# สูตรตำรับ การประเมินผลและการขยายขนาดการผลิตของยาเม็ดเคลือบฟิล์มสารสกัดบัวบก

นางสาว สรญา เฮ้งสวัสดิ์



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต

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# FORMULATION, EVALUATION AND SCALE-UP PRODUCTION OF *CENTELLA ASIATICA* EXTRACT FILM COATED TABLETS

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| Thesis Advisor | Professor Garnpimol C. Ritthidej, Ph.D. |
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สารสกัดบัวบก มีลักษณะเป็นผงสีขาวออกเหลืองเล็กน้อย ละลายน้ำได้น้อยมาก มีคุณสมบัติในการไหลไม่ดี เนื่องจากประกอบด้วยอนุภาคหลายขนาด และหลายรูปร่างโดยอนุภาคส่วนใหญ่มีลักษณะเป็นแท่ง สารสกัดนี้ประกอบด้วยสารสำคัญ 3 ชนิด คือ เอเชียติโคไซด์ กรดมาเดคาสสิก และกรดเอเชียติก การทดสอบการสลายตัวของสารสำคัญเหล่านี้ในสภาวะเร่ง พบว่าสารเหล่านี้สลายตัวด้วยกรด ด่าง และไฮโดรเจน เปอร์ออกไซด์ เมื่อทดสอบความเข้ากันได้กับสารช่วยอื่นๆ ในตำรับในสภาวะเร่งที่ 45±2° ซ, ความชื้นสัมพัทธ์ 75±5% เป็นเวลา 4 เดือน พบว่า สารสกัดบัวบกเมื่อผสมกับ สเปรย์ดรายแลคโตส, ทัลคัม, ซิลิคอน ไดออกไซด์ และ แมกนีเซียม สเตียเรท พบว่าการสลายตัวลดลง แต่เมื่อผสมกับโซเดียมสตาชไกลโคเลท หรือพรีเจลาติไนซ์ สตาชนั้นปริมาณสารสำคัญในสารสกัดบัวบกสลายตัวไปมากกว่าสารวัตถุดิบเล็กน้อย ยาเม็ดสารสกัดบัวบกเตรียมโดยวิธีการตอกโดยตรง แล้วเคลือบด้วยสารก่อฟิล์มได้แก่ ไคโตแซน ไฮดรอกซีโพรพริล เมทิลเซลลูโลส และโพลีเมทราไคลเลท ด้วยปริมาณการเคลือบต่างๆกัน ผลการประเมินพบว่ายาเม็ดแกน และยาเม็ดเคลือบฟิล์มที่เตรียมได้นั้นมีลักษณะทางกายภาพที่ดี โดยยาเม็ดแกนมีสีขาว ผิวเรียบ มัน และยาเม็ดที่เคลือบด้วยไฮดรอกซีโพรพริล เมทิลเซลลูโลส และโพลีเมทราไคลเลท นั้นมีสีขาว ผิวเรียบ มันเงา ส่วนยาเม็ดที่เคลือบด้วยไคโตแซน จะมีสีเหลืองอ่อน มันเงา จากการตรวจสอบคุณสมบัติทางเคมี-ฟิสิกส์ของยาเม็ดแกนและยาเม็ดเคลือบฟิล์ม พบว่า ความแข็ง ความกร่อน ความแปรปรวนของน้ำหนักเม็ดยา การแตกกระจายตัวของเม็ดยา ความสม่ำเสมอของปริมาณตัวยาและปริมาณตัวยาสำคัญของยาเม็ด มีคุณสมบัติเข้าตามมาตรฐานเภสัชตำรับของอเมริกา 25 ส่วนการปลดปล่อยตัวยาของยาเม็ดทุกสูตรไม่น้อยกว่า 70% (Q) และเมื่อศึกษาความคงตัวของยาเม็ดเหล่านี้ที่สภาวะปกติ และสภาวะเร่ง เป็นเวลา 4 เดือน พบว่าการเคลือบฟิล์มและการเพิ่มปริมาณสารก่อฟิล์มบนผิวเม็ดยา มีแนวโน้มที่จะเพิ่มความคงตัวของยาเม็ด เมื่อใช้สารก่อฟิล์มในปริมาณที่เท่ากัน สูตรยาเม็ดที่เคลือบด้วยโพลีเมทราไคลเลท จะมีความคงตัวดีที่สุด นอกจากนี้ยังพบว่าเมื่อเก็บภายใต้สภาวะเร่ง สารก่อฟิล์มเป็นปัจจัยสำคัญที่มีผลต่อความแข็ง การแตกกระจายตัวของเม็ดยาและการปลดปล่อยตัวยาจากยาเม็ดเคลือบฟิล์ม โดยเฉพาะยาเม็ดเคลือบฟิล์มไคโตแซนจะมีความแข็งของเม็ดยาและการปลดปล่อยตัวยาลดลง ส่วนการแตกกระจายตัวของเม็ดยาใช้เวลานานขึ้น และนอกจากนี้การเปลี่ยนแปลงสีของเม็ดยาที่เคลือบด้วยไคโตแซนจะเข้มขึ้นโดยสัมพันธ์กับระยะเวลาในการเก็บ ในขณะที่ยาเม็ดเคลือบฟิล์มด้วยไฮดรอกซีโพรพริล เมทิลเซลลูโลสในปริมาณสูงมีการปลดปล่อยตัวยาลดลง แต่ไม่มีการเปลี่ยนแปลงลักษณะทางกายภาพ ส่วนยาเม็ดที่เคลือบด้วยโพลีเมทราไคลเลทไม่พบการเปลี่ยนแปลงทั้งลักษณะทางกายภาพและคุณสมบัติทางเคมี-ฟิสิกส์ จากการศึกษาปัจจัยในการเคลือบฟิล์มที่ต้องเปลี่ยนแปลงเมื่อขยายขนาดการผลิตพบว่า การลดความเร็วในการหมุนของหม้อเคลือบลง จึงจะให้ได้เม็ดยาที่มีลักษณะทางกายภาพที่ดี และผลการทดสอบความแข็ง ความกร่อน ความแปรปรวนของน้ำหนักเม็ดยา และการแตกกระจายตัวของเม็ดยาเปรียบเทียบกับขนาดการผลิตเล็กพบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ

ภาควิชา เภสัชอุตสาหกรรม ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชอุตสาหกรรม ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

##4576611633 MAJOR : MANUFACTURING PHARMACY
KEYWORD : *CENTELLA ASIATICA* / FILM COATING/ TABLETS/ STABILITY/ PHYSICOCHEMICAL PROPERTIES/ ASIATICOSIDE

SORAYA HENGSAWAS: FORMULATION, EVALUATION AND SCALE-UP PRODUCTION OF *CENTELLA ASIATICA* EXTRACT FILM COATED TABLETS. THESIS ADVISOR: PROFESSOR GARNPIMOL C. RITTHIDEJ, Ph.D., THESIS CO-ADVISOR: JITTIMA CHATCHAWALSAISIN, Ph. D., 180 pp. ISBN 974-53-1930-9.

*Centella asiatica* dry extracts were white to pale yellow powder. They were very slightly soluble in water. They exhibited a poor flow property because they had various sizes and shapes. However, most of them were in rod shape. The extracts consisted of asiaticoside, madecassic acid and asiatic acid. They were degraded by acidic, basic agents and hydrogen peroxide at the stress condition. Compatibility test of these compounds at a accelerated condition, 45±2 °C, 75±5% RH, for 4 months found that the stability of Centella extract were improved by spray dried lactose, tulcum, silicon dioxide and magnesium stearate. On the other hand, with sodium starch glycolate or pregelatinized starch the active constituents were degraded slight lower the raw material. The core tablets were prepared by direct compression method and coated with various coating levels of chitosan, hydroxypropyl methylcellulose (HPMC) and polymethacrylate. The core and coated tablets exhibited good physical appearances the core tablets had white with smooth surface. The tablet coated with HPMC and polymethacrylate were white and glossy, while the tablet coated with chitosan were yellowish and glossy. The physicochemical properties of Centella tablets such as hardness, friability, weight variations, disintegration, content uniformity and drug content conformed to the specificication of official USP 25. In addition, the drug dissolutions were more than 70% (Q). The stability study at ambient and accelerated conditions for 4 months found that the film coating and increasing of the coating level tended to increase the stability of the tablets. At the same coating level, the tablets coated with polymethacrylate showed the greatest stability. Moreover, under the accelerated condition the film former, particularly chitosan mainly affected the hardness, tablet disintegration, and the drug release characteristics of the film coated tablet. Either hardness or drug release of the chitosan coated tablets were decreased while the tablet disintegration time was increase. In addition, the higher intensity of the color of tablets coated with chitosan was related to the storage period. The drug releases of tablets coated with HPMC at high coating level were decreased; however, there was no change in physical appearance. Tablets coated with polymethacrylate were unchange in the physicochemical properties. Scaling-up of coating process found that the pan speed should be decreased in order to obtain good physical appearance of film coated tablets. There were no significant differences of hardness, friability, weight variation and disintegration between small scale and scale up batch.

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

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

Academic year 2004 Co-advisor's signature.....

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# ABBREVIATIONS

| | | |
|---|---|---|
| AA | = | asiatic acid |
| AS | = | asiaticoside |
| °C | = | degree Celsius |
| cm | = | centimeter |
| $cm^2$ | = | square centimeter |
| CST | = | centella selected triterpene |
| CV | = | coefficient variation |
| D&C | = | determination applied in USA to dyes permitted for use in drugs and cosmetics |
| e.g. | = | *exemplit gratia,* 'for example' |
| et al. | = | *et alii,* 'and others' |
| FDA | = | Food and Drug Administration |
| FD&C | = | determination applied in USA to dyes permitted for use in foods, drugs and cosmetics |
| g | = | gram |
| glu | = | glucose |
| HPLC | = | high performance liquid chromatography |
| i.m. | = | intramuscular |
| i.v. | = | intravenous |
| kg | = | kilogram |
| kN | = | kilonewton |
| kp | = | kilopound |
| $LD_{50}$ | = | a dose lethal to 50% of the specified animals or microorganism |
| MA | = | madecassic acid |
| mg | = | milligram |
| min | = | minute |
| ml | = | milliliter |
| mm | = | millimeter |
| mPa·s | = | millipascal |
| M.W. | = | molecular weight |

| | | |
|---|---|---|
| N | = | normal (concentration) |
| NMR | = | nuclear magnetic resonance |
| pH | = | the negative logarithm of the hydrogen ion concentration |
| $r^2$ | = | coefficient of determination |
| RH | = | relative humidity |
| rham | = | rhamnose |
| rpm | = | revolutions per minute |
| s.c. | = | subcutaneous |
| S.D. | = | standard deviation |
| sec | = | second |
| $T_g$ | = | glass transition temperature |
| TLC | = | thin layer chromatography |
| µl | = | microliter |
| µm | = | micrometer |
| USP | = | The United States Pharmacopoeia National Formulary |
| vs. | = | versus |
| v/v | = | volume by volume |
| WHO | = | World Health Organization |
| WVP | = | water vapour pressure |
| w/w | = | weight by weight |

# CHAPTER I
# INTRODUCTION

Since prehistoric times, *Centella asiatica* is a medical plant that has been in use. The therapeutic use of this herbal remedy with its wide range of applications has been well known in South East Asia and India for centuries. This plant continues to be used within the framework of folk medicine as an effective remedy. *Centella asiatica* is located at the interface between traditional and modern, scientifically oriented medicine (Brinkhaus et al., 2000). Different uses are claimed for the plant, the more common being its use as a wound healing agent, constituent of brain tonics for the mentally retarded. The active ingredients of *Centella asiatica* were determined to be triterpenoids the constituents of which include: asiatic acid, asiaticoside, madecassic acid and madecassoside. These are pentacyclic triterpenes, found to display chronic venous insufficiency, varicose vein and wound healing properties (Inamdar, Yeole, Ghogare, and De Souza, 1996). The chemical structure, the functional group of ester and alcohol, of these constituents could be degraded by hydrolysis and oxidation, particularly. Moreover, *Centella asiatica* extracts also consist of vallerlin, a bitter taste compound. Centella preparations used in conventional medicine are prepared in oral form (tablets and drops), topical form (ointments and powder) and in the form of injections (s.c., i.m. and i.v.) (Brinkhaus et al., 2000).

The tableting of formulations containing high dosed extracts 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. A number of authors 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 (Eggelkraut-Gottanka et al., 2002).

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 was stated that for optimal dissolution, the tablet had to disintegrate into its primary particles as quickly as possible (Davies, 2001).

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

Film coated tablet is the pharmaceutical dosage form which is deposited upon the tablet surface with a thin plastic-like material consisting of polymer. Polymeric film coating have been applied to pharmaceutical dosage forms 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 prevent 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 hydroxypropyl methylcellulose (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 controlled drug release depending on characteristics of selected films that are 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).

From the excellent clinical treatment outcomes of *Centella asiatica* extract in the oral dosage form as well as the advantages of direct compression method and film coated tablet, thus; the film coated tablet of *Centella asiatica* extracts was interesting. In this investigation, it was aimed to develop the formulation of *Centella asiatica* extract film coated tablet for prevention of the bitter taste and getting the good stability, to evaluate the physicochemical properties and to test the products which storage under the recommended conditions followed the Thai FDA guidance.

**The objectives of the present study were:**

1. To evaluate some physicochemical properties of *Centella asiatica* extract.
2. To develop and evaluate the formulation of *Centella asiatica* extract film coated tablets using chitosan, HPMC and polymethacrylate as film former.
3. To study the stability of *Centella asiatica* extract film coated tablets under accelerated condition (45±2°C and 75±5% RH) compared with the ambient condition for 4 months.
4. To study the factors involved in the scale-up production of *Centella asiatica* extract film coated tablet.

# CHAPTER II
# LITERATURE REVIEW

## I. *Centella asiatica* (Linn.) Urban

*Centella asiatica* (Linn.) Urban, the synonym *Hydrocotyle asiatica* Linn., belonging to the Umbelliferae family, is a small perennial herbaceous creeper growing to 50 cm with leaves rounded to reinform, the petioles elongated; flowers umbels with three sessile flowers (Figure 1). The leaves are thin and soft, with palmate nerves, hairless or with only a few hairs, and measure about 2 to 5 cm in diameter. It has been used as a traditional herbal medicine in Asiatic countries for hundreds of years. In Thailand, locally known as bua-bok, is widely distributed in open or damp shaded places (Sribusarakum, 1997; Cheng and Koo, 2000).

**Figure 1** *Centella asiatica* (Linn.) Urban

Traditionally both the entire plant with roots and leaves of *Centella asiatica* can be used medicinally to treat various skin conditions ranging from slow healing wounds and lesions, to leprosy. Additionally traditional uses of *Centella asiatica* include heart disease, high blood pressure, rheumatism, fevers, nervous disorders, bronchitis, asthma, and syphilis (Duke, 1985).

Through dacades, there has been increasing interest in various compounds obtained from *Centella asiatica.* The different compounds report may due to places of origin of the materials or to the differences in varieties of the plant. At present, the active principles of the plant *Centella asiatica* are asiatic acid, madecassic acid and asiaticoside. The chemical structures were presented in Table 1. Chemically these compounds are pentacyclic triterpenes, belonging to the β-amyrin ursolic acid group. These compounds are known their major clinical indications for the treatment of wounds, venous insufficiency of the limbs, varicose vein, certain mycobacterial infections and cellulitis (Inamdar, Yeole, Srivastava and De Souza, 1996)

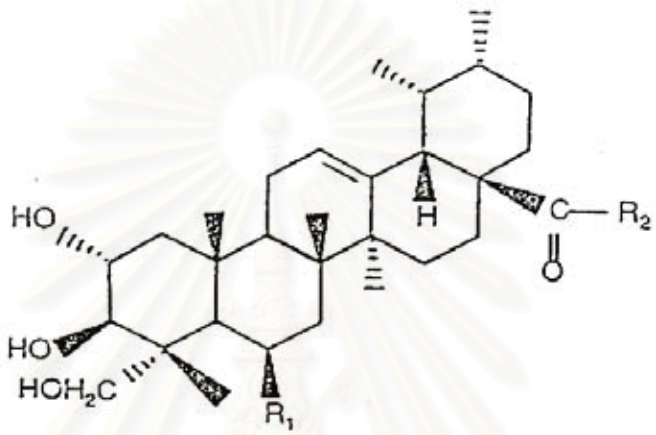
The extracts of *Centella asiatica* were studied on the following extracts: tritrated extract of *Centella asiatica* (TECA), total triterpene fraction of *Centella asiatica* (TTFCA). The TECA is combination comprising asiatic acid 30%, madecassic acid 30% and asiaticoside 40%. The TTFCA comprises asiatic acid and madecassic acid 60% in a ratio that is not clearly defined, in combination with asiaticoside 40%.

The Centella preparations used in conventional medicine are employed in oral form (tablets and drops), topically (ointment and powder) and in the form of injections (s.c., i.m. and i.v.) (Brinkhaus et al., 2000). A summary of commercial products available in the market is shown in Table 2.

Total triterpenic fraction of *Centella asiatica* (TTFCA) is effective in improving venous wall alterations in chronic venous hypertension and in protecting the venous endothelium. TTFCA is active on connective tissue modulation, improves the synthesis of collagen and other tissue proteins by modulating the action of fibroblasts in the vein wall, and stimulates collagen remodeling in and around the venous wall. Studies have indicated the role of TTFCA on the synthesis of specific venous wall elements cell cultures of human embryonal fibroblasts. TTFCA is active on the microcirculation in venous and diabetic microangiopathy. Signs and symptoms of venous hypertension and edema are improved by treatment. The remodeling on collagen synthesis could be one of the possible mechanisms of action of TTFCA in

the remodeling of echolucent (soft; therefore, with risk of thrombosis and embolization) plaques at the carotid and femoral bifurcation. (Incandela et al., 2001)

**Table 1** Chemical structure of asiaticoside, madecassic acid, and asiatic acid (Sribusarakum, 1997)




| Compound | Structure | Molecular formula | M.W. |
|---|---|---|---|
| Asiaticoside | $R_1$, = H<br>$R_2$ = O-glu-glu-rham | $C_{48}H_{78}O_{19}$ | 958 |
| Madecassic acid | $R_1$ = OH<br>$R_2$ = OH | $C_{30}H_{48}O_6$ | 504 |
| Asiatic acid | $R_1$, = H<br>$R_2$, = OH | $C_{30}H_{48}O_5$ | 488 |

**Table 2** Pharmaceutical preparation containing *Centella asiatica* extract (Martindale, 1993; Sribusarakum, 1997; Brinkhaus et al., 2000)

| Name | Manufacturer | Dosage form | Ingredients | Indications |
|---|---|---|---|---|
| Blastoestimulina® | Funk, Spain | injection, eye drops, topical powder | *C. asiatica* | wounds, burns, eye disorder, ulcers |
| Blastoestimulina® | Funk, Spain | ointment, medicated dressing | *C. asiatica*, neomycin sulphate | burns, ulcers, skin infections, wounds |
| Blastoestimulina® | Funk, Spain | pessaries | *C. asiatica*, neomycin sulphate, chlordantoin, metronidazole, polymyxin B sulphate | vulvovaginal infections |
| Blastoestimulina® | Funk, Spain | topical aerosol | *C. asiatica*, amethocaine | wounds, burns, ulcers |
| Centellase® | Aventis Pharma, Italy | ointment, tablets, drops, powder | *C. asiatica* | circulatory disorders |

**Table 2** Pharmaceutical preparation containing *Centella asiatica* extract (cont.) (Martindale, 1993; Sribusarakum, 1997; Brinkhaus et al., 2000).

| Name | Manufacturer | Dosage form | Ingredients | Indications |
|---|---|---|---|---|
| Emdecassol® | Rhone-Poulenc Rorer, France | ointment | asiaticoside | skin disorders |
| Madecassol® | Roche, Belgium | ointment, powder, tablet | *C. asiatica* | burns, cellulitite, keloids, ulcers, venous insufficiency |
| Madecassol C® | Sanofi Winthrop, Mexico | - | *C. asiatica*, metronidazole, nitrofurazone | bacterial vaginosis, tricomoniasis |
| Madecassol Neomycin Hydrocortisone® | Nicholas, France | ointment | *C. asiatica*, neomycin sulphate, hydrocortisone acetate | wounds, skin disorders |
| Madecassol Tulgras® | Nicholas, France | ointment | *C. asiatica* | venous, ulcers |

**Pharmacokinetics**

The pharmacokinetics of the extract of *Centella asiatica* has been investigated in a single study both in animal experiments and in humans. Grimaldi et al. (1990) investigated the absorption behavior of an asiaticoside mixture (TTF) following both single and repeated oral doses. In animal experiments, an *in vivo* transformation of asiaticoside into asiatic acid was established. It was demonstrated that the maximum plasma concentration of asiatic acid increased significantly with increasing dose administrated, while the time point of maximum plasma concentration and the elimination half-life did not significantly change with increasing dose. In comparison with a single oral administration, repeated oral dose have been shown to significantly increase the maximum plasma concentration and the elimination half-life. The possible mechanism behind this accumulation phenomenon is a modification of asiaticoside metabolism induced by the prolonged administration of total triterpene fraction (TTF).

A comparison of figures measured after oral and subcutaneous administration of madecassoside, asiaticoside, asiatic acid, and madecassic acid in rats revealed a bioavailability varying between 30% and 50% (Brinkhaus et al., 2000).

**Pharmacodynamics**

The pharmacodynamic effects of *Centella asiatica* have been investigated in numerous animal experiments and *in vivo* studies. Demonstrated actions include, in particular, a wound healing, ulcer-protective, psychoneuro-pharmacological, antimicrobial and antiviral effect of the Centella or asiaticoside extract. TTFCA has important pharmacological characteristics as a modulator of collagen synthesis and has been used in traditional oriental medicine for the treatment of vascular diseases and in plastic and dermatologic surgery. Microcirculatory methods can be used to evaluate the changes in the microcirculation induced by TTFCA in venous and diabetic microangiopathy. TTFCA may also modify the tone of veins by a structure action on the collagen of vein walls (Cesarone et al., 2001).

Brinkhaus et al. (2000) reviewed the investigation of the effect of TECA on skin lesions induced by repeated mechanical stressing of skin in rats. The orally administered mixture of asiaticosides (TECA) was shown to accelerate wound healing. At the same time, TECA suppressed the post-traumatic edema of the tissue observed to occur after repeated mechanical injury of the skin. The mechanical underlying this phenomenon is possible the regulative effect of the mixture of asiaticosides on the metabolic processes taking place in abnormal skin condition. However Dermarderosiaun and Beutler (2002) reported that the topically applied TECA had a greater effect on wound healing than oral administration. Moreover they found that the glycoside madecassoside has anti-inflammatory properties, while asiaticoside appears to stimulate wound healing.

Vogel et al. (1990) reported that after oral administration of the major saponin containing triterpenic acids (asiaticoside, madecassoside, asiatic acid and madecassic acid) for investigating the wound healing effect, the asiatic acid and madecassic acid both proved to have a greater effect on the parameters investigated than the glycosides. With respect to molecular weight, however, all the isolated fractions were about equally effective.

**Adverse events/Contraindications**

Adverse reactions following the use of *Centella asiatica* include gastric complaints and nausea after oral administration (Brinkhaus et al., 2000; Capasso et al. 2003; Philp, 2004).



## II. Varicose veins and chronic venous insufficiency

### A. Varicose vein

Varicose vein are dilated, tortuous superficial veins that result from defective structure and function of the valves of the saphenous veins, from intrinsic

weakness of the vein wall, from high intraluminal pressure, or, rarely, from arteriovenous fistulas. Varicose veins can be categorized as primary and secondary. Primary varicose veins originated in the superficial system and occur two to three times as frequently in women as in men. Approximately half of patients have a family history of varicose veins. Secondary varicose veins result from deep venous insufficiency and incompetent perforating veins or from deep venous occlusion causing enlargement of superficial veins that are serving as collaterals (O'Rourke and Braunwald, 2001).

Patients with venous varicosities are often concerned about the cosmetics appearance of their legs. Symptoms consist of a dull ache or pressure sensation in the leg after prolonged standing; it is relived with leg elevation. The legs feel heavy, and mild ankle edema develops occasionally. Visual inspection of the legs in the dependent position usually confirms the presence of varicose veins (O'Rourke and Braunwald, 2001).

Varicose veins can usually be treated with conservative measures. Symptoms often decrease when the legs are elevated periodically, when prolonged standing is avoided, and when elastic support hose are worn. Small symptomatic varicose veins can be treated with sclerotherapy, in which a sclerosing solution is injected into the involved varicose vein and a compression bandage is applied. Surgical therapy should be reserved for patients who are very symptomatic, suffer recurrent superficial vein thrombosis, and/or develop skin ulceration. Surgical therapy may also be indicated for cosmetic reasons (O'Rourke and Braunwald, 2001).

The investigation of the biochemical action of an extract of *Centella asiatica* in the patients with severe varicose veins in the leg was established. The main target parameters were changes in the serum levels of uric acid and of three lysosomal enzymes involved in the mucopolysaccharide metabolism. Following the treatment, there was a significant reduction in the serum levels of uric acid and the lysosomal enzyme. It could interpret that the reduction was the evidence of a positive effect of Centella extract on varicose veins (Arpaia et al., 1990).

In addition, Montecchio et al (1991) investigated the effect of *Centella asiatica* triterpenic fraction (CATTF) on the reduction of the number of cells described as endothelial cell (EC) in patients with post-thrombotic syndrome, in comparison with healthy subjects. It was conducted that the CATTF-induced reduction in the number of endothelial cells has a protective effect on the integrity of the intima of the veins (Brinkhaus et al., 2000).

## B. Chronic venous insufficiency

Chronic venous insufficiency may result from deep vein thrombosis and/or valvular incompetence. Following deep vein thrombosis, the delicate valve leaflets become thickened and contracted so that they cannot prevent retrograde flow of blood; the vein becomes rigid and thick-walled. Although most veins recanalized after an episode of thrombosis, the large proximal veins may remain occluded. Patients with venous insufficiency often complain of a dull ache in the leg that worsens with prolonged standing and resolves with leg elevation. Examination demonstrates increase leg circumference, edema, and superficial varicose veins. Cellulitis may be a recurring problem. (O'Rourke and Braunwald, 2001).

Three major types of treatment are now used in chronic venous insufficiency (Incandela et al., 2001):

1. surgery and sclerotherapy (in associations or sequence) ;
2. elastic compression (usually with stockings, sometimes with bandages or pneumatics and or sequential compression) ;
3. drug treatment (with drugs mainly acting on edema and capillary filtration).

## C. Clinical picture

Chronic venous insufficiency is among the most common conditions affecting human. Ten to fifteen percent of men and 20-25% of women are affricated. It is more than a mere "cosmetic problem" because patients often require hospital

admission and surgical treatment. At least two thirds of leg ulcers have evidence of venous disease in the affected limb. One of the most common manifestations of venous insufficiency is varicose veins. Varicose veins are largely the result of the destruction of the network of proteoglycans in the elastic tissue of the vein wall by lysosomal enzymes. This situation causes an abnormal dilatation of the vein and a passage of electrolytes, proteins and water through the venous well and then oedema formation. Varicose veins are treated with sclerotherapy (injection of sodium tetradecyl sulfate into the vein which usually occludes the veins) or surgery (Capasso et al., 2003)

Herbal medicines used in the treatment of varicose vein may provide only relief of the unpleasant symptoms by increasing capillary resistance and venous tone but reverse changes in organic structures. These include horse chestnut, butcher's broom, hydrocotile, bilberry and witch hazel. By increasing venous tone, these herbal medicines are also useful to treat hemorrhoids (Capasso et al., 2003).

There are several studies carried out the effect of *Centella asiatica* extract on chronic venous insufficiency. The results of treatment were evaluated both subjectively by the patient on the basis of symptom and also objectively by means of plethysmography. The significant improvements in both the subjective and plethysmographic parameters were observed. Moreover, the significant improvement in vascular tone, venous capacity, venous reflux, venous flow and the reduction in venous pressure were reported in many researches as well (Brinkhaus et al., 2000).

