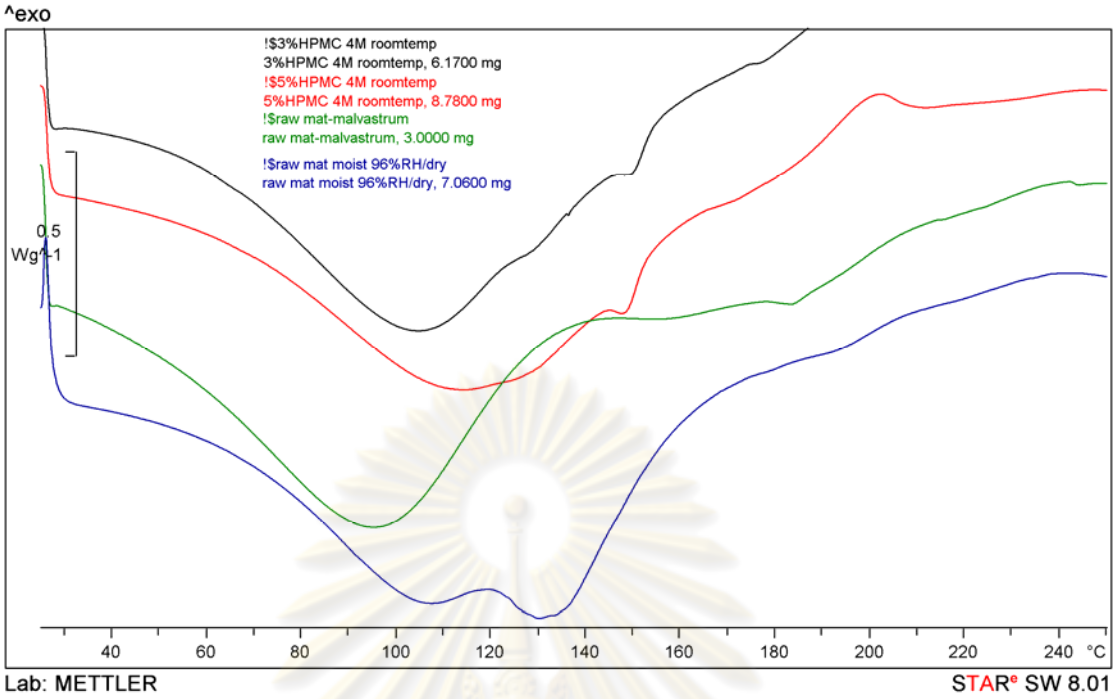

Figure 34. The thermogram of core tablets and film coated tablets of 3% HPMC and 5% HPMC at ambient condition