*Centella asiatica* has been reported to improve the blood circulation in the lower limbs through a stimulation of collagen and mucopolysaccharides synthesis in the vein wall. As a consequence there is an increase in vein elasticity and reduction in the capacity of the vein to distend. *Centella asiatica* is also useful in case of cutaneous ulcers of venous origin, surgical wounds, fistulas, gynaecological wounds. This herbal medicine is stated to act like cortisone with respect to wound healing (Capasso et al., 2003). Table 3 shows clinical studies of *Centella asiatica* extract in the conventional dosage forms.

**Table 3** Clinical studies of *Centella asiatica* extract in conventional dosage forms (Brinkhaus et al., 2000; De Sanctis et al., 2001).

| Author | Indications | No. of patients | Medication | Treatment duration |
|---|---|---|---|---|
| Monteverde et al., 1987 | Venous insufficiency of the legs | 40 | TECA 30mg/day or O-(β-hydroxyethyl)-rutoside 500 mg/day | 30 days |
| Pointel et al., 1987 | Venous insufficiency of the legs | 94 | TECA 2 x 30 mg/day or 2 x 60 mg/day | 2 months |
| Arpaia et al., 1990 | Varicose veins | 20 | C. asiatica extract 2 x 30mg/day | 3 months |
| Belcoro et al., 1990 a | Venous hypertension | 62 | TTFCA 30 mg/day or 60 mg/day | 4 months |
| Belcoro et al., 1990 b | Venous hypertension | 44 | TTFCA 60 mg/day | 10 weeks |
| Montecchio et al., 1991 | Postphlebitic syndrome | 30 | CATTF (Centellase oral) 3 x 30 mg/day | 3 weeks |
| Mallol et al., 1991 | Striae gravidarum | 80 | Trofolasin (cream) | Throughout pregnancy |
| De Sanctis et. al., 2001 | Venous hypertension | 62 | TTFCA 3 x 30 mg/day or 3 x 60 mg/day | 4 weeks |

# 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 Figure 2 presents the schematic drawings of direct compression method. 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 trend to be expensive (Davies, 2001).

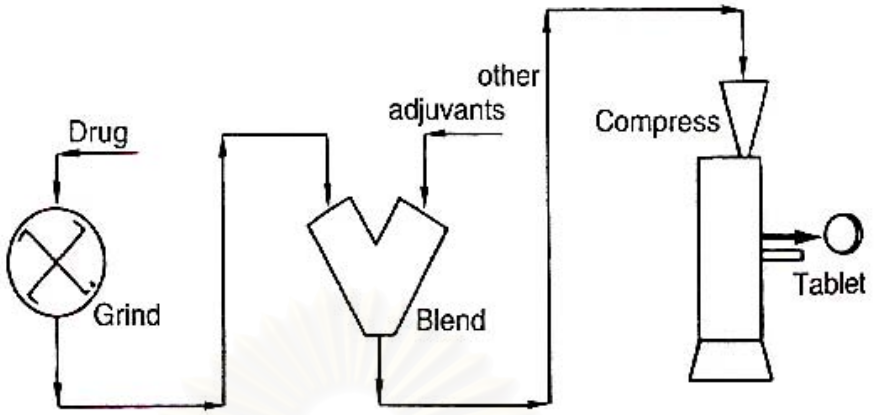



**Figure 2** Direct compression method (Davies, 2001).

## 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 granules hard flakes of glassy appearance, making the granules hard to compress (Bonati, 1991; 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).

Lauro et al. (2002) prepared the fast-release tablets of flavonoids; rutin and quercetin, by direct compression. Moreover, St. John's Wort plant dry extract were studied to prepare as tablets by roller compaction method (Eggelkraut-Gottanka et al., 2002).

## V. Film coated tablets

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; 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, or 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). Each of these uses relies on the integrity of the film and sufficient bonding to the substrate to ensure optimum performance. A single type of film coating may serve more than one of the above uses.

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 use of pH dependent polymers offers a different approach to taste masking than addition of artificial flavors or the use of rapidly disintegrating (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).

Recently, 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). Eudragit® E PO was developed to a novel powder coating process for attaining taste masking and moisture protective film coating on tablets. The delay of drug release in pH 6.8 buffer media provided by the powder coated films can be successfully exploited for taste masking and possible for other controlled release applications (Cerea et al., 2004).

Film coatings 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).

Bajdik et al. (2004) studied the crystal coating of dimenhydrinate by using HPMC as film former to promote the tablet making, increasing the flowability, compressibility, protect from several harmful factors (light, moisture, and heat transition) and prepare 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 coatings 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 or 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 of 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 chitosan, HPMC and polymethacrylate (Eudragit® E100). There are various in the properties as following:

### Chitosan

Chitosan is produced by alkaline *N*-deacetylation of chitin (Nunthanid et al., 2001; Fernández Cervera et al., 2004). Figure 3 illustrates the chemical structure of chitosan. It is a cationic polymer and a cationic polyelectrolyte. Thus the compatibility with cationic and nonionic polymer is good, while multivalent anions will easily crosslink with chitosan to form gels and precipitates.

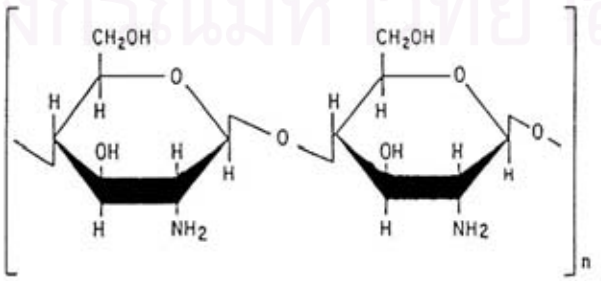



**Figure 3** Chemical structure of chitosan (Phaechamud, 1995)

Chitosan could be used as film former for coated pharmaceuticals. Since it is soluble in diluted acidic medium, attempt to prepare in insoluble form 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 propanolol HCl 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.

Besides, chitosan has been used for the production of herbal medicine controlled release implant systems. The extract of danshen (*Radix Salviae miltiorrhizae*), a medicinal herbal, was developed with chitosan acetate-gelatin as an implant (film) for the promotion of anastomosing and healing on muscles and tissues at the organic incision site in abdominal cavities. The film was degraded completely in rats over 28 days and the animal's wounds of abdominal incision healed well. As the content of gelatin was increased in the following formulations, the film became more soluble and the degradation of the film was accelerated both in vivo and in vitro. (Zhao et al., 2002).

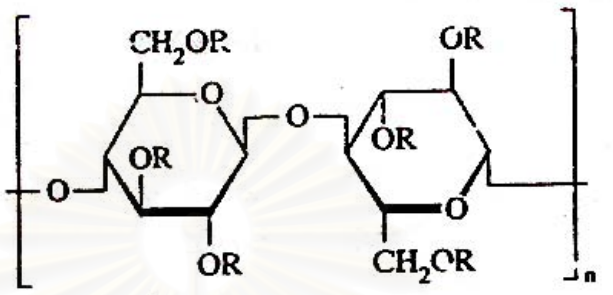
However, the incomplete characterization of chitosans and the variability of commercial chitosans have discouraged the pharmaceutical industry from adopting it as a pharmaceutical excipient or formulation component. The heterogeneity of these chitosans mainly results from the sources of chitin and relatively uncontrolled commercial processing of native chitin involving both N-deacetylation and depolymerization (Fernández Cervera et al., 2004).

**Hydroxypropyl methylcellulose (HPMC)**

Hydroxypropyl methylcellulose (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 4. 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 relative humidity of the surrounding (Kibbe, 2000).




Where R = H, $CH_3$ or $[CH_3CH(OH)CH_2]$

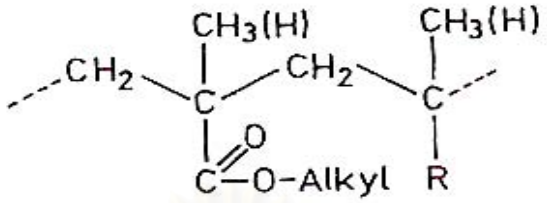
**Figure 4** Chemical structure of hydroxypropyl methylcellulose (HPMC)

**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 4). 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. Given a neutral or slightly alkaline environment, the film swells strongly in water and disintegrates (Kibbe, 2000; Cerea et al., 2004).

For application, Eudragit® E is diluted or dissolved to 4 to 10%. Addition of plasticizer is not necessary, but talc 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).

**Table 4** Chemical structure of polymethacrylates (Bauer et al., 1998)




| Products | R | Substitution (mole) | Function |
|---|---|---|---|
| *Amino*-alkylmethacrylate copolymers (Eudragit® E100) | -CO-$OCH_2CH_2N(CH_3)_2$ | 0.5 | gastrosoluble permeable pH < 5 |

**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 temperatures (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 glass transition temperature (Tg), and an increase in the elongation and flexibility 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; Bauer 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 liquids, 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, (b) 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.

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.

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 increases, 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).

Lin et al. (2000) investigated the effect of the organic esters used as plasticizers on water absorption behavior and adhesive property of Eudragit® 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 indicate that TBC may be the best choice of plasticizer for Eudragit® film, particularly for the Eudragit® E film.

### C. Solvents

Nowadays, 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 isopropanol), esters (ethyl acetate and ethyl lactate),

ketones (acetone) and chlorinated hydrocarbons (dichloromethane and trichloroethane). The polymer should not only be soluble in the chosen solvent but should adopt a conformation in solution that yields the maximum polymer extension, which will result in films with the 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 (Allen, Popovich, and Ansel, 2005).

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).

## VI. Stability study of herbal medicines

To date knowledge, there are few studies regarding the stability study of the herbal medicines. Kleinebudde (2004) reviewed the comparison of the stability of film-coated tablets, prepared from non-compacted *Eschscholtzia californica* Cham. dry extract or prepared from dry granules made of the extract. The different batches of the plant dry extract material showed varying compaction behaviors, which was explained by the different origin and composition mainly different organic acids. Exposing the film coated tablets to different relative humidity showed that tablets prepared with non-compacted extract powder adsorbed more water compared to those produced with compacted material.

Inamdar, Yeole, Srivastava, and de Souza (1996) studied the stability of the active constituents in the *Centella asiatica* extract formulations. It was found that at high temperature (40 °C) *Centella asiatica* extract in creams were stable for 3 months and in tablet dosage form were stable for 6 months under accelerated condition (40 °C, 80% RH). In addition, the different origins of the Centella extracts had an influence on the stability of the product formulations.

# CHAPTER III
# EXPERIMENTAL

## Materials

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

1. **Active Ingredient:**
   Centella Selected Triterpene (Batch No. CST030610, Changzhou Natural Products Development Co., Ltd., China)

2. **Standard materials:**
   - Asiaticoside (Batch No. AS030610, Changzhou Natural Products Development Co., Ltd., China)
   - Madecassic acid (Batch No. MA030501, Changzhou Natural Products Development Co., Ltd., China)
   - Asiatic acid (Batch No.AA030610, Changzhou Natural Products Development Co., Ltd., China)

3. **Commercial Preparation:**
   Centellase® Tablet (Lot No.03001, Aventis Pharma Co., Ltd., Italy)

4. **Tablet Diluents:**
   - Spray dried lactose monohydrate, Super-Tab® (Lot No. 2031104, The Lactose Company of New Zealand, Ltd., New Zealand)
   - Pregelatinized starch, Starch 1500® (Batch No. IN505698, Rama Production Co., Ltd., Thailand)
   - Magnesium stearate USP (Faci Asia Pacific PTE, Ltd.)
   - Talcum (Haichen Talc Powder, China)
   - Sodium starch glycolate, Explotab® (Rama Production, Co., Ltd., Thailand)

- Colloidal silicon dioxide, Aerosil® (Cabot, Holland)

**5. Film formers:**

- Chitosan M.W. 50,000 (Kyowa Technos Co., Ltd., Japan)
- Hydroxypropyl methylcellulose, Methocel® E5LV Premium (Batch No. RD19012404, Colorcon Ltd., England)
- Hydroxypropyl methylcellulose, Methocel® E15LV Premium (Batch No. RD14012406, Colorcon Ltd., England)
- Polymethacrylate, Eudragit® E100, (Lot. No. 8311101177, Rohm Pharma Polymers, Germany)

**6. Miscellaneous:**

- Citric acid, anhydrous AR (Lot. No. 6-23279-1, Srichand United Dispensary Co., Ltd., Thailand)
- Polyethylene glycol 6000 (Lot. No. 427124, Fluka Chemie GmbH, Switzerland)
- Polyethylene glycol 400 NF XVII (Lot. No. PH0901AAEC, Srichand United Dispensary Co., Ltd., Thailand)
- Propylene glycol USP XXII (Lot. No. 7879040502, Srichand United Dispensary Co., Ltd, Thailand)
- Glycerine USP XXII (Lot. No. LDLG192U, Srichand United Dispensary Co., Ltd, Thailand)
- Tributyl citrate (Lot. No. 3193097, Merck-Schuchardt)
- Methanol HPLC (Fisher Scientific UK Limited, UK)
- Acetronitrile HPLC (Fisher Scientific UK Limited, UK)
- Hydrochloric acid (Labscan Asia Co., Ltd., Thailand)
- Sodium Hydroxide (Lot. No. B231098243, Merck, Germany)
- Potassium Dihydrogen Orthophosphate, (Batch No. F2H145, Ajax Finechem, Australia)
- Other solvents were AR grade

## Equipment:

- Analytical Balance (Model A 200 S, Sartorius, Germany)
- Balance (Model SK-20KWP, A&D Co., Ltd., Korea)
- Hot air oven (Type UL 80, Memmert, Germany)
- Hot air oven (Model BM600, Memmert, Germany)
- Frita vibration (Model FT-200M, Fitra, Spain)
- Planetary paddle mixer (Model EB 20 F, Crypto-Peerless, England)
- V-shape mixer (Kan Seng Lee Machinery Ltd., Part., Thailand)
- 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 503S, Watson-Marlow, Limited, England)
- Shaker bath (Model 28L/8/SH/C, Polyscience Co., Ltd.)
- Modified tap density tester (Chanchai Engineering, Thailand)
- Tensile tester (Model LR10K, LLORD Instruments Limited, USA)
- 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)
- pH meter (Model 210 A+, Thermo Orion, Germany)
- High performance liquid chromatography (HPLC) (Model SCL-10A VP, Shimadzu, Japan):
  - Degasser (Model DGU-14A, Shimadzu, Japan)
  - Pump A, B liquid chromatography (Model LC-10AD, Shimadzu, Japan)
  - Auto injector (Model SIL-10A, Shimadzu, Japan)
  - Column oven (Model CTD-10AS, Shimadzu, Japan)
  - UV-VIS detector (Model SPD-10A, Shimadzu, Japan)
  - System controller (Model SCL-10A, Shimadzu, Japan)

# Methods

The experiment was performed in 7 parts as the following:

## 1. Characterization of Centella Selected Triterpene (CST) powder

### 1.1 Morphology Study

A small amount of CST powder was viewed using a scanning electron microscope (SEM). This approach allowed the particles to be observed in their size and shape and thus could provide in-depth morphological information.

### 1.2 Particle size distribution analysis

One hundred grams of CST powder was weighed and analysed for its particle size distribution using sieve analysis method. The Frita vibration apparatus with standard sieves mesh size number 30 (0.6 mm), 35 (0.5 mm), 40 (0.425 mm), 50 (0.3 mm), 60 (0.25 mm), 70 (0.212 mm), 80 (0.180 mm), 100 (0.150 mm), 120 (0.125 mm), 140 (0.106 mm), 170 (0.09 mm), 200 (0.075 mm) and 230 (0.063 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 quality 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 ($\rho_b$) was calculated as the ratio between weight (g) and volume (ml).

To determine the ultimate tapped density ($\rho_t$), the cylinder was tapped on a tap density tester from a height of 1.3 cm until no measurable change in volume was noticed. The constant volume was read and used to calculate the tap 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:

$$\text{Carr's index} = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100 \qquad [2]$$

### 1.4 Determination of angle of 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 paper 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

Ten 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 of g/sec.

### 1.6 Determination of active constituents of *Centella asiatica* extract

#### 1.6.1 Thin layer chromatographic method (TLC)

The commercial raw material, CST powder, received from the supplier, was first determined by TLC method to compare with the standard solution in methanol of asiaticoside, madecassic acid and asiatic acid.

The CST powder was dissolved with methanol. TLC analysis of active constituents was to spot the sample on precoated alumina plate compared to standard solution using the following procedures:

technique : one way, ascending
stationary phase : alumina sheet silica gel 60 F 254
mobile phase : ethylacetate : methanol : water (7.5 : 2.5 : 1)
spray reagent : 10% solution of sulfuric acid in methanol, followed by heating at hot air for 15 minutes

The general parameter used for describing the migration is the Rf value, where

$$\text{Rf value} = \frac{\text{distance moved by the solute}}{\text{distance moved by mobile phase front}} \quad [1]$$

### 1.6.2 High performance liquid chromatographic method (HPLC)

HPLC method for determination of active constituents from *Centella asiatica* was modified from Sribusarakum (1997) and validated as the following:

**HPLC Analysis**

HPLC chromatographic conditions:

Column : Hypersil® BDS (C18) column (250x4.6 mm), 5µm (Thermohypersil, UK) equipped with guard column packed with BDS (C18), 5 µm set at an ambient temperature
Detector : UV detector at 210 nm
Injection volume : 20 µl
Mobile phase : Water – acetonitrile linear gradient conditions are described in Table 5

Mobile phase were filtered through a membrane filter with a pore size of 0.45 μm and degassed for at least 30 minutes prior to use.

**Table 5** Linear gradient condition for HPLC method

| Time (min) | Flow-rate (ml/min) | Pump A Water (%) | Pump B Acetronitrile (%) |
|---|---|---|---|
| 0 | 1.0 | 70 | 30 |
| 12 | 1.0 | 0 | 100 |
| 15 | 1.0 | 70 | 30 |
| 30 | 1.0 | 70 | 30 |

***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 active constituent peaks was determined by the resolution and tailing factor (symmetry factor). The well resolving from the other peaks and symmetry peaks should be obtained.

The mix standard solutions of asiaticoside (AS), madecassic acid (MA) and asiatic acid (AA) in methanol at the concentration 400 μg/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 100 to 1000 μg/ml of each reference standard in methanol were prepared and analyzed. The linear equation of the curve obtained by

plotting the peak area at each level prepared versus the concentrations of each standard was calculated using the least square method.

***Precision***

The *standard preparation* from accuracy section was stepwise diluted with methanol to obtain the final concentration 400 µg/ml. Six replicated injections of this standard solution was analyze. Percentages of coefficient of variation (%CV) were calculated for determination of the precision.

**1.7 Solubility study**

The equilibrium solubility of active components as AS, MA and AA from CST were determined at 37 °C in triplicate as followed:

**1.7.1 Solubility of active components from centella selected triterpene in deionized water**

The excess amounts of CST were added into 100 ml of deionized water in 125- ml stoppered erlenmeyer flask. The mixtures were shaken in the constant temperature shaker bath with the rate of 150 rpm, at 37 °C. Samples were frequently taken until the concentration was constant. The sampling solutions were filtered through a filtering membrane with 0.45 µm pore size and then analyzed by HPLC gradient method.

**1.7.2 Solubility of active components from centella selected triterpene in the mixture of 0.1 N hydrochloric acid : isopropyl alcohol (70:30)**

The method was the same as previously described in 2.4.1 but the used solvent was the mixture of 0.1N hydrochloric acid: isopropyl alcohol (70: 30) instead of deionized water.

### 1.8 Forced degradation study (Stress test)

CST, standard AS, MA and AA were exposed to severe storage conditions according to the Thai FDA guideline (2004) as followed:

#### 1.8.1 Moisture Hydrolysis (method 1)

Fifty milligram each of CST, standard AS, MA and AA were weighed into four separate test tubes and added 3 drops of deionized water. The samples were heated at 80 °C for 3 hours, and left to room temperature. Twenty-five milligram each of the forced CST, and forced standards were accurately weighed into four separate 25-ml volumetric flasks, dissolved, and quantitatively diluted to volume with methanol HPLC grade. The solution were filtered through 0.45 µm membrane filter then injected into HPLC column.

#### 1.8.2 Moisture Hydrolysis (method 2: the modified method)

Fifty milligram each of CST, standard AS, MA and AA were weighed into four separate beakers and stored under 96% RH (desiccator with saturated potassium sulphate salt), 60 °C for 24 hours. The clear solution of the forced degraded powder was prepared for HPLC assay.

#### 1.8.3 Acid hydrolysis

Fifty milligram each of CST, standard AS, MA and AA were transferred into four separate test tubes and added 3 drops of 3N hydrochloric acid. The samples were heated at 80 °C for 3 hours, left to room temperature and then neutralized with sodium hydroxide solution. An accurately weight portion 25 mg of each of neutralized standards, were transferred to each 25-ml volumetric flasks. The drugs were dissolved, adjusted with methanol HPLC grade to volume and thoroughly mixed. The solutions were filtered through 0.45 µm membrane filter and the clear solution was used for HPLC assay.

### 1.8.4 Alkaline hydrolysis

Fifty milligram each of CST, standard AS, MA and AA were transferred into four separate test tubes and added 3 drops of 5N sodium hydroxide. The powder were heated at 80 °C for 3 hours, left to room temperature and then neutralized with hydrochloric acid solution. An accurately weight portion about 25 mg of each of neutralized standards, were transferred to each 25-ml volumetric flasks. The drug were dissolved, adjusted with methanol HPLC grade to volume and thoroughly mixed. The solutions were filtered through 0.45 µm membrane filter and the clear solution was used for HPLC assay.

### 1.8.5 Temperature degradation

Fifty milligram each of CST, standard AS, MA and AA were transferred into four separate test tubes and heated at 80 °C for 3 hours, left to room temperature. The clear solution of the forced powder was prepared for HPLC assay.

### 1.8.6 Oxidation

Fifty milligram each of CST, standard AS, MA and AA were transferred into four separated test tubes and dropped 3 drops of 30% hydrogen peroxide ($H_2O_2$), heated at 80 °C for 3 hours, and left to room temperature. The clear solution of the forced powder was prepared for HPLC assay.

### 1.8.7 Photolysis

Eight clear containers were equally divided to two groups, the controlled group was wrapped with aluminium foil and another test group was used as received. Fifty milligram each of CST, standard AS, MA and AA were transferred into that two groups of containers. Both groups were placed under daylight fluorescent lamp (40 Watts) at ambient temperature and humidity for 7 days. After

that, each of the stressed powders was prepared as the clear solution with methanol for HPLC assay.

## 2. Compatibility Study

Mixtures drug substances with the excipients were prepared in the binary mixture. Then the mixtures and raw material of CST (control) were stored in the same accelerated conditions as stability test that followed the Thai FDA guideline (จุไรรัตน์, 2547), which was at 45±2 °C, 75±5 % RH for 4 months. The tested excipients were spray dried lactose (Super-Tab®), pregelatinized starch (Starch®1500), magnesium stearate, colloidal silicon dioxide (Aerosil®), sodium starch glycolate (Explotab®) and talcum. Ratios of the mixtures between CST and Super-Tab® and Starch®1500 were 1:20. On the other hand, the mixtures between drug and the other excipients were prepared in 1:1 mixtures. The samples were taken every 4 weeks for physical appearance observation and HPLC evaluation.

The powder equivalent to 25 mg of CST was weighed into 25-ml volumetric flask then adjusted with methanol HPLC grade to volume and filtered through membrane filter. The remaining amounts of active constituents were determined by HPLC chromatographic condition as aforementioned. In addition, it was calculate the percentage of degradation with the following equation:

$$\% \text{ of degradation} = \frac{\% \text{ recovery at time 0} - \% \text{ recovery after storage)}}{\% \text{ recovery at time 0}} \times 100 \quad [3]$$

## 3. Formulation of *Centella asiatica* core tablets

The excipients which were tested in section 2 were chosen to include in the tablet formulation. Prior to mixing, the excipients (except CST powder) in the formulations were dried until constant weight for 2-3 hours at 60 °C in hot air oven. CST, Super-Tab®, Starch 1500®, and Explotab® were individually sieved through a 30-mesh screen. On the other hand, talcum, Aerosil® and magnesium stearate were screened through sieve size 80-mesh prior to mixing. The tablets containing 20% of CST were prepared by direct compression method.

A batch size of 500 g was produced by manually mixing in a plastic bag. First, CST was blended in the mixer with Starch 1500®, Super-Tab® and Explotab® by geometric dilution for 10 minutes each to obtain a homogeneous powder mixture. Next, Aerosil®, talcum and magnesium stearate were thoroughly mixed with the first mixture for 5 minutes each. Finally, the lubricated mixture was compressed into 150 mg tablets using a round concave punch 6.5 mm in diameter on a single punch tableting machine that was in a controlled humidity plastic box (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 evaluated following the specification in section 5. The formulations of core tablet are listed in Table 6.

**Table 6** *Centella asiatica* core tablet formulation composition

| Ingredients (%w/w) | Formulations | | | | | | |
|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| Centella selected triterpene | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Super-Tab® | 74.5 | 49.7 | 48.7 | 48.3 | 47.7 | 47.0 | 48.0 |
| Starch 1500® | - | 24.8 | 24.3 | 24.2 | 23.8 | 23.5 | 24.0 |
| Explotab® | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Talcum | 3.0 | 3.0 | 3.0 | 3.0 | 4.0 | 4.0 | 3.0 |
| Aerosil® | - | - | 1.5 | 1.5 | 1.5 | 2.5 | 2.0 |
| Magnesium stearate | 0.5 | 0.5 | 0.5 | 1.0 | 1.0 | 1.0 | 1.0 |

# 4. Formulation of *Centella asiatica* film coated tablets

## 4.1 Preparation of *Centella asiatica* coated tablets

Three types of film coating polymer applied on *Centella asiatica* core tablets in various coating level were studied as film formers. All formulations of the coating solution included talcum as an antiadherant or tackiness reducer. The amount added was equivalent to 0.5 % w/w of the formulation.

### 4.1.1 Preparation of chitosan coating solutions

Chitosan coating solution 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 60 minutes before applying. Pale Yellow solution was obtained for the coating on the core tablets. Sufficient polymers to achieve 3, 5 and 10% weight gain were applied.

### 4.1.2 Preparation of hydroxypropyl methylcellulose (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, 5, 10 and 15% weight gain film coated tablets were applied.

### 4.1.3 Preparation of polymethacrylate coating solutions

A certain amount of Eudragit® E100 was dissolved in the mixture of acetone and isopropyl alcohol (50:50) to obtain 5% w/w concentration and mixed well with the required plasticizer, tributyl citrate, at the concentration of 20% w/w (based on Eudragit® E100) for 60 minutes before coating. The coating levels were 1, 3 and 5% weight gain based on Eudragit® E100.

## 4.2 Coating procedure

The most satisfactory formulation of core tablet in section 3 was prepared in large batch (1,500 g) for film coating. Tablet formulation which had good appearance tablets with consistent weight and hardness was selected. Moreover, that formulation should exhibit smooth processing and was easy to compress. The test tablets (100 g) were mixed with 1,100 g of placebo tablets which were the color tablets for easy 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 7 gives details of the coating process parameters used. Samples were removed every hour and the mean coating weight gain was calculated.

**Table 7** Process parameters and levels for the 15-inch perforated coating pan

| Parameters | Coating solutions | | |
|---|---|---|---|
| | Chitosan | HPMC | Polymethacrylate |
| Tablet load (kg) | 1.5 | 1.5 | 1.5 |
| Spray rate (ml/min) | 5 | 10 | 5 |
| Air pressure (bars) | 3.5 | 3.5 | 2.0 |
| Pan rotational speed (rpm) | 8 | 10 | 8 |
| Inlet air temperature (°C) | 55 | 60-65 | 40-45 |
| Exhaust air temperature(°C) | 45 | 50 | 40 |
| Tablet bed warming (min) | 15 | 15 | 15 |
| Total weight gain (%w/w) | 3, 5, 10 | 3, 5, 10, 15 | 1, 3, 5 |

## 4.3 Preparation and Evaluation of cast film

### 4.3.1 Preparation

#### 4.3.1.1 Preparation of chitosan solution

The chitosan solution 5% w/w containing propylene glycol (25 %w/w based on dried polymer) as plasticizer was prepared as described in section 4.1.1.

#### 4.3.1.2 Preparation of HPMC solution

The HPMC solution 5% w/w containing PEG 6000 (20 %w/w based on dried polymer) as plasticizer was prepared as described in section 4.1.2.

#### 4.3.1.3 Preparation of polymethacrylate solution

The Eudragit® E100 5% w/w solution containing tributyl citrate (20%w/w based on dried polymer) as plasticizer was prepared as described in section 4.1.3.

#### 4.3.1.4 Preparation of cast film

Three types of plasticized polymer film solutions of 5%w/w were prepared as described in section 4.3.1-4.3.3. The films of about 25 μm thickness were obtained by casting technique on the clean smooth surface Petri dish. In the case of polymethacrylate the Petri dish have to adhere with the adhesive plastic tape before casting for ease of peeling. Chitosan, HPMC and polymethacrylate cast film were slowly evaporated at 45 °C in the hot air oven for 12, 12 and 6 hours, respectively. The dried films were kept in a desiccator for further testing.

### 4.3.2 Evaluation

#### 4.3.2.1 Tensile Strength Measurement

The film specimens with the size of 0.5 x 2 $cm^2$ were clamped with 2.54 cm pneumatic grips. The rate of strain was 10 mm/min. The stress-strain tester fitted with a 10 kN 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.

#### 4.3.2.2 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. The films were reweighed every 12 hours until saturate 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 °C 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:

$$\% \text{ Moisture sorption} = \frac{(W_1 - W_2)}{W_2} \times 100 \quad [4]$$

## 5. Evaluation of *Centella asiatica* tablets

The following physicochemical properties of *Centella asiatica* core and film coated tablets were investigated and compared to commercial tablets**.** Number of samples of the commercially available Centella tablets (Centellase® tablet, Italy) used for testing were different from investigated core and film coated tablet because of its limited amount. Three commercial tablets were used for dissolution test and 2 tablets for the other tests. Besides, only core tablets were tested for content uniformity as in section 5.6.

### 5.1 Physical Appearances and Morphology Study

The visual appearances of the tablets were observed every month for four months. At the end of the storage period, tablets storage at both ambient and accelerated conditions were viewed using a scanning electron microscope (SEM).

### 5.2 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.

$$\% \text{ Friability} = \frac{(w_o - w)}{w_o} \times 100 \quad [4]$$

### 5.3 Hardness

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

### 5.4 Average weight and weight variation

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

### 5.5 Disintegration test

The disintegration test was determined on six tablets, using distilled water, maintained at temperature 37$\pm$1°C, as disintegration medium. Analyses were performed in accordance with standard USP25 method without disc. Results were reported as the time require for complete disintegration of the tablets.

### 5.6 Uniformity of dosage unit

Ten tablets were taken by random sampling. Each tablet was placed into a 25-ml volumetric flask with approximately 15 ml of methanol HPLC grade and dissolved with sonicator for 10 minutes. Each solution was adjusted to 25 ml with methanol HPLC grade and mixed well. The sample was filter through 0.45 µm nylon filter. Filtered solutions were injected onto the HPLC column.

### 5.7 Assay

The percentage of labeled content was quantitatively calculated by average of peak area from HPLC gradient method as described in section 1.6.2. The parameters to be considered for validation of HPLC method for the assay of pharmaceutical dosage form are 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 analyze of active components in pharmaceutical dosage forms. Specificity is established by showing that the active components should have no interference from extraneous components and be well resolved from them.

The mix standard solutions of AS, MA and AA in methanol at the concentration 400 µg/ml and sample blank solutions in methanol were prepared and evaluated by using the chromatographic conditions as described in section 1.6.2.

***Accuracy***

The accuracy of the proposed method was performed by analyzing placebos 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 placebos were prepared and analyzed. Three set of the assay were performed.

*Placebo Tablets*

The placebo tablets (tablets without CST) were prepared by direct compression method.

*Procedure*

Ten placebo tablets, accurately weighed, were ground and mixed. Sixty, 90, 120, 150 and 180 mg of the powder were transferred to the separate 25-ml volumetric flasks. Two, 3.0, 4.0, 5.0 and 6.0 ml aliquot of standard solutions of AS, MA and AA were added to each volumetric flask, filled up with methanol approximately 15 ml and sonicated for 10 minutes, followed by adding methanol HPLC grade to make up to volume. The obtained solutions were filtered through the membrane filter and injected by using the chromatographic condition. The percentage of the recovery of each reference standard was calculated.

### 5.7.1 Assay for the pharmaceutical products

#### *5.7.1.1 Standard preparation*

Twenty-five mg each of standard AS, MA and AA were weighed into a 25-ml volumetric flask, dissolved in methanol and diluted to volume. The solution was quantitatively and stepwise diluted with methanol HPLC grade to obtain the final concentration of 400 µg/ml. The solution was filtered through membrane filter and injected into HPLC column.

#### *5.7.1.2 Sample preparation*

Ten tablets were weighed to get the average tablet weight. The samples of the powdered tablets theoretically equivalent to one tablet were transferred to a 25-ml volumetric flask and 15 ml of methanol was added. After 10 minutes of sonication, the volumetric flask was filled up to volume with methanol

and mixed. Solution was filtered through 0.45 µm filter paper. Each sample was determined in duplicate.

### 5.8 Dissolution Study

From the preliminary study, a 100 ml of mixture of 0.1N hydrochloric acid and isopropyl alcohol (70:30), maintained at temperature 37±0.5 °C in the closed system, was selected to use as dissolution medium for tablets. The small size dissolution apparatus (paddle method) were rotated at a speed of 100 rpm. A portion of dissolution sample was withdrawn at 30 minute and assayed by HPLC (gradient system) as described in section 1.6.2. Three tablets of each formulation were determined.

## 6. Stability study

Stability study of Centella tablet was performed according to Thai FDA guideline on stability testing of drug product (จุไรรัตน์, 2547).

The tablets, packed in close amber glass containers, were stored under both accelerated (45±2 °C, 75±5% RH) and ambient conditions for 4 months and randomly sampled every 4 weeks interval for analyzing the percent remaining of drug contents by HPLC (gradient system). The sample preparations were prepared as described in section 5.7.1. Moreover, the physical appearances of the tablets were observed every month and the evaluations of the tablets in section 5 were retested at the end of the storage period, except the test of uniformity of dosage unit in section 5.6.

## 7. Scale-up

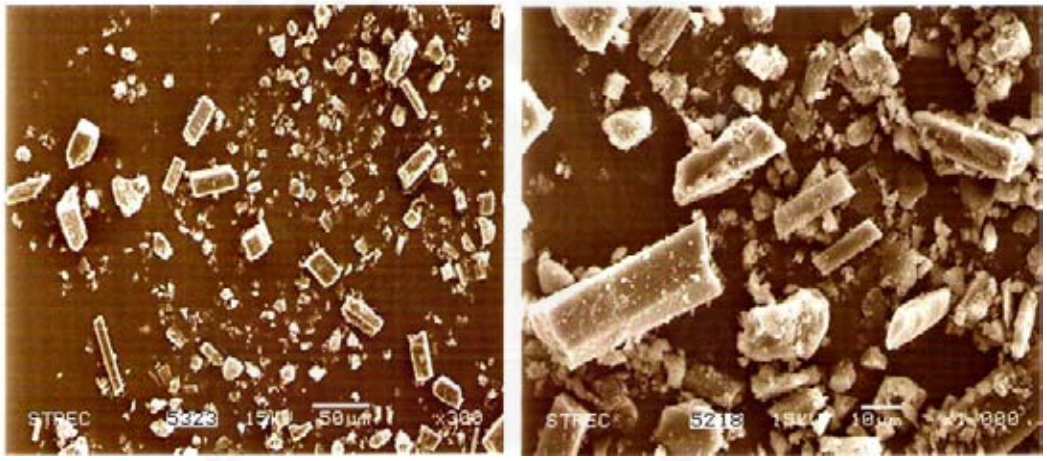
The selected formulations of *Centella asiatica* core and film coated tablet of chitosan, HPMC and polymethacrylate were scale-up in the process of direct compression and film coating. Due to limited amount of CST three kilograms of core tablets were compressed as well as a batch of 3 kilograms of core tablets was coated. The changes of the process were studied. The core tablets were mixed in V- shape blender and compressed by using the single punch tableting machine whereas the coating process still made in Thai coater (15"). After that the tablets were evaluated in the tests of friability, hardness, weight variation, as well as disintegration as described in section 5.

# CHAPTER IV
# RESULTS AND DISCUSSION

## 1. Characterization of Centella selected triterpene (CST) powder

### 1.1 Morphology Study

Figure 5 illustrates the photomicrographs of the CST powders from scanning electron microscope (SEM). They were particles of various shapes with a wide size distribution. However, most of them were in rod shape.



**A** **B**

**Figure 5** SEM photomicrographs of CST powders at magnification of (A) 300x and (B) 1000x

### 1.2 Particle size distribution analysis

The particle size distribution of CST is displayed in Figure 6. The particles were in various sizes but the highest percent of distribution (22.04%) were the particles size range between to 0.106-0.125 mm.

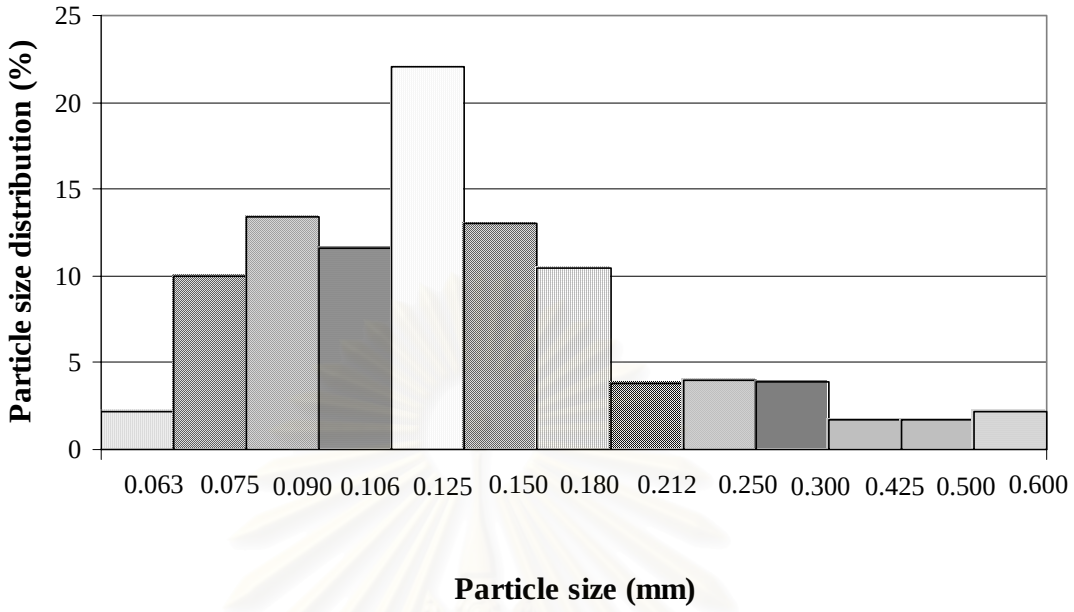



**Figure 6** Particle size distribution of CST

### 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 particle 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 8 (Davies, 2001).

**Table 8** 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, tap density and Carr's compressibility index of CST were 0.40, 0.56 and 28.74, respectively as shown in Table 21, Appendix B. For these results, it could not be clearly concluded about the bulk and tap density. However, from Carr's compressibility index, the CST was classified as poor flow characteristic level. That was correspondingly confirmed with the photomicrographs from SEM and the particle size distribution of this extract. Due to they were in rod shape and in the wide range of particle size distribution so their flow property may be inappropriate.

### 1.4 Angle of repose and flow rate

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 and flow rate of CST 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. It was shown that the powder did not have good flow property.

### 1.5 Determination of active constituents of *Centella asiatica* extract

#### 1.5.1 Thin layer chromatography (TLC)

The active compounds from CST were identified by TLC method. The chromatograms of these compounds compared with the marker compounds are shown in Figure 7. The Rf value were 0.40, 0.70 and 0.75 for AS, MA and AA, respectively. Similar Rf values were also obtained from these compounds from CST sample. Therefore, this TLC chromatogram showed that the tested

commercial available CST powder consisted of the interested active components analogous to AS, MA, and AA.


**Figure 7** TLC chromatograms of AS, MA, AA and CST

### 1.5.2 High performance liquid chromatographic method (HPLC)

Analysis of active consituents from CST has been reported employing various methods such as titration, colorimetry, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). In previous study, TLC in combination with HPTLC scanner was applied for quantitative analysis of AS from various parts of the herbs (Sribusarakum, 1997). However, HPLC is the most suitable method to determine the active constituents of this herbal extract both raw material and pharmaceutical dosage form because of its high sensitivity, specificity and convenience for the research. In the available articles, HPLC gradient method was recommended for the analysis of active constituents from *Centella asiatica* but the time consumed in one cycle is considered troublesome (Inamdar, Yeole, Srivastava and De Souza, 1996; Brinkhaus et al., 2000).

In preliminary study, HPLC isocratic method was tried to establish but to no avail. It could not attain the separation of the active components from the herbal extract because of the much different polarity among the compounds. Consequently, the HPLC gradient method was preferred to be utilized in this investigation. The adjustment of mobile phase composition for linear gradient system was undertaken to obtain the satisfactory single run which showed great resolution of three components. The HPLC chromatograms of standard AS, MA, AA, standard mixture and these active compounds from CST solution were respectively shown in Figure 8 to 9. The chromatogram of MA had a small peak of AA (6.8 $\pm$ 0.4 % of peak area, n=3). This may be obtained from the manufacturing process of MA. Moreover, the AA chromatogram had two small unknown peaks at retention time about 8.8 and 9.5.

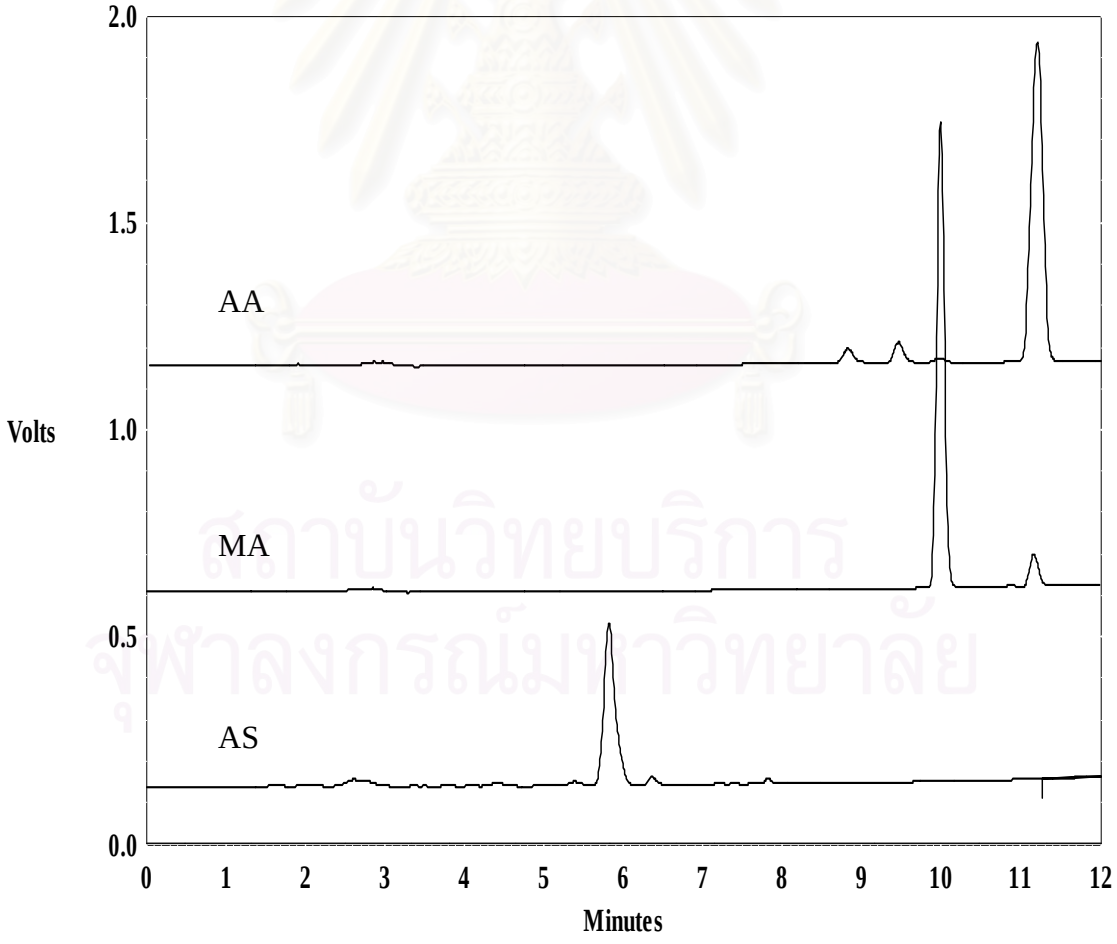



**Figure 8** HPLC chromatogram of standard AS, MA and AA

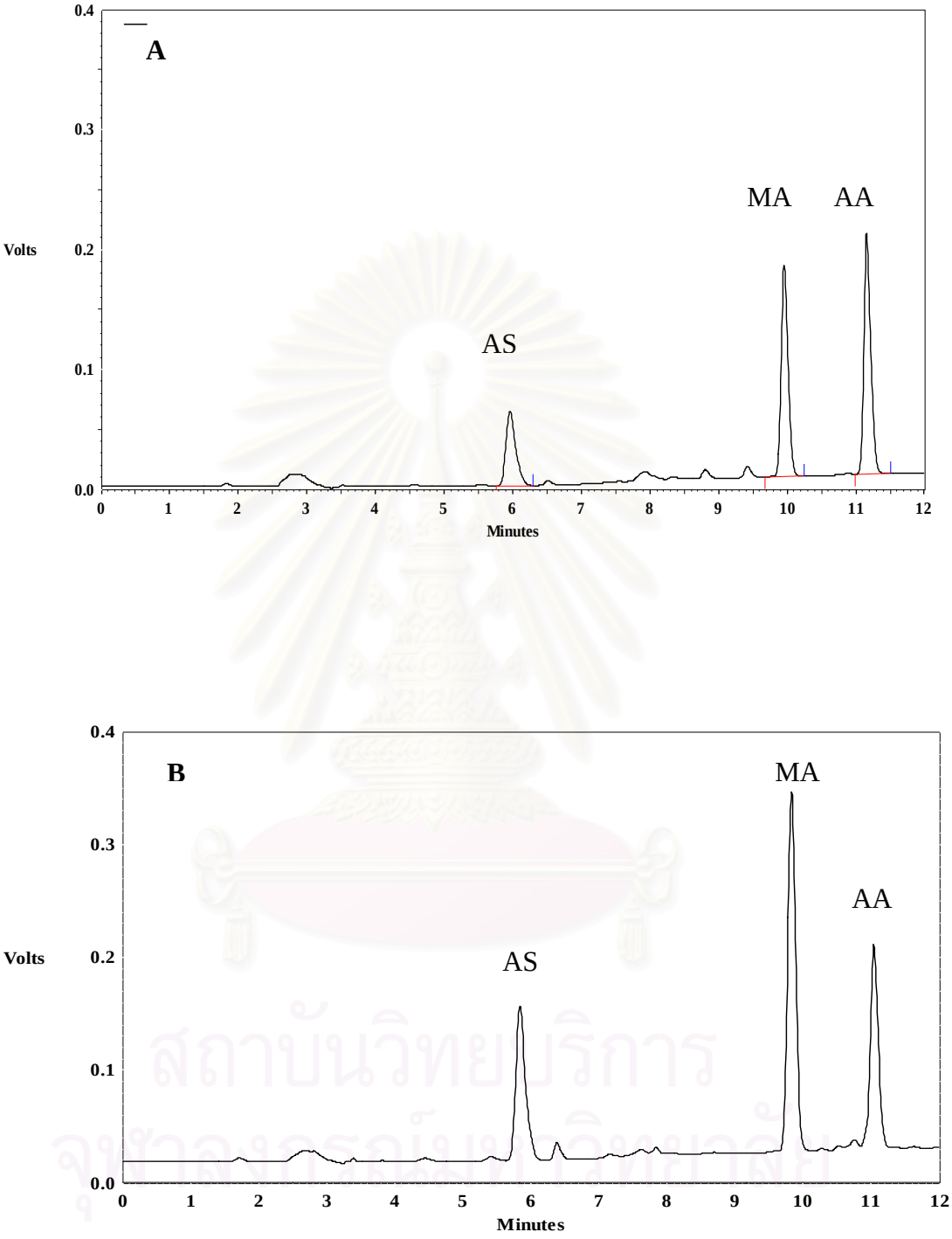



**Figure 9** HPLC chromatograms of standard mixture of AS, MA and AA (A) and CST (B)

***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 AS, MA and AA were about 5.9, 10.0 and 11.2, respectively. The resolution factors were 20.42 and 7.03, respectively and the tailing factors of AS, MA, and AA were 1.09, 1.06, and 1.12, respectively. They showed the satisfactory resolution, symmetry peak and conformed the USP25 specification.

***Linearity***

A linearity study was carried out to determine whether this method could measure accurately different concentrations of AS, MA, and AA. The linearity curves of the peak area versus the concentrations of standard AS, MA, and AA were shown in Figure 58-60 in Appendix A.

The standard concentration that gave linear standard curve was in the range of 100 to 1000 µg/ml. The regression coefficients ($R^2$) for standard curve were 0.9999 for all compounds. This result showed a good linearity of standard concentrations and peak area.

***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 were shown in Table 15-17, in

Appendix A. The %CV of AS, MA, and AA were 0.99, 1.02 and 1.02 respectively. The low %CV indicated the good precision of this method.

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

### 1.5.3 Solubility study

The solubility study was useful to select an appropriate dissolution medium for the comparison of the release characteristics of Centella products because there was no recommended dissolution medium and no monograph of *Centella asiatica* extract tablet in the international pharmacopoeia.

The solubility study of AS, MA and AA from CST was performed in two types of solvent, deionized water and the mixture between 0.1N hydrochloric acid and isopropyl alcohol in the ratio of 70:30.

As shown in Figure 62, in Appendix B the solubility of AS in deionized water at 37 °C was 0.040 mg/ml whereas MA and AA were insoluble. There were no detected peaks of MA and AA in every sampling solution. On the other hand, the mean solubility of these compounds in mixture of 0.1N hydrochloric acid: isopropyl alcohol (70:30) were 0.242, 0.032 and 0.013 mg/ml for AS, MA and AA (Figure 63 - 65, in Appendix B), respectively.

From the data obtained it was found that the solubility of the active constituents from CST in acid alcoholic mixture of 0.1N hydrochloric acid: isopropyl alcohol (70:30) were higher than in deionized water therefore this solvent was chosen to be the dissolution medium in the dissolution test.

### 1.5.4 Degradation of the active constituents of *Centella asiatica* extract

Figure 11 and Table 27 in Appendix B present the summary of the percent remaining of AS, MA and AA from the reference standard and CST after exposure to the stress conditions.

#### 1.5.4.1 Moisture Hydrolysis

After exposure to moisture using two methods, it was found that the percent remaining amount of all of the active constituents from AS, MA and AA reference standards and CST were in the range of 96.0-106.9 % w/w. Moreover, the HPLC chromatograms did not show additional peaks of degradation products (Figure 10).

That meant that these compounds were not sensitive to moisture. Although the chemical structures of AS has the ester group and both MA and AA had the carboxylic groups but the molecule of water was not strong enough to react with the bond in these compounds.

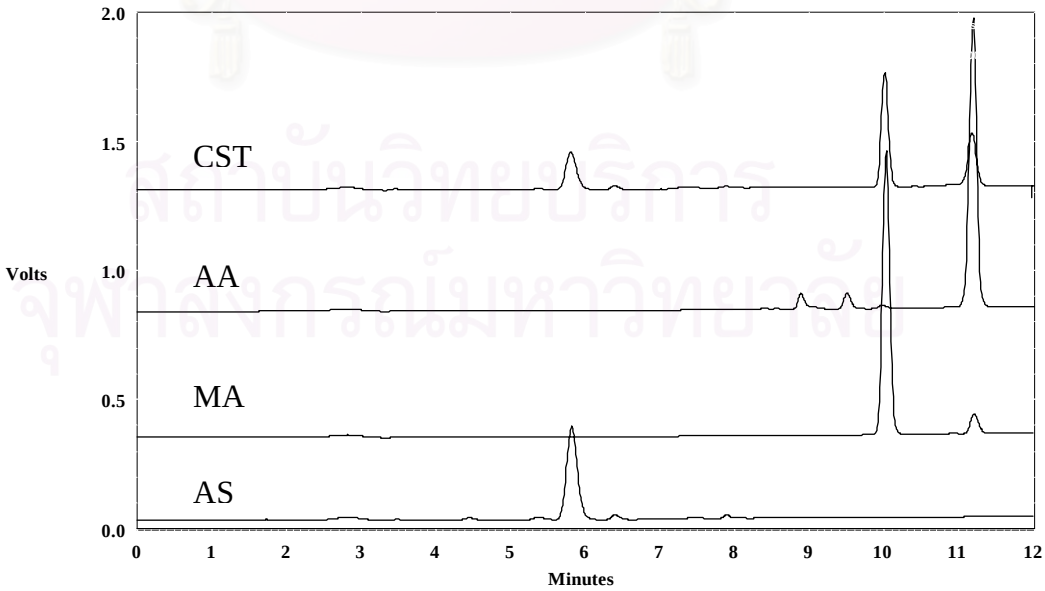



**Figure 10** The HPLC chromatograms of AS, MA, AA and CST after exposure to the stress condition by moisture hydrolysis.

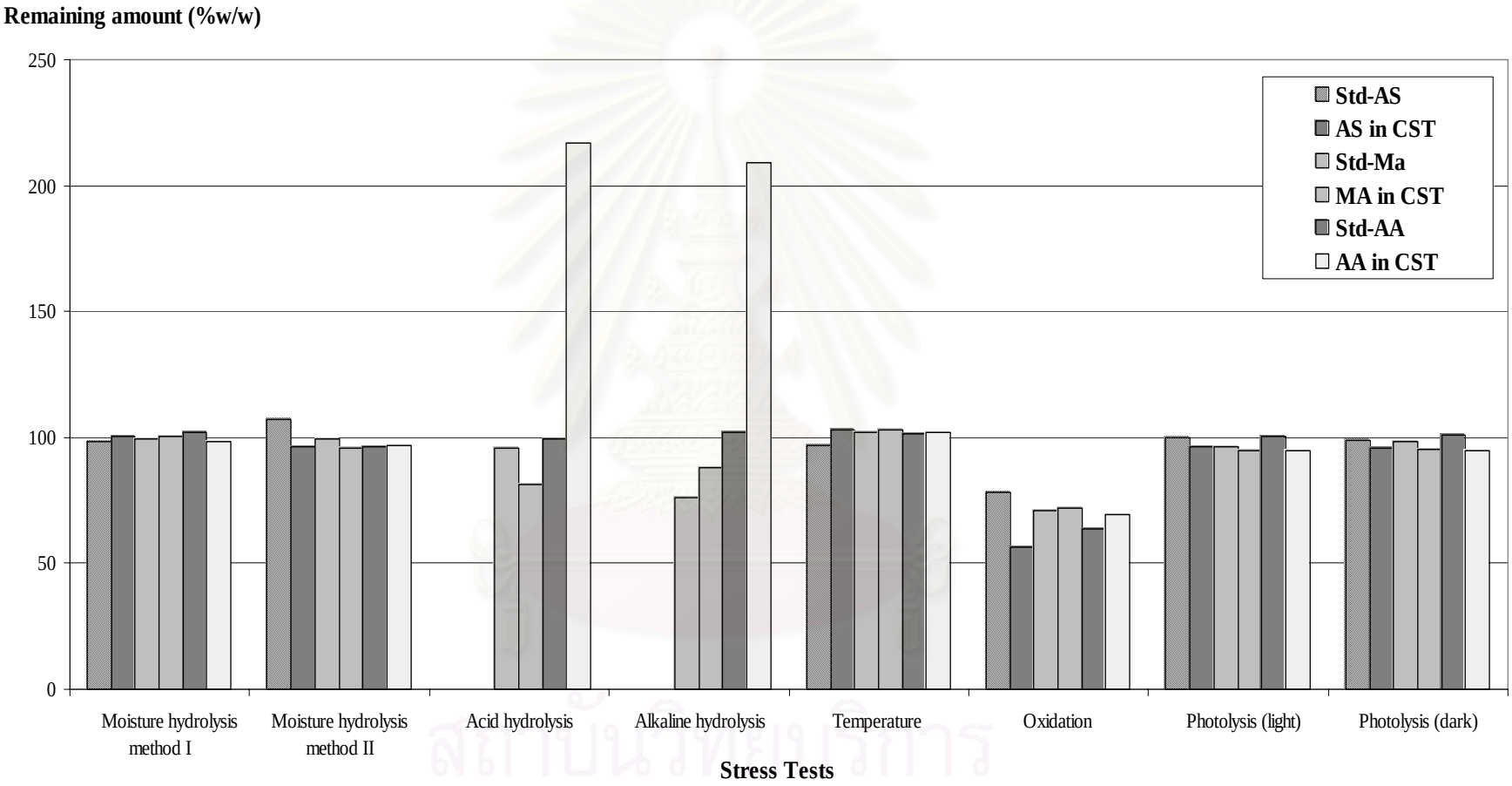



**Figure 11** The Remaining amount (%w/w) of AS, MA and AA from reference standard and CST after exposure to stress conditions.