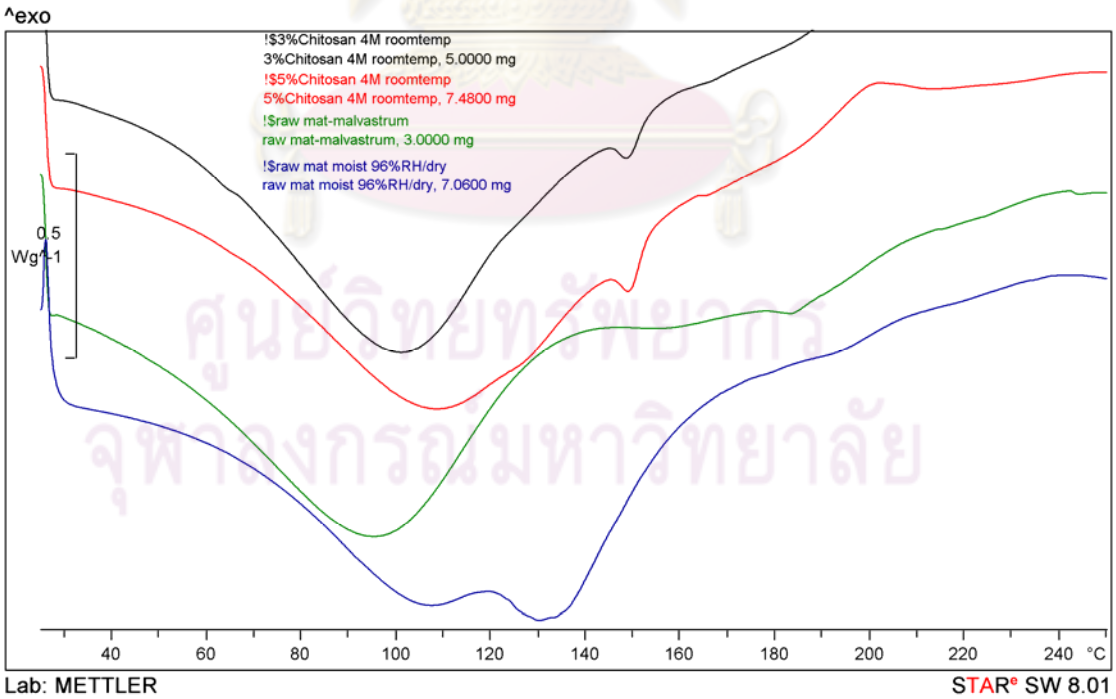

Figure 35. The thermogram of core tablets and film coated tablets of 3 % chitosan and 5% chitosan at ambient condition

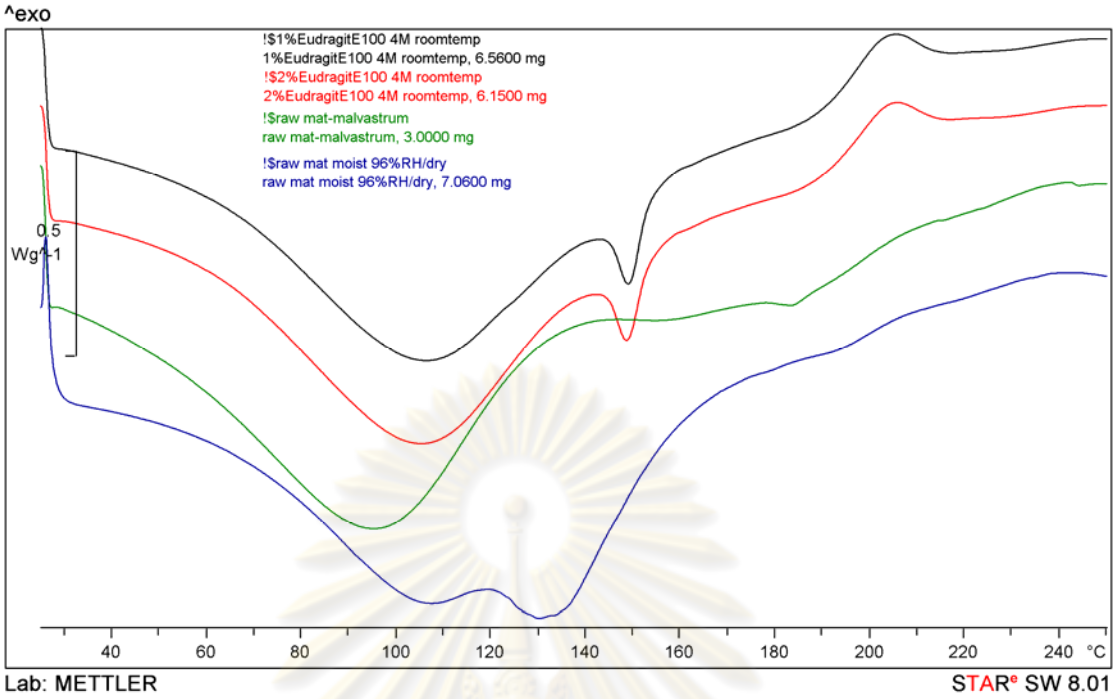

Figure 36. The thermogram of core tablets and film coated tablets of 1 % Eudragit E100 and 2% Eudragit E100 at ambient condition