### 1.5.4.2 Acid Hydrolysis

Visual inspection could be instantly used to identify the physical appearance of standard AS and CST under the stress condition of acid hydrolysis. The standard AS and CST changed in color from white and pale yellow to brown, whereas both MA and AA were unchanged. It was probably indicated that AS had a chemical reaction to acid. It was confirmed by either the percent remaining amount or the HPLC chromatograms. Asiaticoside was completely degraded after the exposure of acid. Interestingly, the HPLC chromatograms of AS and CST showed the disappearance of AS peaks but the appearance of AA peak was noted (Figure 12).

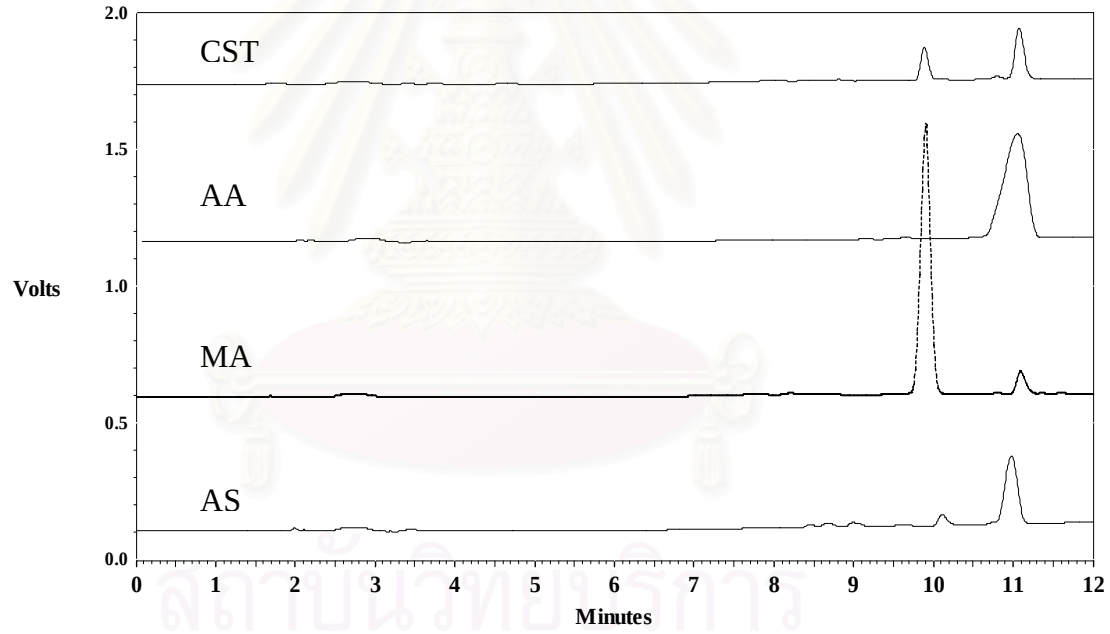



**Figure 12** The HPLC chromatograms of AS, MA, AA and CST after exposure to the stress condition by acid hydrolysis

The percent remaining amount of AA in CST was increased up to 216.8 %. This, therefore, confirmed that acid hydrolysis of AS provided at least one of the degradation products as AA. It could explain that acid was the catalyst in the degradation mechanism of AS. The ester bond in the structure of AS was hydrolyzed by hydronium ion to obtain AA and sugar moiety, glucose and

rhamnose (Figure 13). This result was consistent with the previous studies by Ratsimamanga and Botteau (1960).

In case of MA, the percent remaining in reference standard of MA and CST were 95.5% and 81.5%, respectively. Even though, there was a difference between standard MA and CST, it was possible that MA was not affect by acid hydrolysis. Because CST was mixed with other compounds which may interfere the reaction. Consideration of the structure of MA, it was an acid compound so the reaction with acid should not occur. Similarly, AA was not sensitive to acid so the remaining amount of AA standard was 99.3% after exposure to the stress condition by acid.

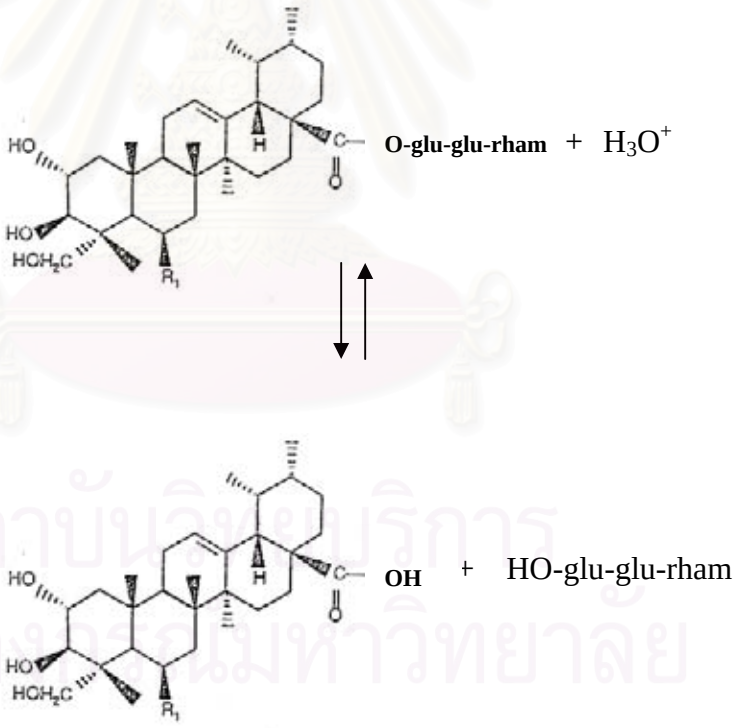



**Figure 13** Hydrolysis of AS by hydronium ion

### 1.5.4.3 Alkaline Hydrolysis

The physical appearance of standard AS, MA, AA and CST under the stress condition of alkaline hydrolysis was observed. Similar to acid hydrolysis, the colors of standard AS and CST changed from white and pale yellow to brown, whereas both MA and AA were unchanged. As summarized in Figure 14, AS was sensitive to alkaline. This finding agreed with Sung et al. (1992). Furthermore, the HPLC chromatogram of AS under alkaline hydrolysis was the same as in acid hydrolysis. It showed that the AS peak from the reference standard AS after stress test on alkaline disappeared, while the AA peak was found. In addition, MA was slightly sensitive to alkaline. Although the degradation peaks were not found, the percent remaining amount of standard MA and MA in CST were decreased. They remained 75.9% and 87.8%, respectively. On the other hand, AA was stable in alkaline because the remaining amount of AA after stress test by alkaline hydrolysis was 102.0 % and 208.9 % for AA standard and CST, respectively. The ester bond in the structure of AS was hydrolyzed by hydroxide ion to obtain AA and sugar moiety, glucose and rhamnose (Figure 15). This result was consistent with the previous studies by Sung et al. (1992).

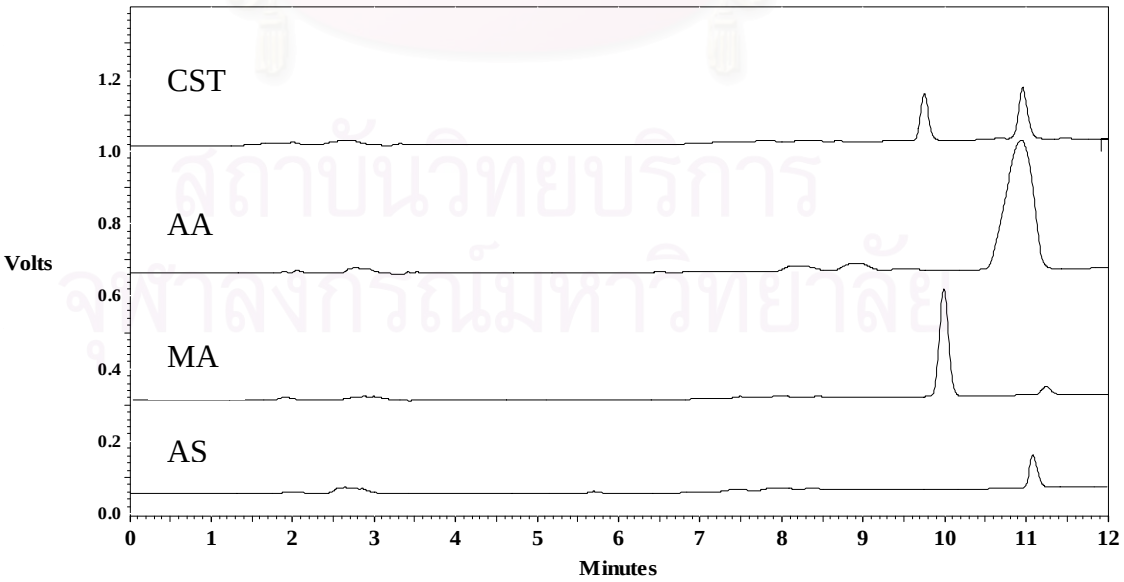



**Figure 14** The HPLC chromatograms of AS, MA, AA and CST after exposure the stress condition by alkaline hydrolysis

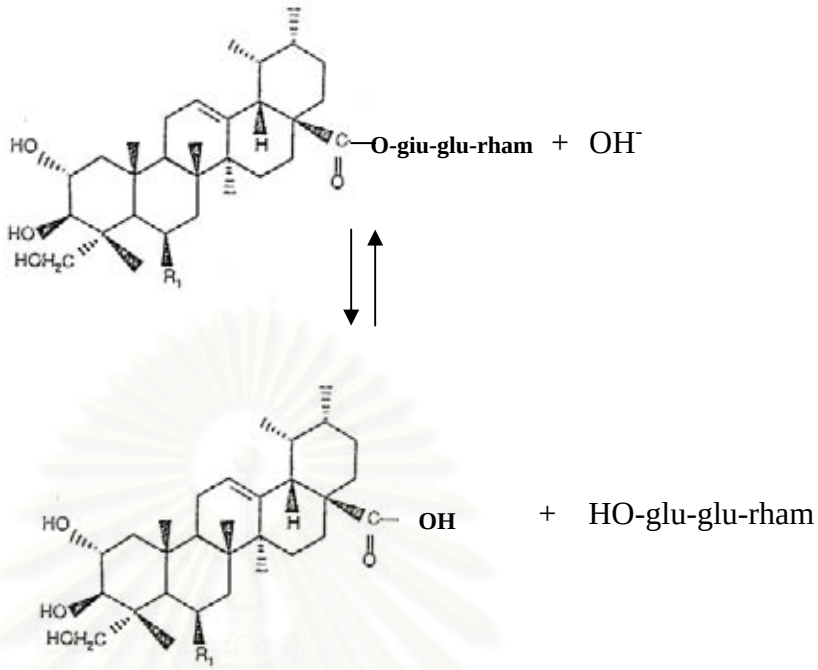



**Figure 15** Hydrolysis of AS by hydroxide ion

### 1.5.4.4 Temperature

After stress test on high temperature, the percent remaining amount of AS, MA and AA from reference standards and CST were in the range of 96.7% to 103.2%. Moreover the HPLC chromatograms did not find any degradation peaks. Thus, these compounds were not sensitive to high temperature.

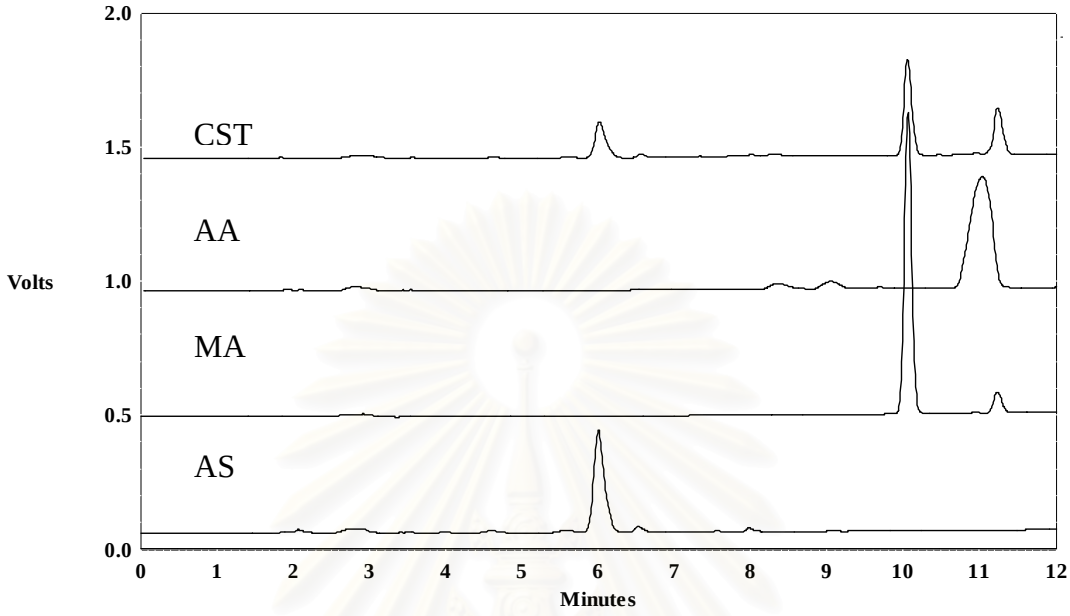



**Figure 16** HPLC chromatograms of AS, MA, AA and CST after exposure to the stress condition by high temperature

### 1.5.4.5 Oxidation

After exposure to stress test on hydrogen peroxide, the percent remaining amount of all compounds in both reference standard and CST were in the range of 56.5% to 78.1% (Figure 11). It expressed that AS, MA and AA were degraded by oxidation mechanism. Interestingly, the chromatogram of AA standard could observe the distinctly increasing of the unknown compound peaks at retention time 8.8 and 9.5 minutes. In addition, the chromatogram of AS was noted the peak at retention time 3.5 minute and also MA was found the peak at retention time 7.7 and 8.4 minute. These peaks were observed in the chromatogram of CST as well. Therefore, these unknown compounds should likely be the degradation products of AA and MA and AS. From the chemical structures of AS, MA and AA, the mechanisms of oxidation were proposed (Figure 18). There were several functional groups such as alkene groups (-C=C-), hydroxyl groups (-OH), carboxylic groups (-COOH) and ester group (-COOR) in the structure of AS, MA and AA.

Consequently, they could be interacted by hydrogen peroxide and degraded to be the other compounds.

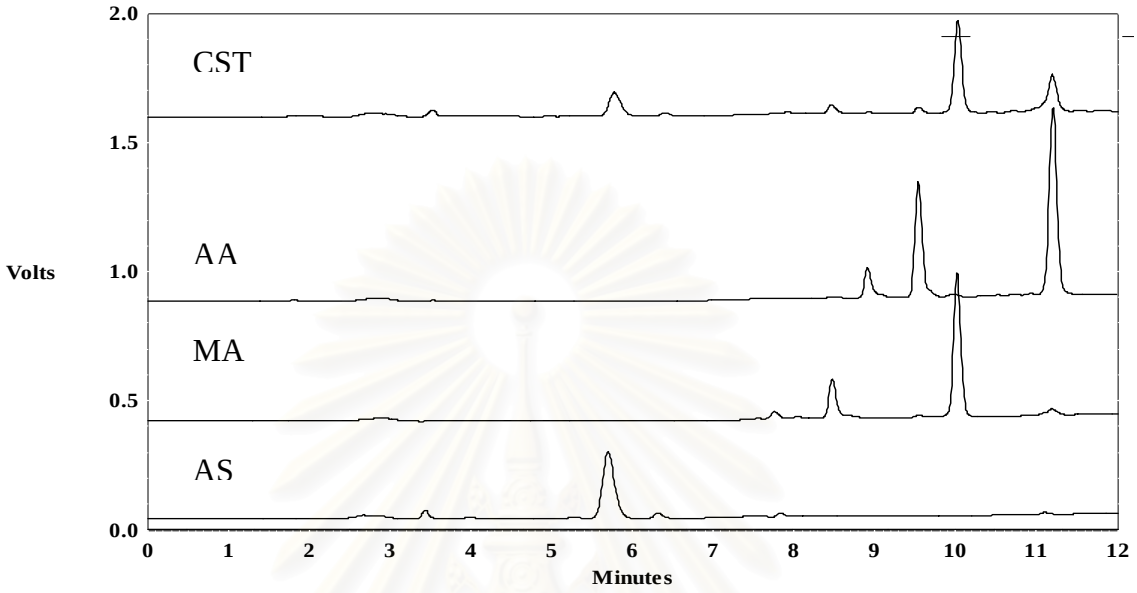



**Figure 17** HPLC chromatograms of AS, MA, AA and CST after exposure to the stress condition by oxidation

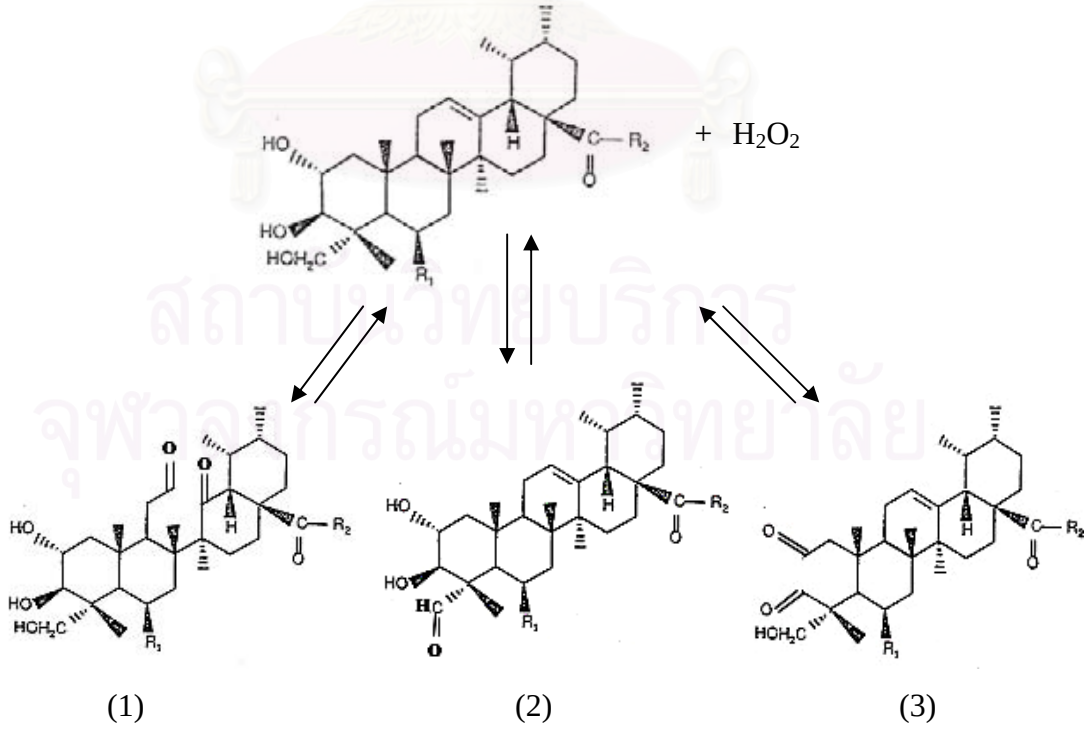



**Figure 18** Proposed mechanism of oxidation of AS, MA and AA

### 1.5.4.6 Photolysis

The percent remaining of the active compounds after exposure to light and dark were in the range 94.5% -100.3% and 94.8%- 100.8%, respectively. There was no difference between the remaining amounts of AS, MA an AA after exposure to light and dark. Accordingly, these compounds were not degraded by photolysis. Furthermore, the HPLC chromatograms showed that there were no degradation peaks.

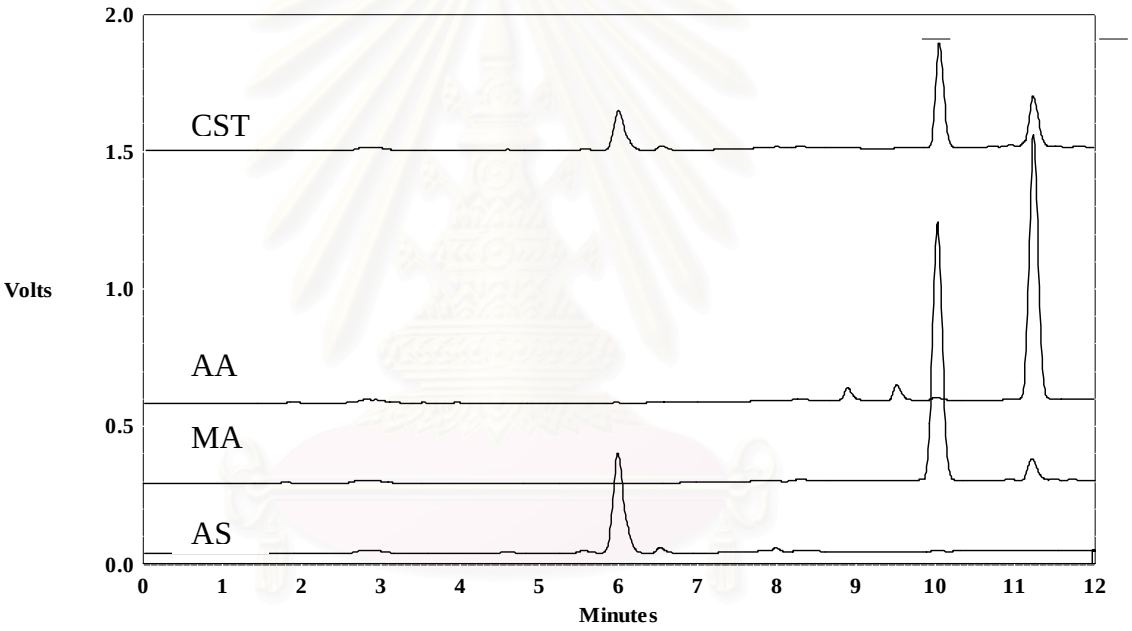



**Figure 19** HPLC chromatograms of AS, MA, AA and CST after exposed the stress condition by photolysis

Concluding the results from stress tests of each constituent as shown in Figure 20-23 it was evident that moisture, temperature and light had modulate effect on degradation mechanism of AS, MA and AA. Moreover, AS showed distinctly sensitive to acid, alkaline and peroxide, while MS was sensitive to alkaline and peroxide. Finally, AA was sensitive to peroxide.

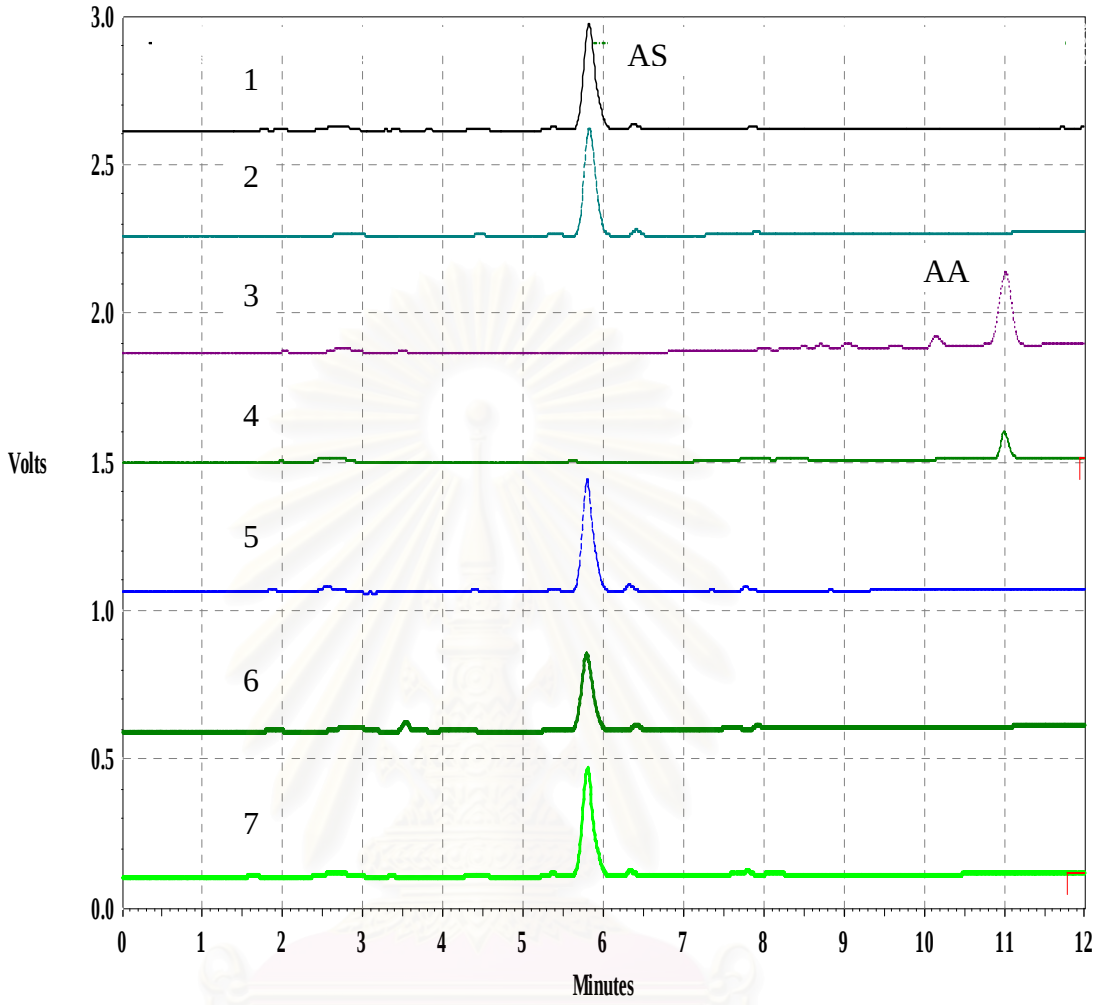



**Figure 20** HPLC chromatograms of AS after exposure to stress conditions

1 = AS standard solution in methanol
2 = moisture hydrolysis
3 = acid hydrolysis
4 = alkaline hydrolysis
5 = temperature
6 = oxidation
7 = photolysis

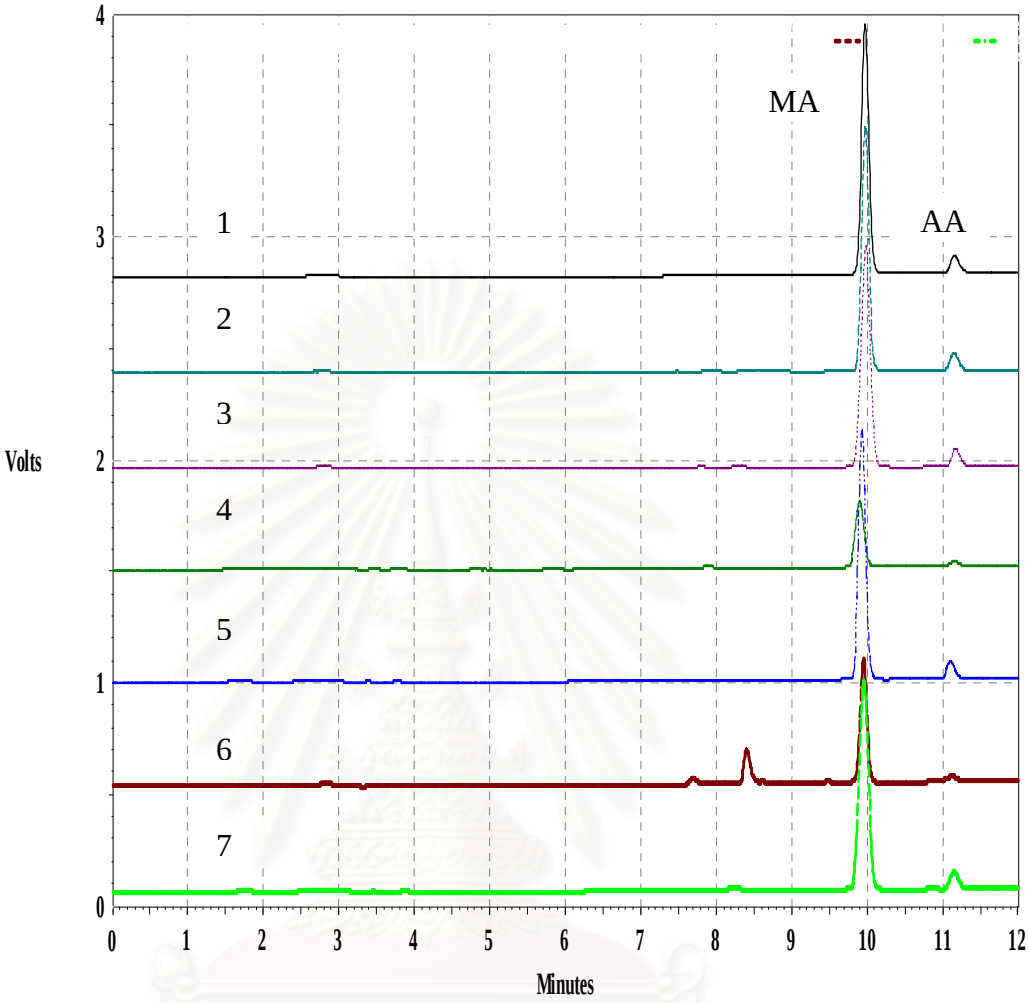



**Figure 21** HPLC chromatograms of MA after exposure to stress conditions

1 = MA standard solution in methanol
2 = moisture hydrolysis
3 = acid hydrolysis
4 = alkaline hydrolysis
5 = temperature
6 = oxidation
7 = photolysis

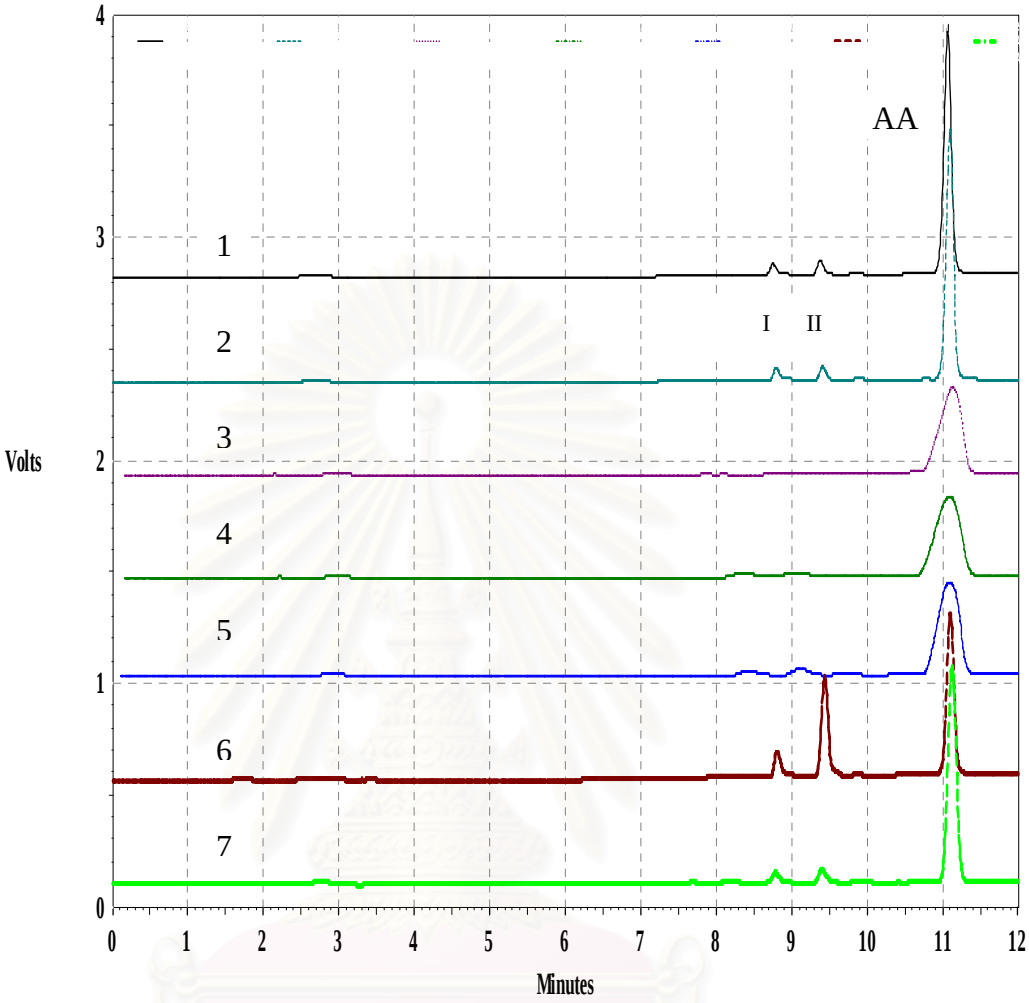



**Figure 22** HPLC chromatograms AA after exposure to stress conditions

1 = AA standard solution in methanol
2 = moisture hydrolysis
3 = acid hydrolysis
4 = alkaline hydrolysis
5 = temperature
6 = oxidation
7 = photolysis

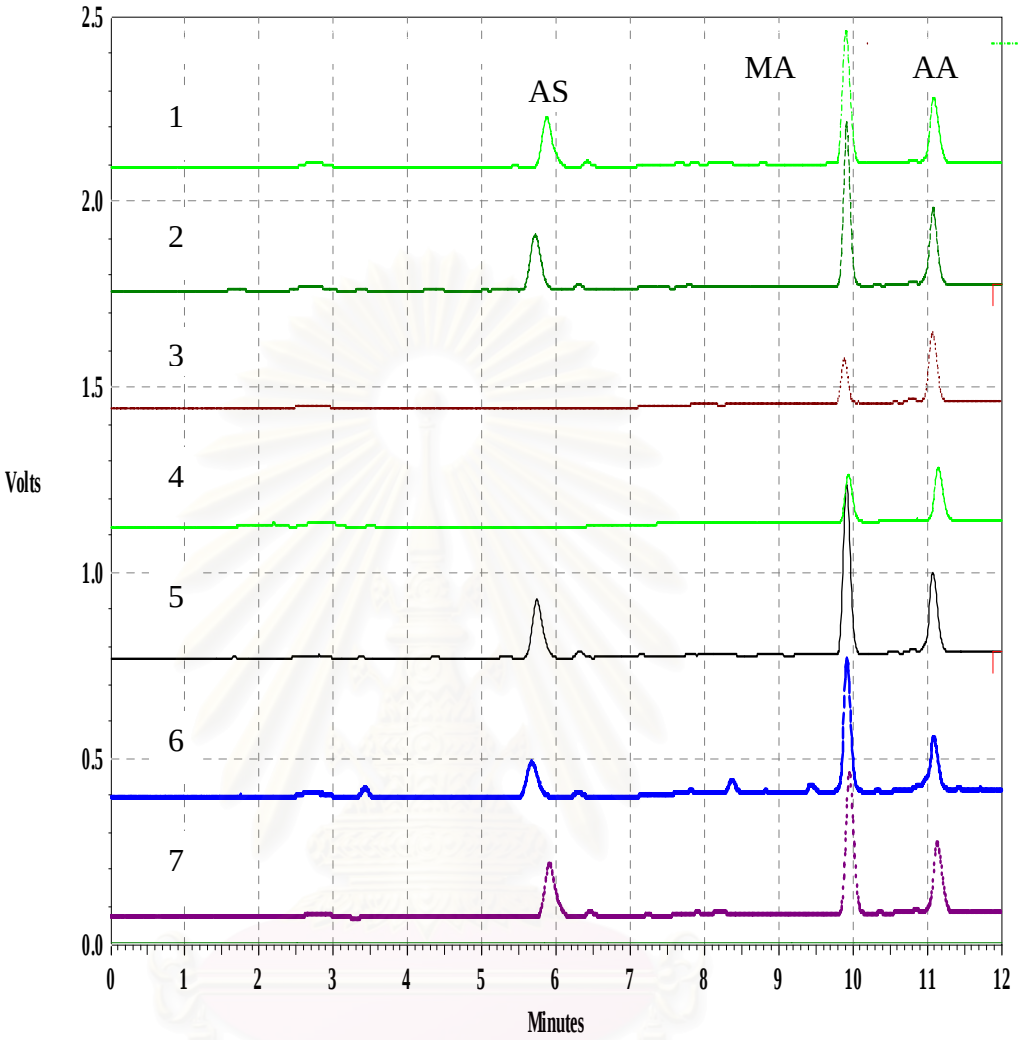



**Figure 23** HPLC chromatograms of CST after exposure to stress conditions

1 = CST solution in methanol
2 = moisture hydrolysis
3 = acid hydrolysis
4 = alkaline hydrolysis
5 = temperature
6 = oxidation
7 = photolysis

## 2. Compatibility Tests

In preformulation studies it is essential to evaluate the possible interactions between the active principle and the excipients, as the choice of the excipients should be performed in relation to the drug delivery, to their compatibility with the same drug and to the stability of the final product (Ceschel et al., 2003). The excipients sometime cause variation in the physicochemical properties of the active component. Accordingly the stability of a formulation depends, among others factors, on the compatibility of the active components with the other ingredients (Rodante et al., 2002).

The HPLC analysis, carried out on binary mixtures of drug and excipient preserved for 4 months under the accelerated condition of 45±2°C, 75±5% RH gave the results shown in Figure 24-27.

**Asiaticoside**

The percent remaining amount of AS of CST of the various binary mixtures after storage at accelerated condition for 4 months were as followed: AS in CST raw material (no additional excipient) – 95.58%, with Super-Tab® - 93.50%, with Starch 1500® - 90.12%, with magnesium stearate – 93.88%, with Aerosil® - 90.73%, with Explotab® - 90.99% and with talcum – 95.30% (Figure 24). Starch 1500®, Aerosil® and Explotab® seemed to have an influence on decreasing the stability of AS more than the other excipients.

**Madecassic acid**

Figure 25 shows the percent remaining amount of MA of CST of the various binary mixtures after storage at accelerated condition for 4 months were as followed: MA in CST raw material (no additional excipient) – 86.24%, with Super-Tab® - 92.94%, with Starch 1500® - 89.34%, with magnesium stearate – 92.11%, with Aerosil® - 89.94%, with Explotab® - 87.28% and with talcum – 94.11%. Stability of

MA in CST could be improved by all excipients. However, the mixture of CST with Explotab® showed the nearly percent remaining to raw material.

**Asiatic acid**

As shown in Figure 26, the percent remaining amount of AA of CST of the various binary mixtures after storage at accelerated condition for 4 months were as followed: AA in CST raw material (no additional excipient) – 84.83%, with Super-Tab® - 96.60%, with Starch 1500® - 87.24%, with magnesium stearate – 92.21%, with Aerosil® - 88.12%, with Explotab® - 85.35% and with talcum – 92.07%. Similar to MA, all excipients could improve the stability of MA in CST as well as the slightly difference of the percent remaining between the mixture of CST with Explotab® and CST raw material was determined.

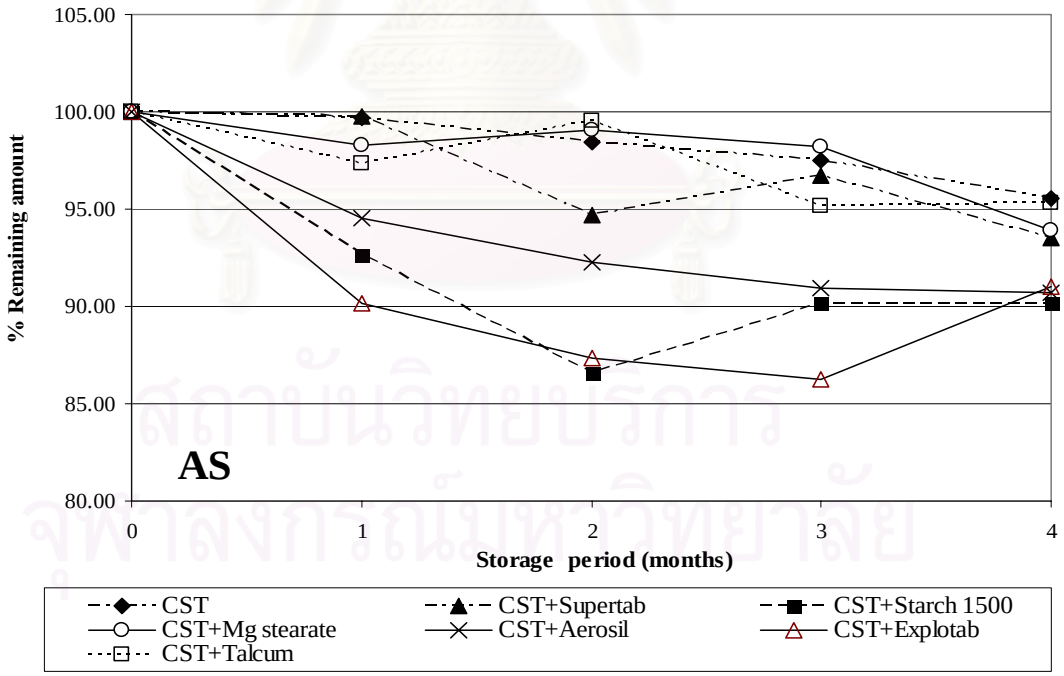



**Figure 24** Compatibility study between AS from CST and the excipients

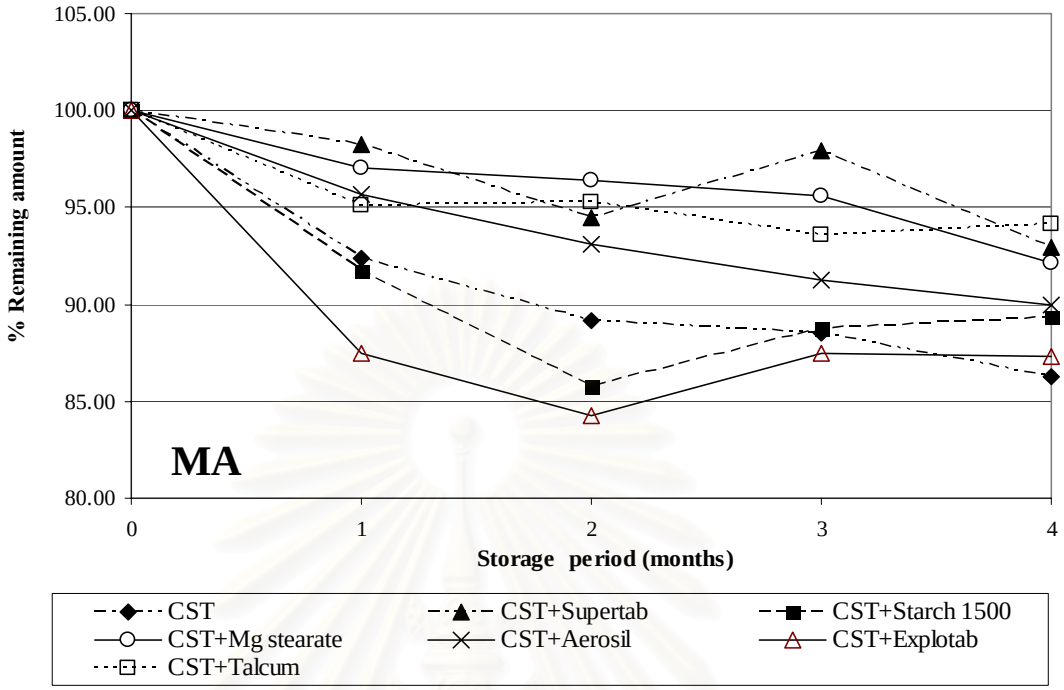



**Figure 25** Compatibility study between MA from CST and the excipients

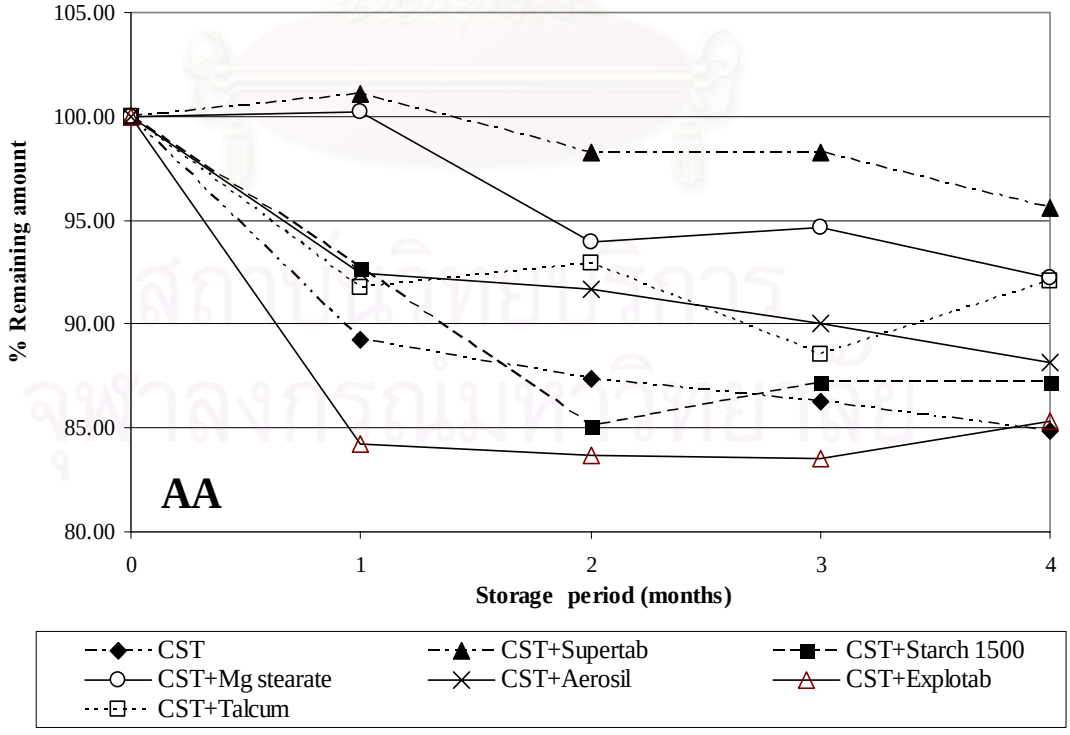



**Figure 26** Compatibility study between AA from CST and the excipients

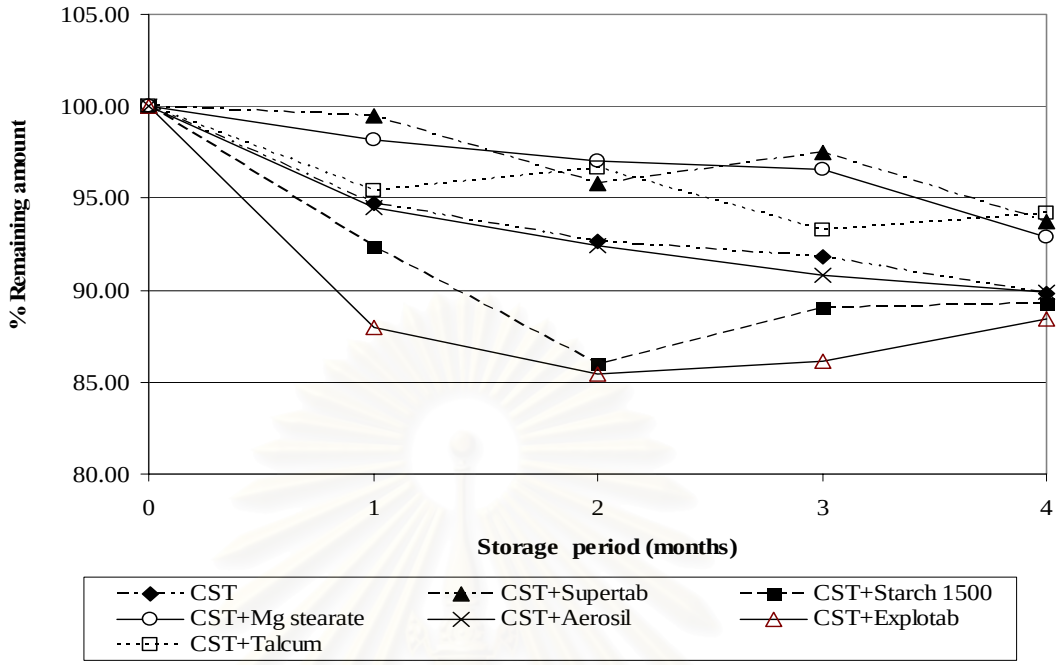



**Figure 27** Compatibility study between the active constituents from CST and the excipients.

Different excipients had an influence on the stability of CST in mixtures to varying extents. The active constituents, AS, MA and AA from CST assay values of the various slurries after storage at accelerated condition for 4 months were as follows: CST raw material (no additional excipient) - 89.84%, with Super-Tab® - 93.71%, with Starch 1500® - 89.25%, with magnesium stearate - 92.89%, with Aerosil® - 89.91%, with Explotab® - 88.47% and with talcum - 94.20%.

The remaining amount of CST in the mixture of CST and Super-Tab®, magnesium stearate, Aerosil® and talcum were higher than CST raw material. In contrast, the remaining amount of CST in the mixture of CST and Starch 1500® and Explotab® were lower than CST raw material. Since Explotab® and Starch 1500®, especially Explotab®, a superdisintegrant, had a disintegrating property so they acted by rapid water uptake. Although, stress test data showed the less effect by moisture hydrolysis to CST, the hydrolysis could happened. The inclusion of a high level of superdisintegrant (ratio of CST: Explotab®, and CST: Starch 1500® = 1:1 and 1: 20) induced the hydrolysis reaction of the active constituents of CST because they were

stored at the high humidity and temperature for a long period. The result was consistent with previous study on the effect of superdisintegrants, sodium starch glycolate or croscarmellose sodium inclusion into tablet formulation of acetylsalicylic acid. It resulted in a substantial increase in free salicylic acid levels (Levina and Cunningham, 2005). In case of Aerosil®, it was a good moisture adsorbent (Bonati, 1991; Kibbe, 2000) so the total percent remaining of AS in CST and total amount of active constituents of CST in the mixture of CST and Aerosil® was slightly lower than CST raw material.

With some excipients, Super-Tab®, magnesium stearate and talcum, the stability of CST was improved. This phenomenon was due to the hydrophobicity of magnesium stearate and talcum themselves. Moreover, both high level and long mixing time with them could result in hydrophobic surface of CST powder thus it was protected from hydrolysis reaction. Although, both magnesium stearate and talcum were weak alkaline but they were mixed with CST in dry state so the alkaline hydrolysis did not occur. For Super-Tab®, the spray dried material which contains about 10% amorphous lactose (Kibbe, 2000), is less water uptake. Moreover, the large amount of Super-Tab® was diluted with small quantities of CST and this may protect CST from the environment.

## 3. Formulation of *Centella asiatica* core tablets

### 3.1 Flow rate and angle of repose

The mean of flow rate as well as the angle of repose of the powder mixture of each formulation are presented in Figure 28. As a result of statistic charge, the glass funnel was inappropriate to use. Thus it was measured by using paper funnel with an aperture of 1.5 cm.

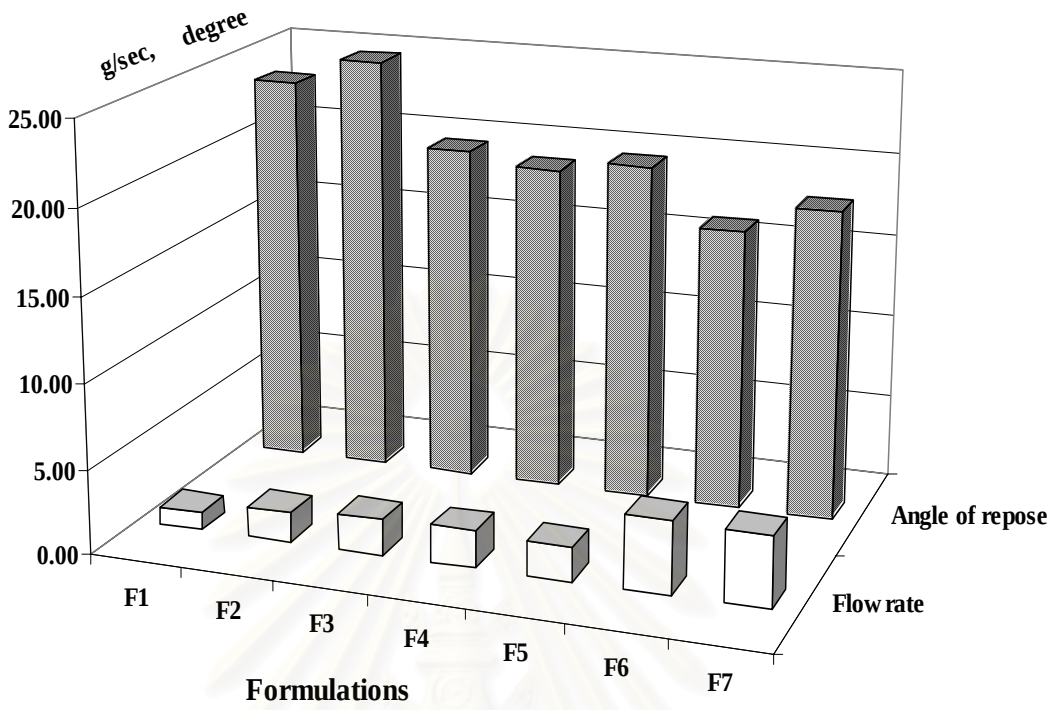
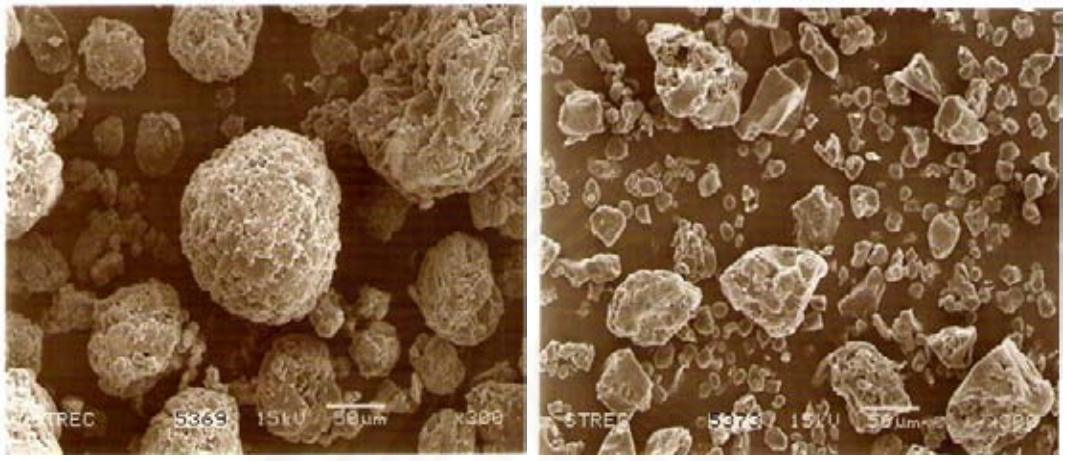



**Figure 28** Flow rate and angle of repose of Centella core tablet formulations

Due to the diluents in the formulations the flowability of CST was improved. Figure 29 presents the SEM photomicrographs of the diluents that of Super-Tab® and Starch 1500®. They were in round shape and rather consistent size so they had good flowability.