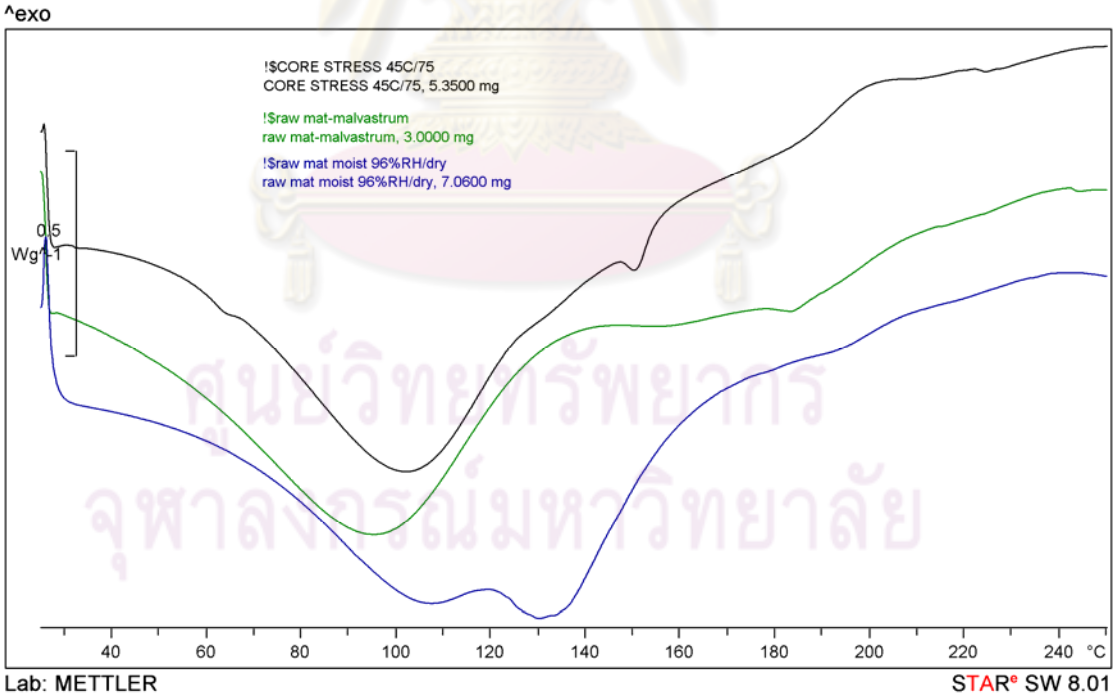

Figure 37. The thermogram of MCS and core tablets at accelerated condition

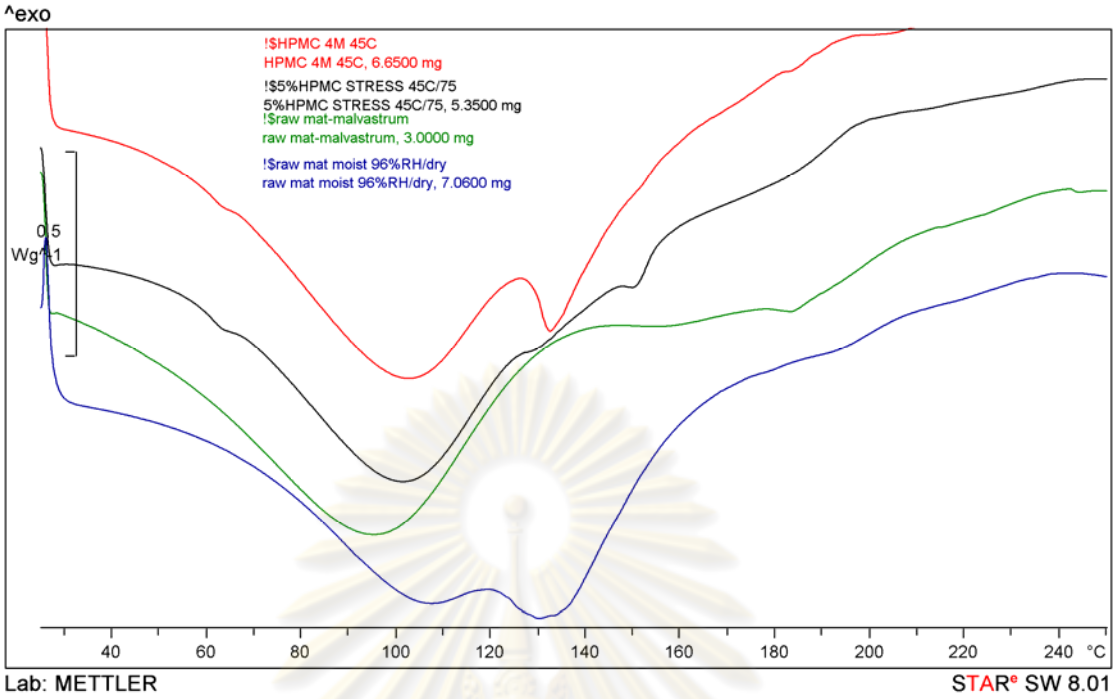

Figure 38. The thermogram of core tablets and film coated