The flow rates of the formulations were in the range of 1.06-4.28 g/sec. They could be ranked as F6 > F7 > F4 > F3 > F5 > F2 > F1. The formulation F6 contained with the highest percent of talcum, Aerosil® and magnesium stearate, 4.0%, 2.5% and 1.0% w/w respectively so it showed the greatest flow rate (4.28$\pm$0.17 g/sec). The formulation F7 was the next in the rank of the amount of Aerosil®, talcum and magnesium stearate, 2.0, 3.0 and 1.0 % w/w respectively so its flow rate was in the second (4.17$\pm$0.23 g/sec). In case of formulations F3, F4 and F5 the flow rates were 2.11$\pm$0.90, 2.19$\pm$0.40 and 2.01$\pm$0.67 g/sec, respectively. Their flow rates were very similar because their compositions were slightly different. On the other hand,

formulation F1 and F2 the poor flow because they consisted of the rather low amount of glidant and lubricant.



**A** **B**

**Figure 29** The SEM photomicrograph of Super-Tab® (A) and Starch 1500® (B).

The mean of angle of repose of all formulations were less than 25 degree and could be ranked as F6 < F7 < F4 < F3 ≅ F5 < F1 < F2. Formulation F6 and F7 exhibited the small angle of repose (16.7±2.0° and 18.3±2.9°). It was shown that this formulation had a good gliding property. Formulation F6 consisted of the highest percent of glidant as Aerosil®, talcum and magnesium stearate of 2.5, 4.0 and 1.0 %, respectively. While formulation F2 and F1 presented of 25.0±12.5° and 23.2±12°. Their angles of repose were larger than other formulations. The lower amount of talcum and magnesium stearate of 3.0 and 0.5 %w/w in both formulations caused the higher angle of repose. The angle of repose of formulation F3, F4 and F5 were 20.0±5.0°, 19.3±1.2° and 20.0±0.0°, respectively. They were also similar to the value of flow rate. In conclusion, formulation F6 showed the greatest flow rate and angle of repose. It could explain that formulation F6 had the best gliding property.

### 3.2 Tablet Formulation

The data obtained from section 2 showed the physicochemical properties of CST that was used as active compound of the formulations.

These properties were useful to choose the additives and tablet making process. In this study, the acidic, basic agents and strong oxidizing agents had to be avoided.

In agreement with Palma et al. (2002), this study found that the dry plant extract do not currently exhibit the appropriate flowability and compressibility required to be processed by direct compression. Numerous reports have addressed techniques to solve this kind of problems, 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).

The SEM photomicrographs of CST showed the inconsistent particle sizes and shapes. Mostly, they were in rod shape so the flowability of the extract was poor. The direct compression excipients could improve the flow property of the drug (Connolly et al., 1990). The data obtained from compatibility tests found that Explotab® and Starch 1500® seemed to have an influence on the stability of Centella extract. However, the use of Explotab®, superdisintegrant, in such small quantities in tablet formulation could not have as a detrimental effect on tablet quality. For Starch 1500®, there was no significant difference between CST raw material and the mixture of CST and Starch 1500® ($P > 0.05$). Therefore, it could be used in the tablet formulation. The other excipients, Super-Tab®, Aerosil®, magnesium stearate and talcum were compatible with Centella extract. Moreover, they could improve the stability of the drug as well.

Table 6 shows the formulations of Centella core tablets that were produced by direct compression method. In preliminary, formulation F1 consisted of

CST, Super-Tab®, Explotab®, talcum and magnesium stearate for 20.0, 74.5, 2.0, 3.0 and 0.5%, respectively could not be made as tablet because the powder was poorly flow. It was not able to fill up the die. Moreover, formulation F2-F5 including Super-Tab® and Starch 1500® as diluents in the ratio of 2:1 and adding of talcum, Aerosil® and magnesium stearate for 4.0, 1.5 and 1.0%w/w, respectively could be made as tablets but the picking problem was found shortly after the operation of compression process. The powder stuck on the punch so the tablet surface was rough. Frequently, dry powdered extracts did not exhibit the physical-chemical properties required for processing by direct compression (Egglkruat-Gottanka et al., 2002). To overcome the hygroscopic problem, silicon dioxide, a high porosity excipient, was substantially used (Bonati, 1991). The increasing up to 2.5% w/w of silicon dioxide in formulation F6 could solve this technical problem. Not only this formulation could produce tablets of good appearance but also the in-process evaluation results such as friability, hardness, and weight variation were 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 4-7 kp and weight variation was in the range of $\pm$ 7.5% of the average weight. The amounts of talcum and silicon dioxide were decreased to 3.0% and 2.0% w/w in Formulation F7. However, smooth tableting process and the satisfactory tablet were still obtained. Accordingly, F7 was the most suitable formulation of *Centella asiatica* extract core tablets because it could be produced the satisfactory tablets by using the lower amount of the additives. It was evaluated and used as substrates in the step of film coating development.

## 4. Formulation of *Centella asiatica* film coated tablets

In order to protect the *Centella asiatica* core tablets from the environmental factors, the film coating process was utilized. Furthermore, *Centella asiatica* extract consists of a bitter taste compound, namely, vallerin thus the taste masking is another reason for film coating. In this study, there are 3 types of interested polymer; chitosan (M.W. 50,000), HPMC (Methocel® E5 and E15) and polymethacrylate (Eudragit® E100). Similarly, they have the good protective and taste masking properties (Bauer et

al., 1998; Li et al. 2002). 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 chitosan or HPMC were prepared in aqueous based system. On the contrary, polymethacrylate, the representative of acrylic polymer was prepared in organic based system.

## 4.1 Film coating

### 4.1.1 Film coating process

During coating process, the tackiness problem was found with both chitosan and polymethacrylate film coating formulations, but easily cascade in coating pan after drying. The problem was attributed to the adhesiveness in nature of the chitosan and polymethacrylate film themselves (Lehmann and Bőssler, 1983; Kusonwiriyawong, 1994; Phaechamud, 1999; Lin et al., 2000). In preliminary study, it was found that film rupture could occur and this could have a damaging effect on the tablet properties. To overcome such the problem, the spraying rate was decreasingly adjusted and the spaying pattern was changed to be intermittent, as a consequence, this could lead to much consuming process time. Although this problem could be improved by adding talcum the antiadherent in the film coating solution, it was still endure. The higher levels of film coating were applied, the more tablet aggregation was produced. 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. Fine, discreet film coated tablets were obtained.

The tackiness problem was consistent with the study of Eudragit® film former by Lehmann and Bőssler (1983). It was recommended that the tablet had not to become wet because, in a thick layer, slowly drying lacquer films remained soft for some time and 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 be

briefly interrupted until the cores were once again dry. Blow drying with warm air should then be implemented for about five minutes with the coating pan rotating at a reduced 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. In addition, Lin et al. (2000) reported that the Eudragit® E film exhibited more adhesive property than the Eudragit RL or RS film at higher concentrations of plasticizer. However, dibutyl phthalate and tributyl citrate induced less tack and adhesion for Eudragit® films.

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

## 4.2 Evaluation of Cast film

### 4.2.1 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 chitosan citrate cast film were yellowish transparent and glossy soft film while a colorless and transparent HPMC solution yielded an off-white opaque and glossy cast film. The polymethacrylate film was colorless transparent and glossy.

### 4.2.2 Mechanical Strength

The tensile strength and percent elongation at break of the cast film were illustrated in Figure 30. The tensile strength of cast plasticized films of HPMC, chitosan and polymethacrylate were between 17.41, 6.86 and 0.78 $N/mm^2$,

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 6.33%, 170.09% and 209.38%, respectively and could be ordered as HPMC < chitosan < polymethacrylate. These data showed that HPMC cast film was harder and stronger than chitosan and polymethacrylate cast film. The polymethacrylate cast film showed the lowest tensile strength and the highest elongation at break, which indicated that this polymer yielded the softest film.

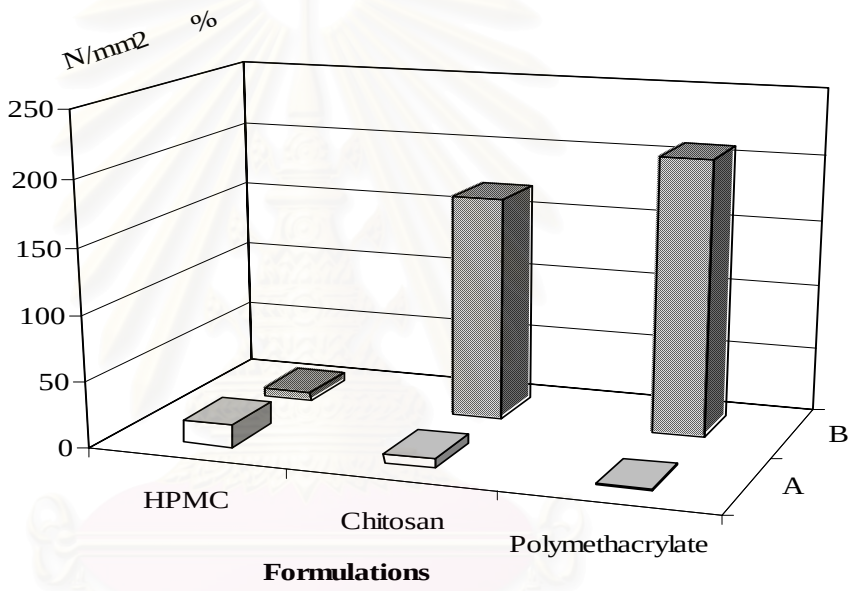



**Figure 30** Tensile strength (A) and elongation at break (B) for cast films.

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 (Lin et al., 2000). 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-Methocel® E5, inserted between the long chains of Methocel® 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 from 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 chains 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). Lin et al (2000) concluded that tributyl citrate seems to be a best 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 tributyl citrate, plasticizer, was appropriate to reduce the glass transition temperature (Tg) of the polymethacrylate film so the increasing of the flexibility of film was obtained.

### 4.2.3 Moisture sorption test

From the data obtained in Figure 31 degree of moisture sorption of the cast films were between 0.42-9.51% and could be ranked as followed: chitosan citrate > HPMC > polymethacrylate. It may indicate that polymethacrylate film is the best film to protect the tablets from the moisture.

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.

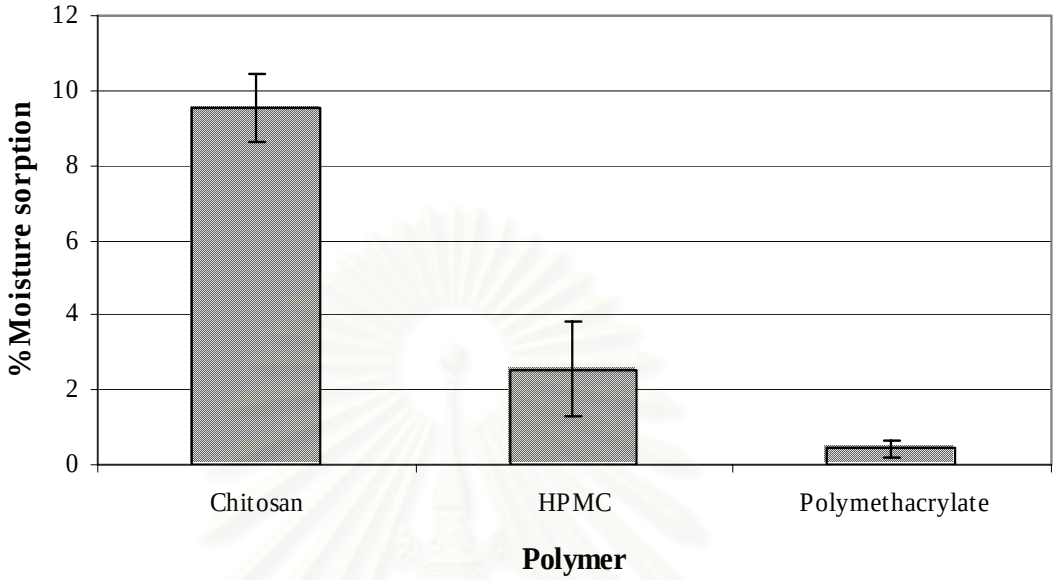



**Figure 31** The percentage of moisture sorption of cast films.

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 that of higher amount than the inclusion of PEG 6000 and tributyl citrate 20% w/w as plasticizers in HPMC and polymethacrylate films. The higher amount of hydrophilic plasticizer made the plasticized chitosan cast film was the highest moisture sorption film. Propylene glycol and PEG 6000 could easily dissolve from the film surface and then the water could suddenly penetrate through the pore occurred after plasticizer dissolving. Molecular differences between propylene glycol and PEG 6000 were probably responsible for the different sorption rate of film plasticized with them. Propylene glycol and PEG 6000 were polyols with similar straight-chain molecules;

however, propylene glycol molecule was smaller (molecular weight of 76.1) and had three hydroxyl groups while PEG 6000 (molecular weight of 6000) had two hydroxyl groups (Kibbe, 2000). Propylene glycol presented more hydroxyl groups to interact with water by hydrogen bonds. Moreover, the chances of PEG6000 to interact with the polymer chains were higher, thus, PEG 6000-containing films presented higher intermolecular forces and showed a lower capacity to interact with water. This finding was consistent with Sothornvit and Krochta (2001) that 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. This is also true in the case of hydrophobic polymer and plasticizer. Polymethacrylate film with tributyl citrate as plasticizer was unsurprisingly resulted in the film with lesser degree of moisture sorption. The chemical structure of Eudragit ® E100 had several long chain of hydrophobic group that of ester and aminoalkyl groups. Moreover, tributyl citrate had the hydrophobic property as well. It consisted of long chain butyl groups that made it practically insoluble in water (Kibbe, 2000). The hydrophobicity of the film former and plasticizers played the important role on water uptake of the film. This finding agrees with the previous study by Lin et al. (2000). That was water absorption of Eudragit® films was dependent on type of Eudragit® polymer and plasticizer used. Triacetin, a plasticizer with high affinity for water, induced slight water absorption for Eudragit® E film. But diethyl phthalate, dibutyl phthalate or tributyl citrate, due to limited solubility in water, caused the little water uptake for the Eudragit® E film. Moreover, Eudragit® RL film plasticized with any of the above plasticizers took up water a hundred times its weight and this may be attributed to the higher hydrophilic property of Eudragit® RL. And due to the relative hydrophobicity of Eudragit® RS, it was difficult to hydrate this film even using triacetin and the film exhibited a minimal degree of water absorption.

## 5. Evaluations of *Centella asiatica* tablet

All of the tablet formulations after freshly prepared and storage at both ambient and accelerated conditions for 4 months were evaluated following this section. Excluding, the test of uniformity of dosage unit was determined only for core tablets after freshly prepared. The abbreviation of the film coated tablets formulations were shown in Table 9.

**Table 9** The abbreviations of the film coated tablet formulations

| Abbreviations | Formulations |
|---|---|
| CS3 | tablet coated with chitosan at 3% coating level |
| CS5 | tablet coated with chitosan at 5% coating level |
| CS10 | tablet coated with chitosan at 10% coating level |
| HPMC3 | tablet coated with HPMC at 3% coating level |
| HPMC5 | tablet coated with HPMC at 5% coating level |
| HPMC10 | tablet coated with HPMC at 10% coating level |
| HPMC15 | tablet coated with HPMC at 15% coating level |
| PMC1 | tablet coated with polymethacrylate at 1% coating level |
| PMC3 | tablet coated with polymethacrylate at 3% coating level |
| PMC5 | tablet coated with polymethacrylate at 5% coating level |

### 5.1 Physical appearance

#### 5.1.1 The color of tablet

The physical characteristics of *Centella asiatica* core tablet were white and glossy. After freshly prepared, all formulations of coated tablet had smooth and glossy surface, but with different shade of color. Both HPMC and polymethacrylate film coated tablets were white. On the other hand, chitosan citrate film coated tablets were yellowish. Likewise, the commercial tablets, Cetellase® tablets, were pale yellow (Figure 32). After storage at ambient condition, the degree

of yellow color of chitosan citrate film coated tablet was slightly increased. In the other words, after exposure to accelerated condition for 4 month the chitosan citrate film coated tablet was brown or dark brown (Figure 33 and 34). The degree of color intensity was increased as a function of storage time and coating level. That was the longer storage period was studied and the higher coating level was applied, the more intensity of the color was dominantly detected. Lim 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 films were changed. In contrast, neither HPMC nor polymethacrylate film coated tablet was changed in color under any conditions (Figure 35 and 36).


**Figure 32** The appearance of core tablets, tablets coated with chitosan, HPMC and polyethacrylate at 5% coating level after freshly prepared and Centellase® tablet immediately after received

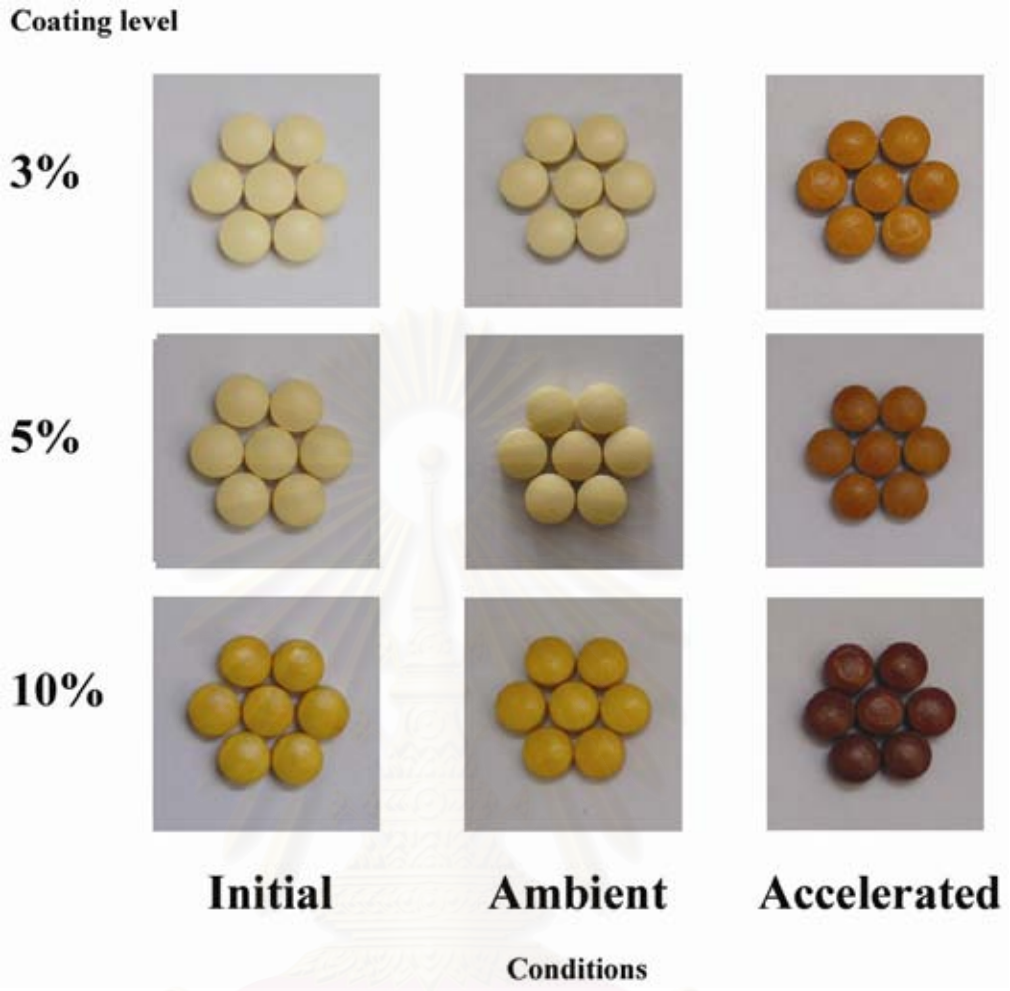



**Figure 33** The appearance of tablet coated with chitosan citrate at various coating level and storage conditions for 4 months.

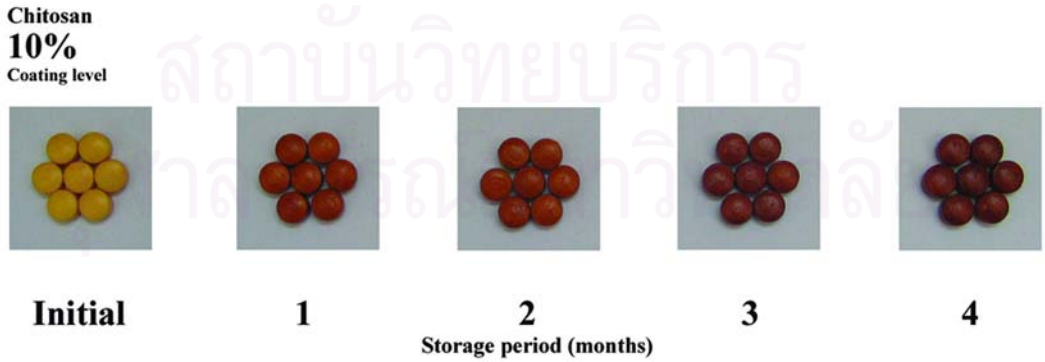



**Figure 34** The appearance of tablet coated with chitosan citrate at 10% coating level after storage at accelerated condition for 4 months.

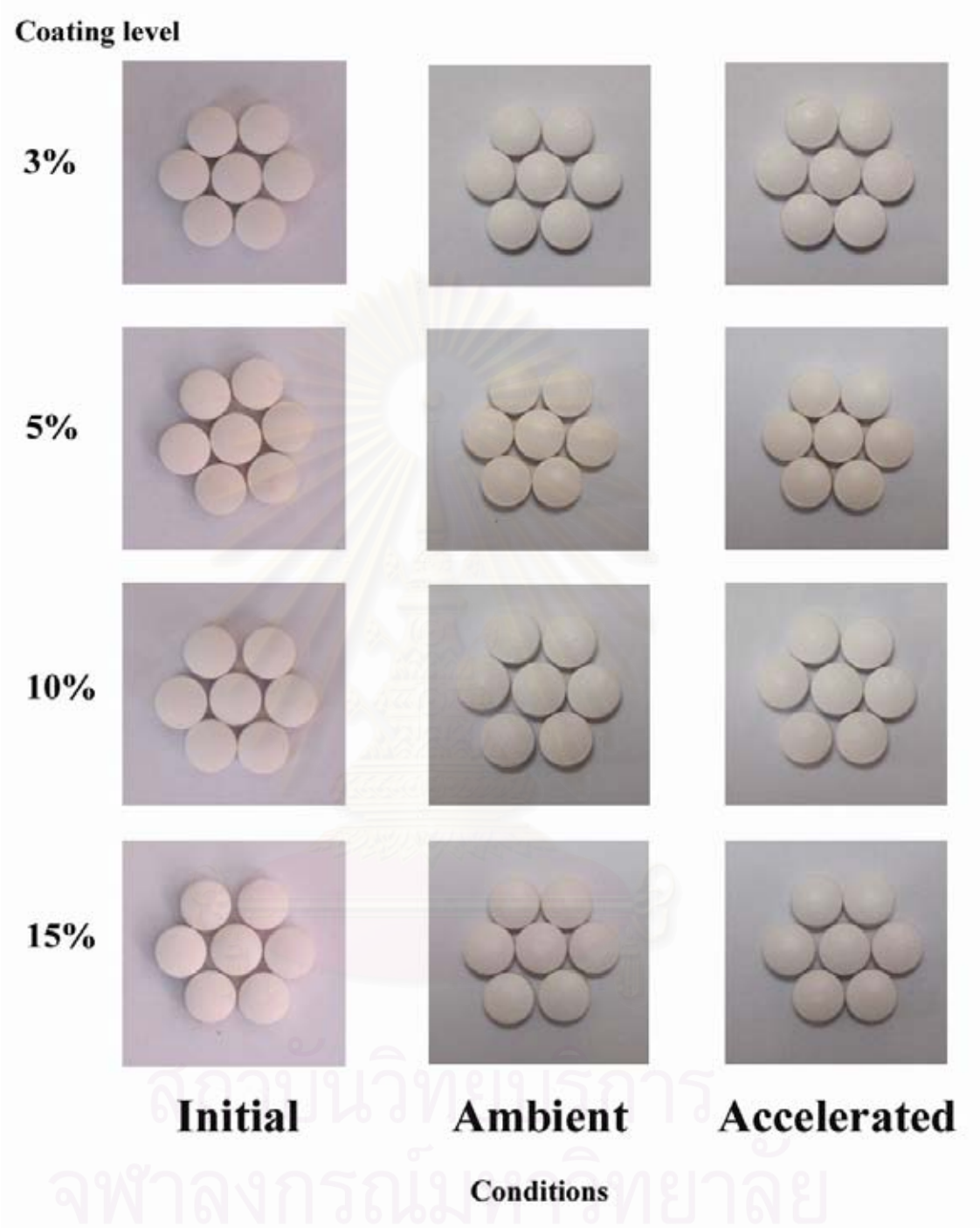



**Figure 35** The appearance of tablet coated with HPMC at various coating level and storage conditions for 4 months.

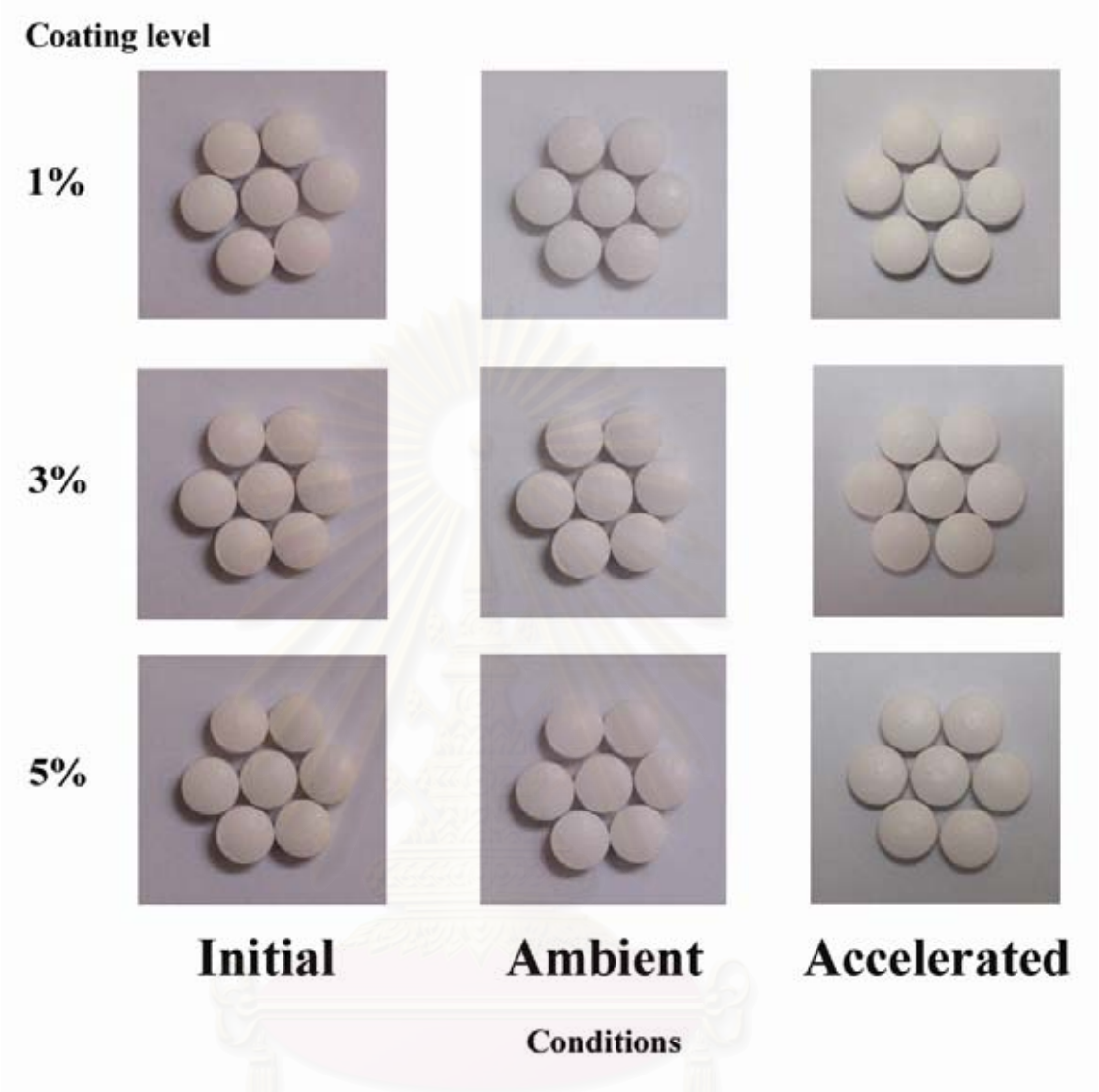


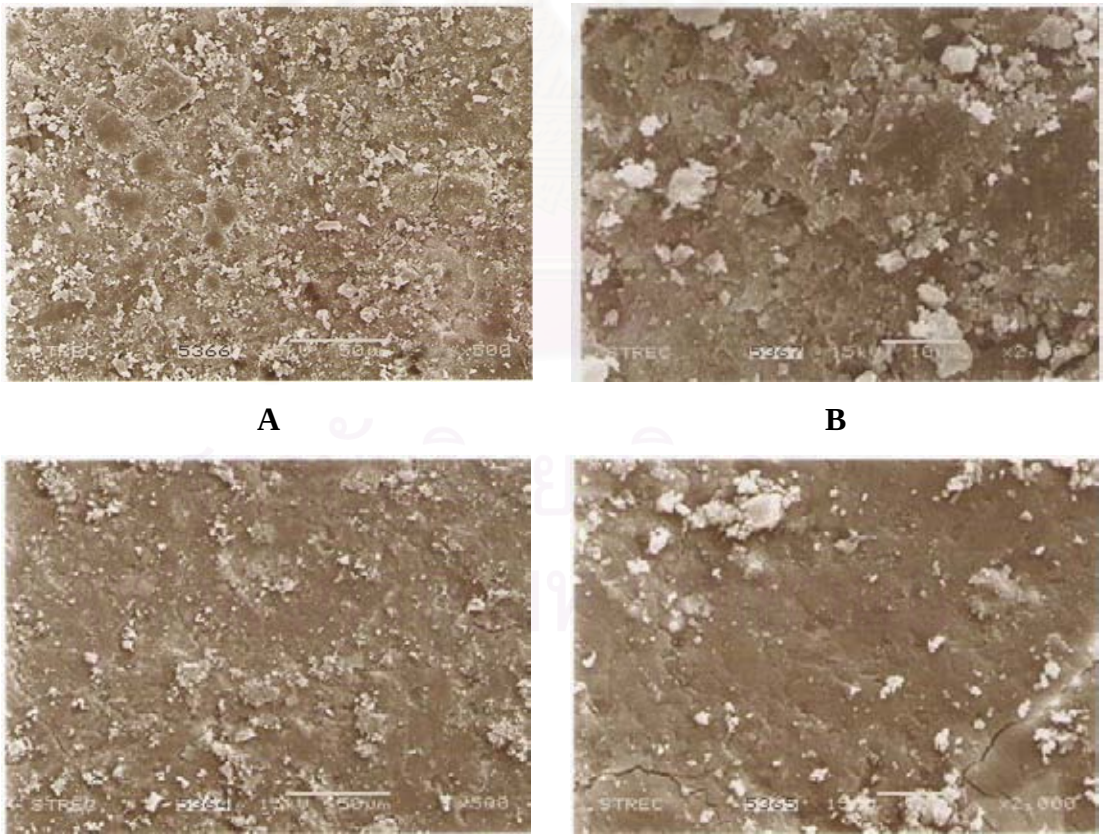

**Figure 36** The appearance of tablet coated with polymethacrylate at various coating level and storage conditions for 4 months.

### 5.1.2 Surface topography

Scanning electron micrographs showing the surface topography of Centellase® tablet, core and coated tablets were illustrated in Figure 37-40. The surfaces at crown area at magnification 500 and 2000 and edge areas at magnification 500 for tablets coated with chitosan and HPMC as well as magnification 150 for tablets coated with polymethacrylate were depicted.

#### 5.1.2.1 Core tablet and Centellase® tablet

The surface topography of core tablet and Centellase® tablet were illustrated in Figure 37. Both of them had the smooth surfaces but Centellase® tablet was found to exhibit cracking in some area.



**A** **B**

**C** **D**

**Figure 37** The photomicrograph of core tablet (A, B) and Centellase® tablet (C, D) (A, C = 500x, B, D = 2000x)

#### 5.1.2.2 Coated tablets

Area of coated tablet were sampled and photographed. Figure 38-40 were illustrations of coated tablets using chitosan, HPMC and polymethacrylate as film former respectively after exposure to ambient condition and accelerated condition.

**Chitosan**

The photomicrographs of the surface of tablet coated with chitosan citrate exhibited rather smooth surface without any pores (Figure 38). The surface of this coated tablet after exposure to accelerated condition was rather smooth at high magnification (2000x). However, from cross-section photos, the films of the coated tablet after storage at both conditions were extremely dense and homogeneous. Additionally, the plasticized chitosan film showed the good adhesion on the core tablet surface.

**HPMC**

The microscopic appearance of tablets coated with HPMC showed that the surface was rather rough (Figure 39). The surface smoothness after exposure to both ambient and accelerated condition was still alike. The cross-section views presented the film with some porous, while it had the satisfactory adhesive property.

**Polymethacrylate**

Figure 40 depicts the surface of tablets coated with polymethacrylate. It was rather rough with some pore. Similar to HPMC, the surface smoothness of the tablets after exposure to both ambient and accelerated condition was still alike. From the cross-section photos, it was shown that polymethacrylate film

was thick and had less density than chitosan and HPMC film. However, it showed the good adhesion on the surface of tablet.

In conclusion, the SEM photographs of the coated tablets showed the difference in degree of smoothness. At the same coating level, the degree of smoothness and density could be ranked as: chitosan > HPMC > polymethacrylate. The degree of the film thickness was ordered as: polymethacrylate > HPMC ≅ chitosan.

The differences of smoothness and thickness were probably contributed to the difference in drying of the film coating formulations during the coating process. The slow drying was believed to yield the high degree of smoothness (Seitz et al., 1986). Although the spraying pattern of both chitosan and polymethacrylate were intermittent but the surface of tablets coated with chitosan was rather smooth. It was probably due to the rate of evaporation of the solvent in coating solution. The organic solvent in polymethacrylate solution was rapidly evaporated so the film former was also rapidly dried when it attached on the tablet surface without spreading. From this reason, the polymethacrylate film was overlapped in many layers. Thus the film become thick and had a lot of pores inside. As shown in the photomicrographs chitosan film seemed smoother than HPMC film. Due to the slower spray rate of chitosan coating solution, the drying and spreading of the coating solution would be better than the high spray rate of HPMC.

After exposure to both conditions, all types of plasticized films showed the good adhesion on the tablet surface. In an earlier study conducted by Fung and Parrott (1980), the effects of relative humidity on polymer adhesion were investigated. Some tablet formulations exhibited an increase in adhesion with increased humidity, whereas other coated tablets showed the opposite behavior. Furthermore, 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 was the primary type of interfacial bonding mechanism between the tablet surface and

polymer. Dipole-dipole and dipole-induced dipole interactions also occurred, however, to lesser extent. Factors which affect the type or the number of bonds formed between the polymer and the tablet surface would influence film adhesion (Felton and McGinity, 1999).

### 5.1.3 Defect of the coated tablet

After preparation and storage at ambient and accelerated conditions, the chitosan citrate and polymethacrylate film coated tablets were tackiness. The defect of picking was slightly found in chitosan film coated tablet whereas HPMC and polymethacrylate film coated tablet could be separated without any defect. Table 10 showed the percentage of defected tablets coated with chitosan citrate by manual coating from 100 tablets. The accelerated condition and the coating level apparently increased the tackiness. That was the chitosan citrate film coated tablet at accelerated condition showed more defects than that after preparation and storage at ambient condition. Due to the high humidity of accelerated condition, the tablets coated with chitosan citrate would up taken the more moisture from the environment. So they became tacky and had more defect than the tablets storage at ambient condition. The higher percent coating level was applied, the more moisture absorption and defects were increased.

**Table 10** The percent defect of Centella tablets coated with chitosan citrate

| Conditions | The percent defect of Centella film coated tablets | | |
|---|---|---|---|
| | CS3 | CS5 | CS10 |
| Ambient, at $4^{th}$ month | 0 | 0 | 2 |
| Accelerated, at $4^{th}$ month | 7 | 11 | 16 |

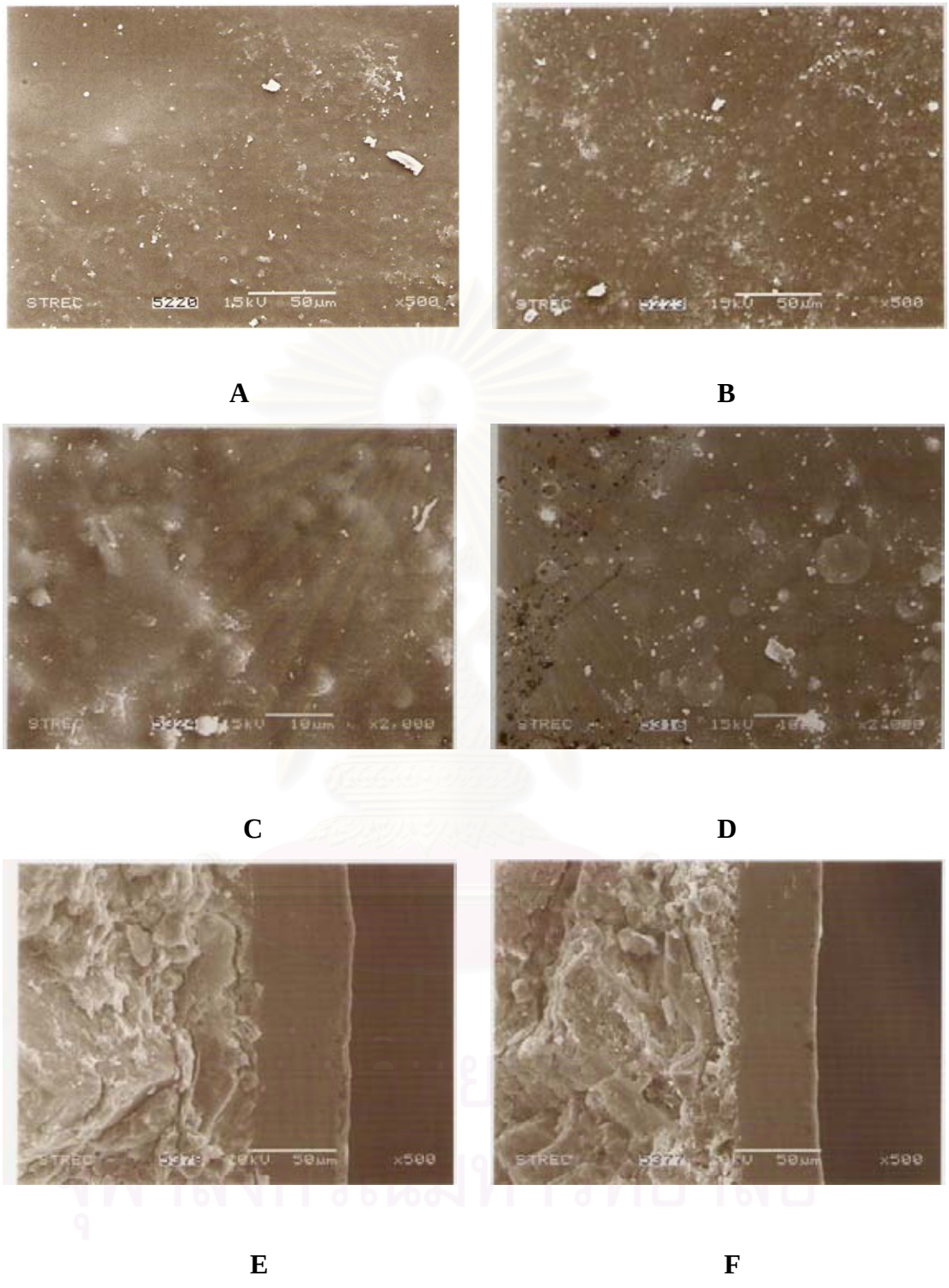



**Figure 38** The photomicrograph of tablet coated with chitosan at 3% coating level after exposure to ambient (A, C, E) and accelerated (B, D, F) conditions for 4 months (A, B, E, F = 500x, C and D = 2000x), (A, B, C, D = surface and E, F = edge)

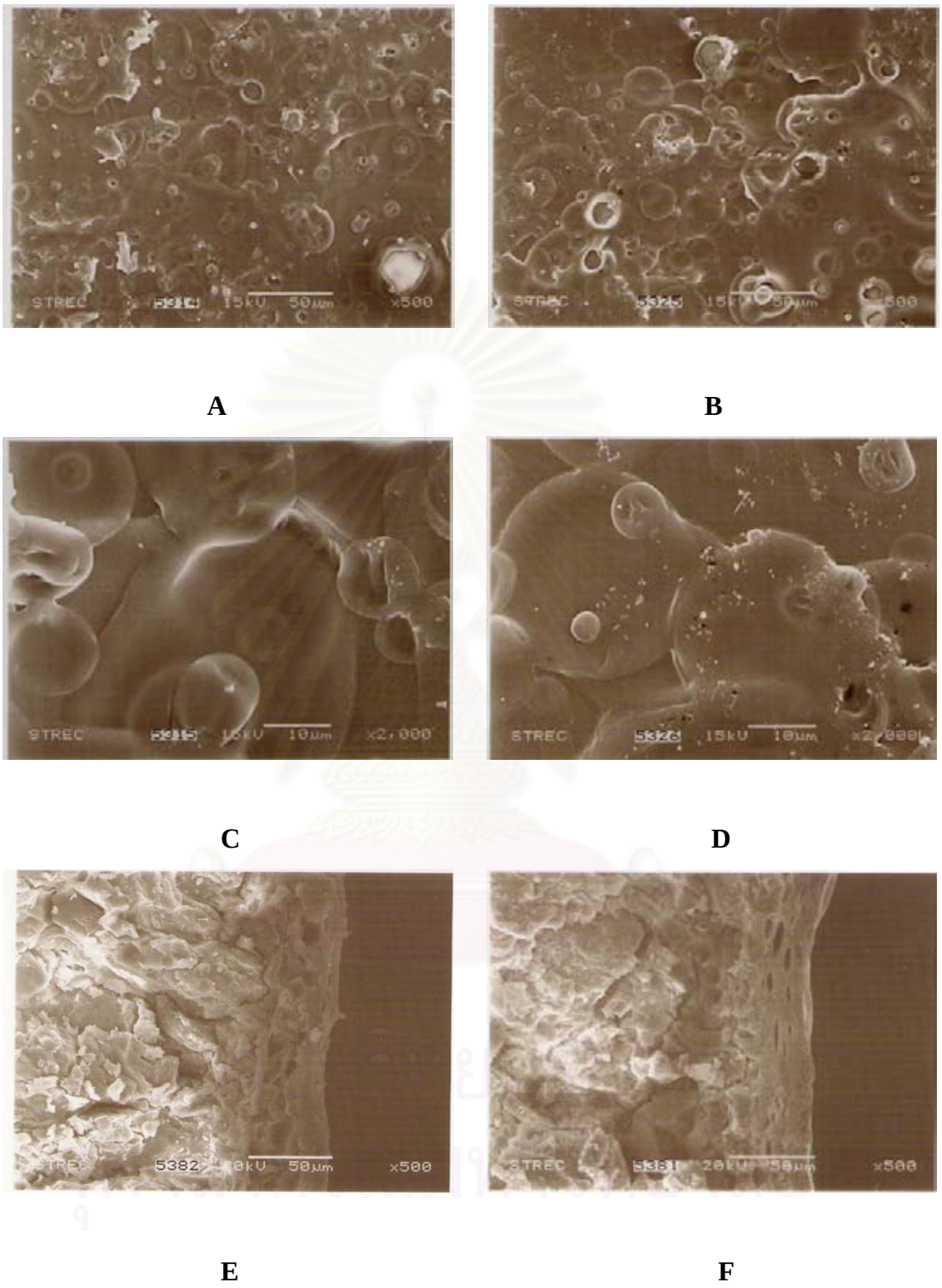



**Figure 39** The photomicrograph of tablet coated with HPMC at 3% coating level after exposure to ambient (A, C, E) and accelerated (B, D, F) conditions for 4 months (A, B, E, F = 500x, C and D = 2000x), (A, B, C, D = surface and E, F = edge)

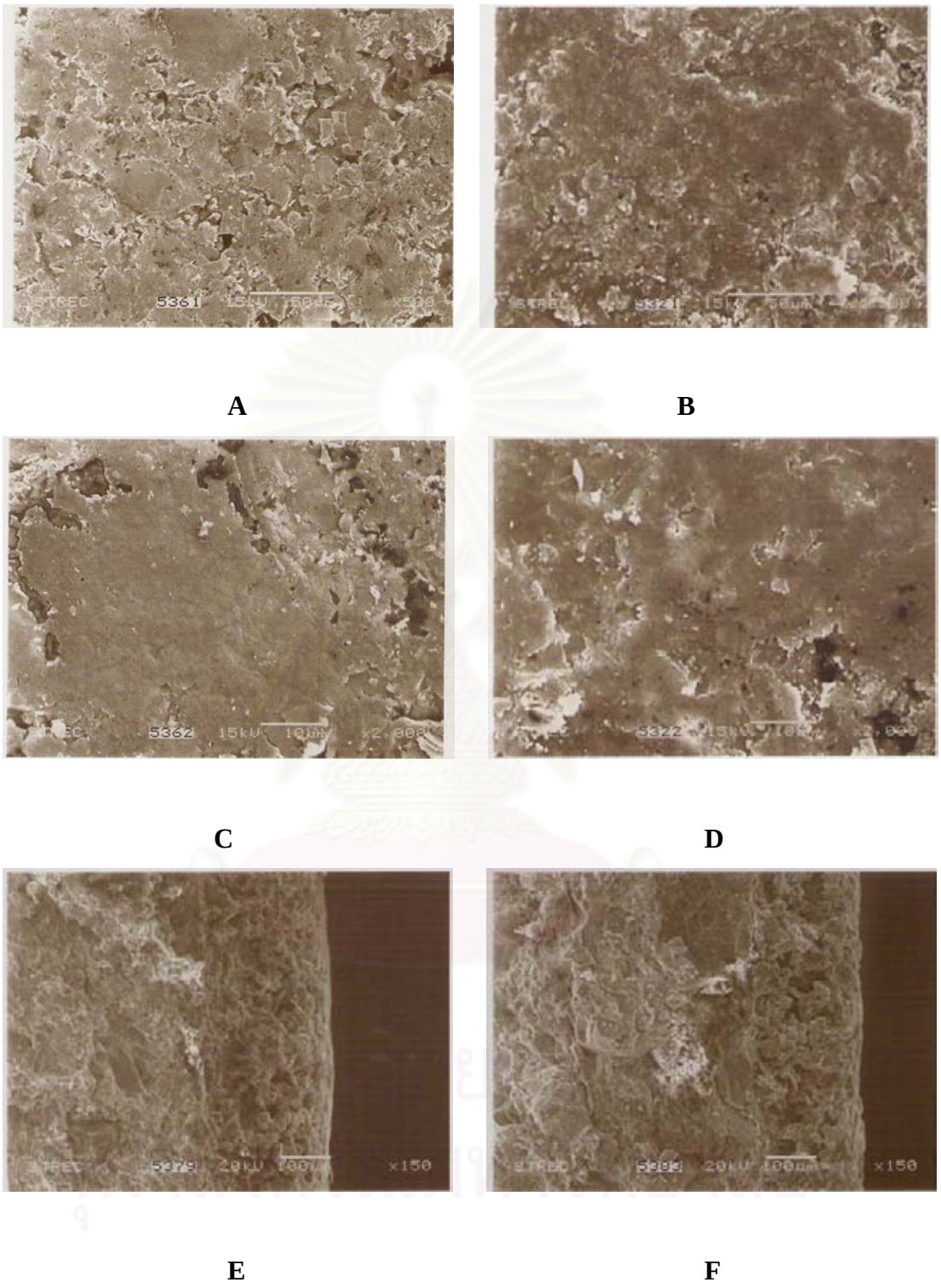



**Figure 40** The photomicrograph of tablet coated with polymethacrylate at 3% coating level after exposure to ambient (A, C, E) and accelerated (B, D, F) conditions for 4 months (A, B = 500x, C, D = 2000x, E, F =150x), (A, B, C, D = surface and E, F = edge)

### 5.2 Friability

The percentage of friability of core tablets and coated tablets (after preparation, after storage under accelerated condition for 4 month) are presented in Table 32-34 in Appendix C. 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 tablet after compression and storage under accelerated condition were 0.47 and 0.66, respectively. Due to the moisture absorption by the surface of core tablet the percentage of friabitity after exposure to accelerated condition was more than that after compression. 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. Banker et al. (1990) reported that the tablets made with spray dried lactose generally show better physical stability such as hardness and friability than regular lactose. In addition, Starch 1500® as diluent in direct compression had the excellent compactibility at low pressure, high dilution capacity (Davies, 2001).

Most of the formulations of coated tablets were not friable and showed that the weight were unchanged. It was the coating could improve the friability of the core tablets. However, the percent friability of tablets coated with chitosan citrate at 5% and 10% coating level after freshly prepared had surprising negative values. This may be attributed to the moisture sorption of coating surface during friability test. In conclusion, the friability of all formulations were conformed the USP 25 specification (less than 1.0%).

### 5.3 Hardness

The mean and standard deviation of hardness are displayed in Figure 41. The hardness of tablet coated with chitosan citrate and HPMC were higher than that of core tablets about 2.3-15.1 kp. An enhancement of coating level enhanced the hardness of tablets coated with HPMC and chitosan citrate. On the other hand, the

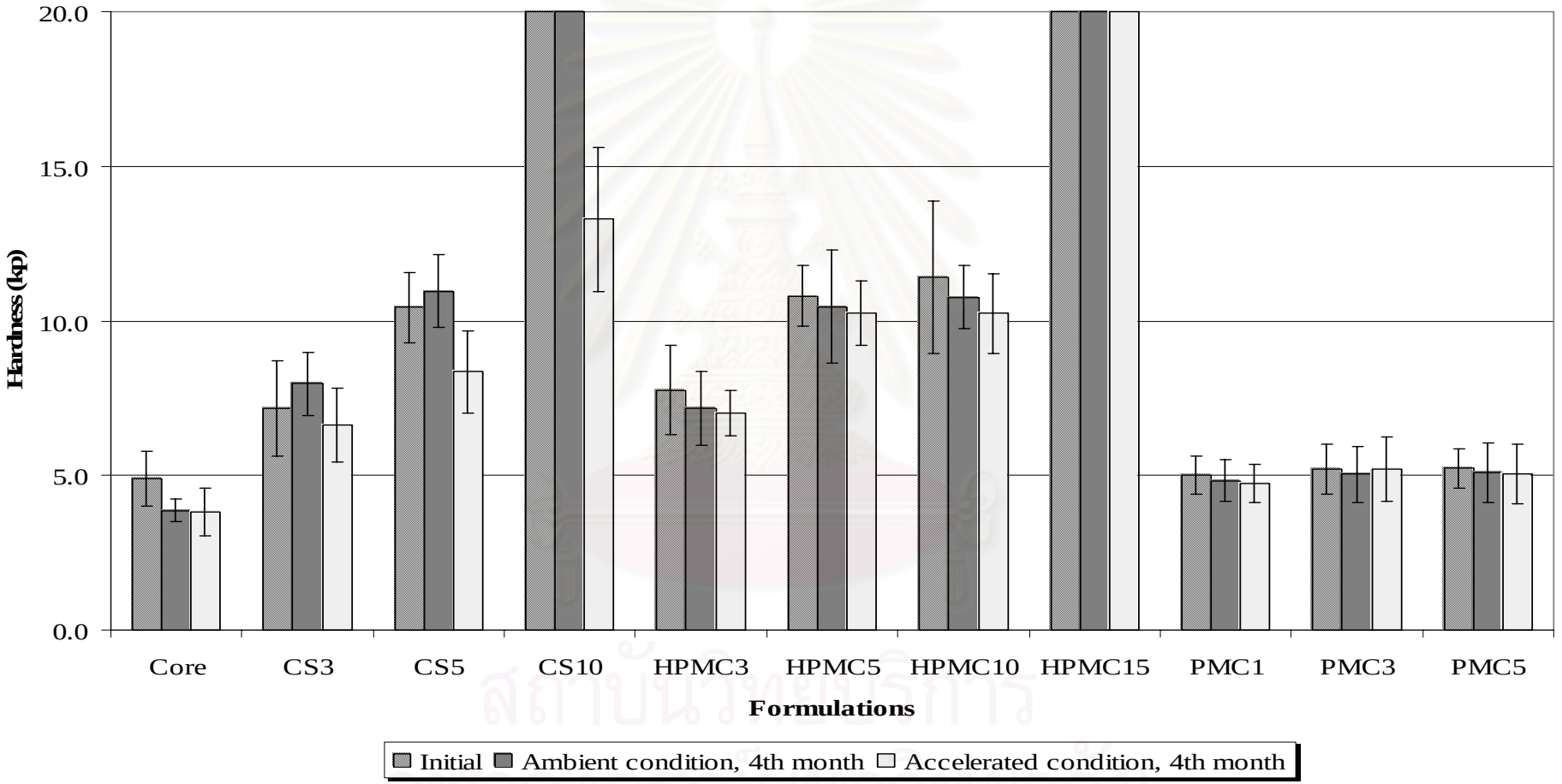



**Figure 41** Hardness of core and film coated tablet formulations after preparation, storage under ambient and accelerated conditions for 4 months

hardness of tablet coated with polymethacrylate was not much difference from core tablet.

After exposure to the ambient and accelerated conditions for 4 months, the hardness of tablets coated with HPMC and polymethacrylate were slightly decreased. In case of the hardness of tablets coated with polymethacrylate at 3% and 5% coating level were not markedly altered after storage at any conditions. It indicated that this film had a satisfactory protective property. Whereas the hardness of core tablets and tablet coated with chitosan citrate storage at accelerated condition for 4 month were greater decreased. Particularly at 10% coating level of tablets coated with chitosan citrate, the hardness was decreased up to 6.7 kp. Regarding moisture sorption study, it was found that 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 and core tablet decreased of tablet hardness. While the HPMC and polymethacrylate films were uptake less moisture so they had greater protection of the tablets from the moisture. However, mean and standard deviation of the tablet hardness of some formulations, the tablet coated with chitosan citrate at 10% coating level after preparation and after storage at ambient condition for 4 month and HPMC at 15% coating level could not be calculated because some obtained values exceeded the maximum limit of the apparatus of 20 kp. At the same coating level, the hardness of coated tablets could be ordered as followed: polymethacrylate < chitosan < HPMC. It was related to the mechanical property that of the plasticized HPMC film was the hardest and strongest film while the plasticized film of polymethacrylate was the softest film.

### 5.4 Weight variation

Table 39-41 in Appendix C shows the weight variation of all formulations. The weight variation was negligible in case of tablet storage under ambient condition for 4 months. The weight variations of all formulation were conformed to the specification in official standard USP 25 (average difference of less than 7.5%). 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 and angle of repose 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.