tablets of 3% HPMC and 5% HPMC at accelerated condition

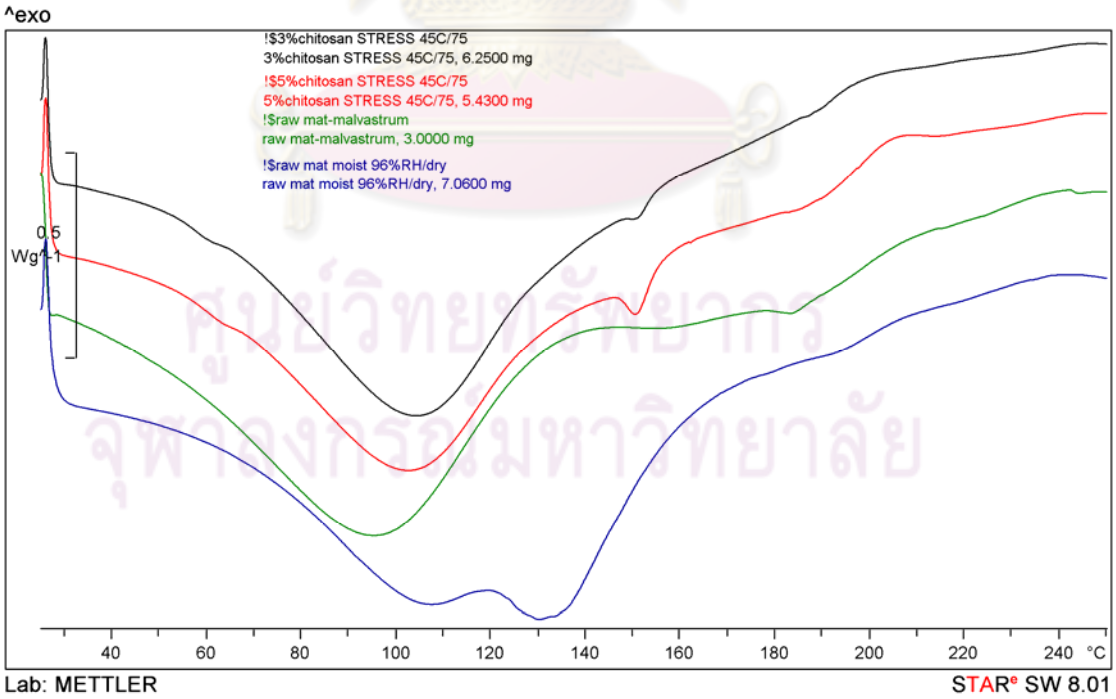

Figure 39. The thermogram of core tablets and film coated tablets of 3 % chitosan and 5% chitosan at accelerated condition

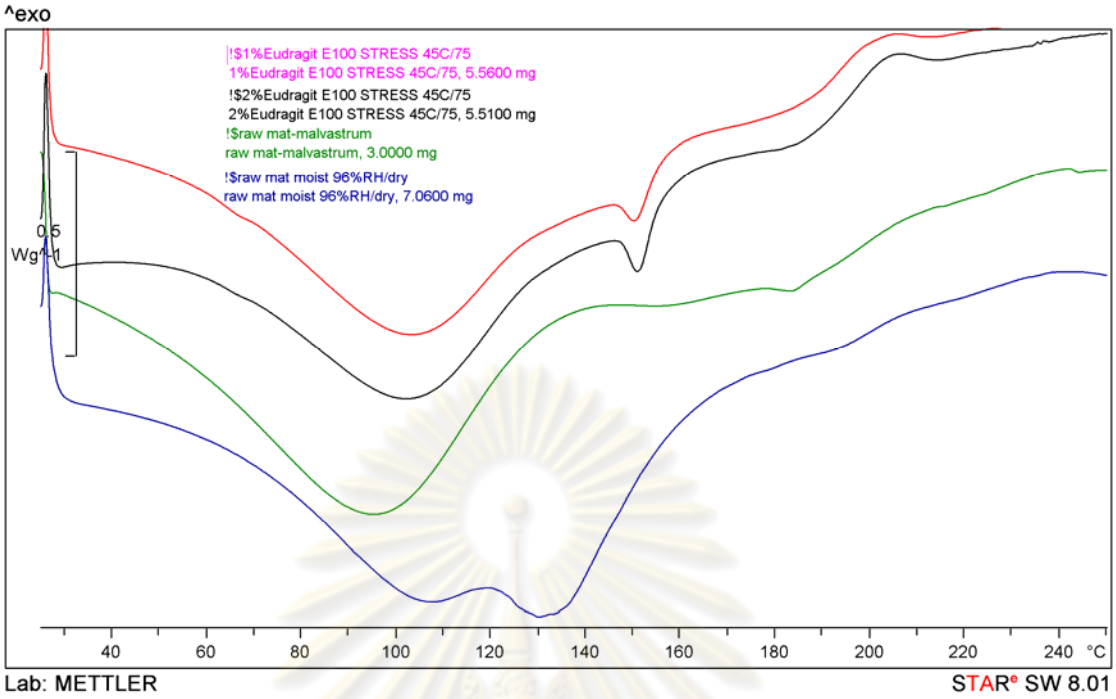

Figure 40. The thermogram of core tablets and film coated tablets of 1 % Eudragit E100 and 2% Eudragit E100 at accelerated condition.

Descriptives

content

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	72.9833	1.45056	.83748	69.3799	76.5867	71.51	74.41
2	3	86.6533	2.41177	1.39244	80.6622	92.6445	85.08	89.43
3	3	89.4100	.96892	.55940	87.0031	91.8169	88.35	90.25
4	3	85.1167	.93431	.53942	82.7957	87.4376	84.05	85.79
5	3	90.3200	.68462	.39526	88.6193	92.0207	89.90	91.11
Total	15	84.8967	6.57341	1.69725	81.2564	88.5369	71.51	91.11

Test of Homogeneity of Variances

content

Levene Statistic	df1	df2	Sig.
2.459	4	10	.113

ANOVA

content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	584.534	4	146.133	71.626	.000
Within Groups	20.402	10	2.040		
Total	604.936	14			

Post Hoc Tests

Multiple Comparisons

Dependent Variable:content

	(I) formulation	(J) formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Games-Howell	1	2	-13.67000*	1.62489	.010	-21.7794	-5.5606
		3	-16.42667*	1.00713	.001	-21.2619	-11.5915
		4	-12.13333*	.99617	.003	-16.9780	-7.2886
		5	-17.33667*	.92607	.002	-22.4409	-12.2325
	2	1	13.67000*	1.62489	.010	5.5606	21.7794
		3	-2.75667	1.50060	.498	-11.5757	6.0623
		4	1.53667	1.49327	.830	-7.3598	10.4332
		5	-3.66667	1.44745	.324	-13.1871	5.8537
	3	1	16.42667*	1.00713	.001	11.5915	21.2619
		2	2.75667	1.50060	.498	-6.0623	11.5757
		4	4.29333*	.77712	.024	.8362	7.7505
		5	-.91000	.68496	.695	-4.1381	2.3181
	4	1	12.13333*	.99617	.003	7.2886	16.9780
		2	-1.53667	1.49327	.830	-10.4332	7.3598
		3	-4.29333*	.77712	.024	-7.7505	-.8362
		5	-5.20333*	.66874	.009	-8.3207	-2.0859
	5	1	17.33667*	.92607	.002	12.2325	22.4409
		2	3.66667	1.44745	.324	-5.8537	13.1871
		3	.91000	.68496	.695	-2.3181	4.1381
		4	5.20333*	.66874	.009	2.0859	8.3207

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
content	21	51.2%	20	48.8%	41	100.0%

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent

Descriptives

			Statistic	Std. Error
content	Mean		87.2361	.88189
	95% Confidence Interval for Mean	Lower Bound	85.3965	
		Upper Bound	89.0757	
	5% Trimmed Mean		87.0984	
	Median		87.1100	
	Variance		16.332	
	Std. Deviation		4.04132	
	Minimum		81.14	
	Maximum		95.85	
	Range		14.71	
	Interquartile Range		7.20	
	Skewness		.397	.501
	Kurtosis		-.658	.972

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
content	.119	21	.200*	.955	21	.429

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Oneway

Test of Homogeneity of Variances

content

Levene Statistic	df1	df2	Sig.
4.136	6	14	.013

ANOVA

content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	240.187	6	40.031	6.482	.002
Within Groups	86.457	14	6.176		
Total	326.645	20			

Post Hoc Tests

Multiple Comparisons

Dependent Variable:content

	(I) formulation	(J) formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Games-Howell	1	2	-6.89048*	.62030	.029	-12.1781	-1.6028
		3	-7.10382	1.76651	.167	-19.2277	5.0200
		4	-.93382	.88596	.915	-5.3546	3.4870
		5	1.31952	.83684	.705	-2.8699	5.5089
		6	-5.58715	1.53794	.193	-15.4942	4.3199
		7	-6.56382	2.99197	.506	-30.4858	17.3581
	2	1	6.89048*	.62030	.029	1.6028	12.1781
		3	-.21333	1.65713	1.000	-14.7202	14.2935
		4	5.95667*	.64066	.042	.4850	11.4284
		5	8.21000*	.57081	.017	3.3709	13.0491
		6	1.30333	1.41095	.937	-11.0279	13.6346
		7	.32667	2.92872	1.000	-25.3887	26.0420
	3	1	7.10382	1.76651	.167	-5.0200	19.2277

	2	.21333	1.65713	1.000	-14.2935	14.7202
	4	6.17000	1.77376	.221	-5.8554	18.1954
	5	8.42333	1.74975	.118	-3.9501	20.7968
	6	1.51667	2.17407	.985	-9.4773	12.5106
	7	.54000	3.36351	1.000	-18.8770	19.9570
4	1	.93382	.88596	.915	-3.4870	5.3546
	2	-5.95667*	.64066	.042	-11.4284	-.4850
	3	-6.17000	1.77376	.221	-18.1954	5.8554
	5	2.25333	.85204	.306	-2.0273	6.5340
	6	-4.65333	1.54627	.279	-14.4790	5.1723
	7	-5.63000	2.99625	.606	-29.4473	18.1873
5	1	-1.31952	.83684	.705	-5.5089	2.8699
	2	-8.21000*	.57081	.017	-13.0491	-3.3709
	3	-8.42333	1.74975	.118	-20.7968	3.9501
	4	-2.25333	.85204	.306	-6.5340	2.0273
	6	-6.90667	1.51866	.123	-17.0287	3.2153
	7	-7.88333	2.98210	.394	-32.0535	16.2868
6	1	5.58715	1.53794	.193	-4.3199	15.4942
	2	-1.30333	1.41095	.937	-13.6346	11.0279
	3	-1.51667	2.17407	.985	-12.5106	9.4773
	4	4.65333	1.54627	.279	-5.1723	14.4790
	5	6.90667	1.51866	.123	-3.2153	17.0287
	7	-.97667	3.24929	1.000	-21.0767	19.1233
7	1	6.56382	2.99197	.506	-17.3581	30.4858
	2	-.32667	2.92872	1.000	-26.0420	25.3887

	3	-.54000	3.36351	1.000	-19.9570	18.8770
	4	5.63000	2.99625	.606	-18.1873	29.4473
	5	7.88333	2.98210	.394	-16.2868	32.0535
	6	.97667	3.24929	1.000	-19.1233	21.0767

VITA

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