### 5.5 Disintegration

In this study the rapidly disintegrating film coated tablets were produced. Therefore the tablet should disintegrate rapidly in the disintegration medium. Chitosan citrate and HPMC are the water soluble films, but polymethacrylate film can not dissolve in pure water (Bauer et al., 1998; Kibbe, 2000; Ritthidej et al., 2002). Lehmann and Bőssler (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. Incorporating adequate quantities of disintegrating agents in the tablet core would afford film coated tablets which would disintegrate in water within a few minutes.

The disintegration time of core and coated tablets are presented in Figure 42. The disintegration time was ranked as: core tablet < polymethacrylate < HPMC < chitosan. The disintegration time of core tablets in deionized water at 37 °C were within minute. It is probable due to the addition of sodium starch glycolate, Explotab®, the high efficiency disintegrant and the producing method of direct compression. The disintegration times of film coated tablets in deionized water were longer than that core tablets. The increasing of coating level showed a tendency to prolong the disintegration time. The thicker of the barrier as the coating level was increased, the longer was the disintegration time of the film coated tablet. Because the thicker film would require more time in order to be dissolved.

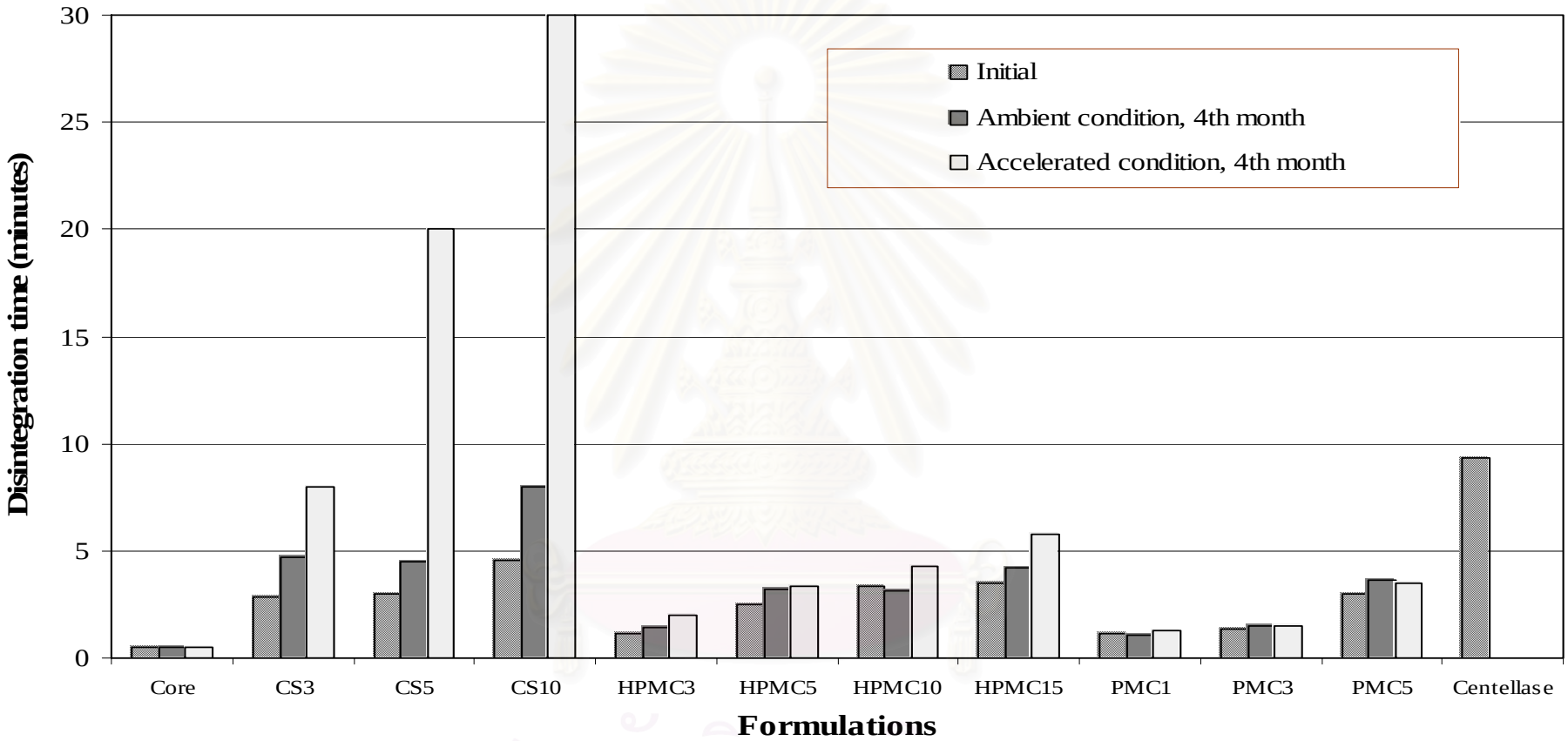



**Figure 42** Disintegration time of film coated tablet formulations in deionized water at 37°C

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 tablets coated with 10% coating level chitosan citrate fims was 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 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. Consisting of 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, could, however, partially compensate for the loss of available hydroxyl groups by giving, especially, highly hydrophilic carbonyl groups. According to a previous study, cross-linking with citric acid of HPMC films resulted in a decrease of the affinity of the natural polymer toward water and showed a non water solubility of films associated with an improvement of water vapor barrier properties (Coma et al., 2003; Sebti, 2003). While there was a slight change of disintegration time of film coated tablet after storage at ambient condition for 4 months. The changed values were in the range of 0.09-3.45 minutes.

### 5.6 Uniformity of dosage unit

The content uniformity of *Centella asiatica* core tablets, freshly prepared, is shown in Table 11. There were in the range of 97.6-102.7 % of the label amount. The percentage of coefficient variation (%CV) was 1.82.

The results passed the specification of general monograph of USP 25, in which the content uniformity of the tablets was within the range of 85.0-115.0% of the label amount and the percentage of coefficient variation (%CV) was less than 6.

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.

**Table 11** Content uniformity of *Centella asiatica* core tablet after freshly prepared

| Tablet No. | Amount (mg) | | | Total (mg) | %label amount |
|---|---|---|---|---|---|
| | AS | MA | AA | | |
| 1 | 13.08 | 11.36 | 6.07 | 30.51 | 101.7 |
| 2 | 13.18 | 11.51 | 6.11 | 30.80 | 102.7 |
| 3 | 12.53 | 10.93 | 5.82 | 29.28 | 97.6 |
| 4 | 12.85 | 11.21 | 6.00 | 30.06 | 100.2 |
| 5 | 13.03 | 11.29 | 6.07 | 30.39 | 101.3 |
| 6 | 12.61 | 11.01 | 5.91 | 29.53 | 98.4 |
| 7 | 12.85 | 11.24 | 6.05 | 30.14 | 100.5 |
| 8 | 12.86 | 11.28 | 6.06 | 30.20 | 100.7 |
| 9 | 12.51 | 10.91 | 5.88 | 29.30 | 97.7 |
| 10 | 12.58 | 10.93 | 5.89 | 29.40 | 98.0 |
| Average | 12.81 | 11.17 | 5.99 | 29.96 | 99.9 |
| SD | 0.24 | 0.21 | 0.10 | 0.55 | 1.82 |
| %CV | 1.89 | 1.87 | 1.70 | 1.82 | 1.82 |

### 5.7 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 active components. The chromatogram was presented in Figure 61 in Appendix A.

***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 18-20, in Appendix A. The ranges of percentage of recovery were 98.4-102.8%, 98.1-101.9% and 97.9-102.3% for standard AS, MA and AA, respectively. Hence this HPLC gradient method was accurate for these compounds assay.

The consequences of the assay are shown in Figure 43. The assay content of the tablet formulations were in the range of 25.8-28.5 mg/tablet or 86.1-95.0 % label amount. The total content of *Centella asiatica* extracts in Centellase® tablets that assay when received was 24.9 mg/tablet or 82.9% of the label amount. At the same coating level, the assay amount of the coated formulations could be ranked as polymethacrylate > chitosan > HPMC. Although the tackiness problem was found with both polymethacrylate and chitosan, polymethacrylate showed the less tacky. Therefore, the period of coating process of polymethacrylate was shorter than chitosan. The tablets coated with polymethacrylate were less affected by the environment. Besides, it was possible that polymethacrylate solution was prepared in organic solvent so it used the low inlet temperature in the coating process. For HPMC, it may be the use of high inlet temperature and aqueous system coating process. The obtained values from assay amount of tablets coated with 3 and 10% coating level of HPMC were lower than 90.0 % of the label amount. It was probably due to fall in the coating and analytical error.

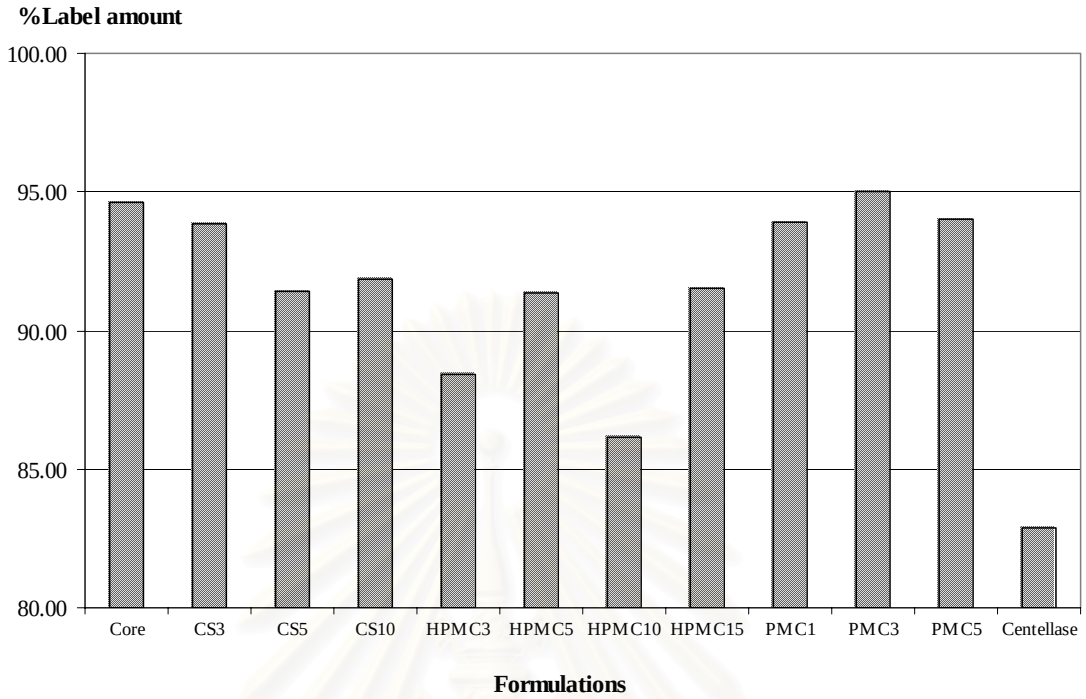



**Figure 43** The assay content of the tablets formulations

### 5.8 Dissolution study

#### 5.8.1 Selection of dissolution medium

The solubility data of all active ingredients in section 2.3 indicated that these constituents were poorly soluble in water whereas they could soluble in the acid alcoholic solvent system.

Several studies recommended the partially organic dissolution medium, particularly alcohol-water solvent system, for testing the pharmaceutical dosage forms (Walkling et al., 1979; Dodge and Gould, 1987; and Corrigan, 1991). From preliminary study, the chosen dissolution medium for this investigation was the mixture of 0.1N hydrochloric acid and isopropyl alcohol as the ratio of 70: 30. Isopropyl alcohol of 10, 20, 30, 40 and 50% of were mix with 0.1N hydrochloric acid to prepare the dissolution medium. The optimum percent of isopropyl alcohol was 30% of total volume because all film coated tablets formulations could disintegrate and dissolve in order to give the highest detected peak area of all constituents.

Whereas at the level of 40 and 50% of isopropyl alcohol, chitosan films seemed to less dissolve. After 30 minutes the tablets coated with chitosan citrate were still similar appearance to the initial. This may be due to the nature of chitosan have a limit to dissolve in water-alcohol mixture (Sandford, 1989). As a consequence of a small volume medium, the paddle apparatus and the dissolution vessels were agreeable. This selected medium was used for comparing the dissolution of all formulations.

### 5.8.2 Dissolution of the tablets

Figure 45 illustrates the percent dissolution at 30 minutes of the formulations 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 initial of the storage, after storage at ambient condition and accelerated condition for 4 months were in the range of 76.1- 88.4%, 73.2-81.2% and 64.7-78.1% label amount, respectively.

There were significant differences by using an analysis of variance (ANOVA) and Scheffe test for post-hoc comparisons of core tablets between the tablets after freshly prepared and accelerated condition and between the tablets after storage at ambient condition and accelerated condition ($P < 0.05$). It was probably due to the decreasing of the amount of active constituents of CST 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 ($P > 0.05$). Nonetheless, this study did not investigate the comparison of dissolution profiles of the formulations. Thus the release pattern of drug dissolution was unknown. However, it noticed that the percents dissolution at 30 minutes of all formulations after preparation were sufficiently high of 70 % (Q).

The effect of storage conditions on drug dissolution was investigated. From the data obtained, the percent dissolution tended to be decreased 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 still nearly to the previous shape. There were statistical significant differences of the tablets coated with 3% and 10% coating level of chitosan citrate between after freshly prepared and after storage at accelerated condition for 4 month ($P < 0.05$). Meanwhile, tablet coated with 5% coating level of chitosan citrate were not significant difference. However, the drug release of one tablet of 5% coating level of chitosan was only 58.1%.

This was probably due to the delay time of the tablet disintegration from the coating films. This finding agreed with previous studies by Phaechamud et al. (2000) in that the dominantly 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 at 30 minutes interval, 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 this study the longer storage period at accelerated condition was investigated so the greater of this problem was found. Coma et al. (2003) introduced cross-linking film of HPMC with citric acid for great advantages in improving the moisture barrier properties and decreasing water solubility. High temperature (190 °C, 15 minutes) was used in the process of curing this cross-linking material. That could indicate that the cross-linking mechanism of cellulose with citric acid have an influence on the solubility of films.

In case of tablets coated with 10% and 15% coating level of HPMC film there were significant differences by using an analysis of variance (ANOVA) and Scheffe 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 form 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 the drug release was slightly retarded by the hydrated film. The hydration and gel forming properties of chitosan in the presence of citric acid in matrix formulations had been reported by Nigalaye et al. (1990).

For polymethacrylate, there were no significant differences of the dissolution between before and after storage. It was noticed that this acrylic polymer has a great solubility in this kind of dissolution medium which containing both of acid and alcoholic solvent.

Interestingly, the dissolution of commercial tablets (Centellase®) at any condition of storage was extremely low at time interval either 30 or 45 minutes (Figure 44). Visually, they were very slightly abrasive. Furthermore, the disintegration time in deionized water of Centellase® tablets was 9.33 minutes. It may be their interaction mechanism was unknown or this dissolution medium that might not appropriate for using to compare with this kind of tablet.

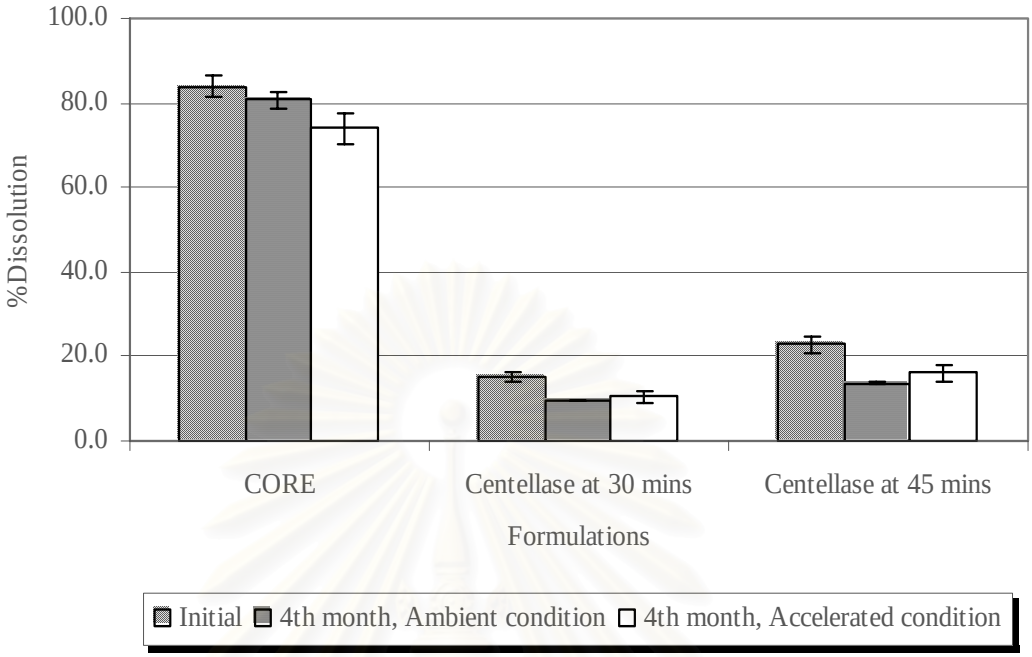



**Figure 44** The percentage of dissolution of core tablet at 30 minutes and commercial Centellase® tablet at 30 minutes and 45 minutes ($P < 0.05$).

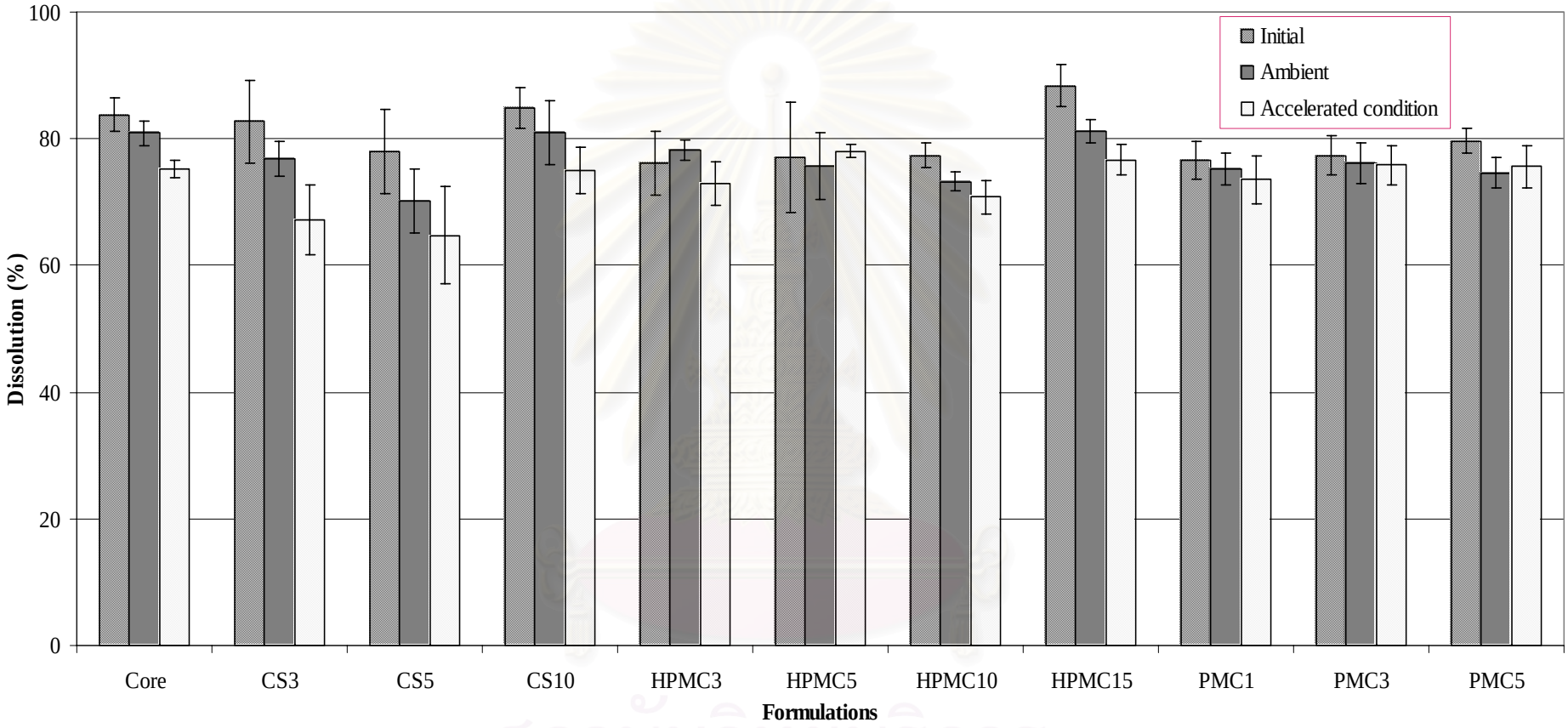



**Figure 45** The percentage of dissolution of *Centella asiatica* film coated tablets at 30 minutes

## 6. Stability study

### Chemical stability study

Stability study of *Centella asiatica* tablets, the storage condition and storage period was performed according to Thai FDA guideline 2004. The percentages of the total remaining contents are more than 90% and less than 110% of the initial.

The percent content of active constituents of *Centella asiatica* both total and individual are summarized in Figure 46-52. The residual contents were calculated by comparing the corresponding values with initial.

From the residual percent content, stability of tablet containing *Centella asiatica* extracts would be expressed 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). This result agreed with a previous study that investigated the stability of *Centella asiatica* extracts in two dosage form, cream and tablet (Inamdar et al., 1996). The formulations containing *Centella asiatica* extracts from different origins were prepared. It was found that one from four origins of *Centella asiatica* could be made stable tablet and cream formulations. The tablet dosage form was stable at all conditions in that study as followed: air conditioned (25$\pm$1 °C), room temperature, 40 °C and accelerated condition (40 °C, 80%RH) for 6 months. The residual content was more than 90% of the initial amount. Moreover, after storage at the accelerated condition (40 °C, 80%RH) for 3 months the percent remaining of the active compounds was 97.15% of the initial content. It showed a slightly decreasing of the active constituents from *Centella asiatica* extracts. The other two origins of *Centella asiatica* extracts were stable in tablets form at the accelerated condition (40 °C, 80%RH) for 3 months and another origin was not stable at any conditions (the percent drug content was less than 85% of the initial after storage at accelerated condition for 1 month).

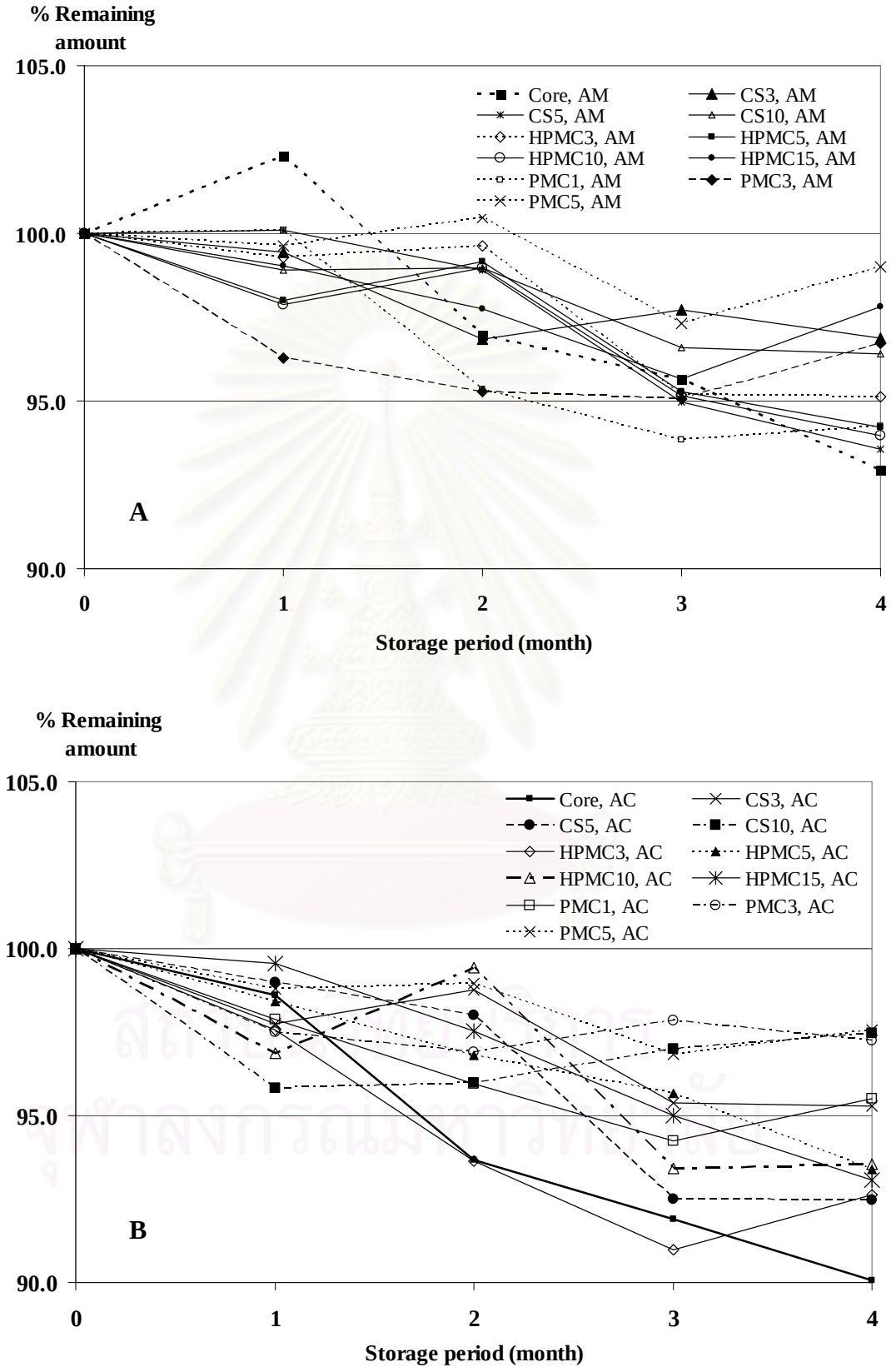



**Figure 46** Residual total percent content in *Centella asiatica* tablet formulations (A = after storage at ambient condition, B = after storage at accelerated condition)

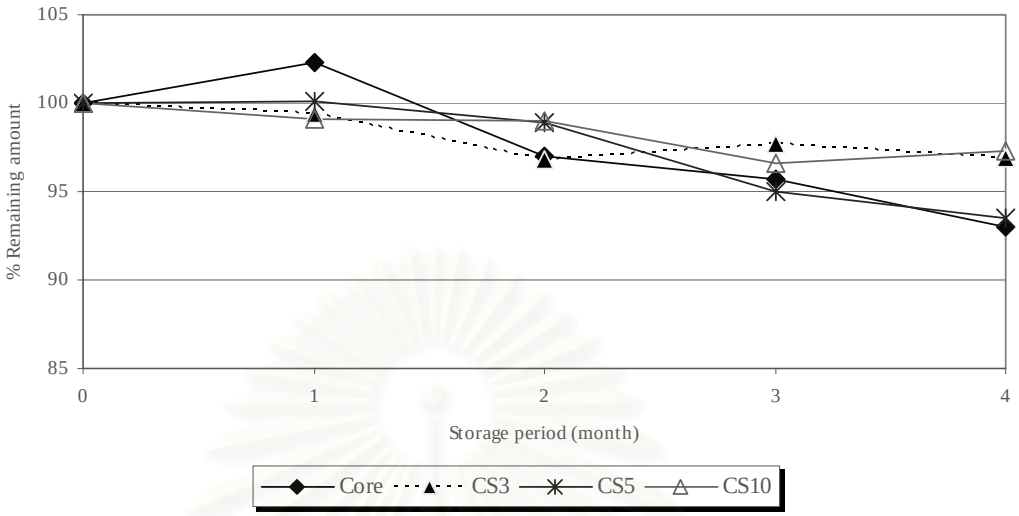



**Figure 47** Residual total percent content in *Centella asiatica* tablets coated with chitosan citrate after storage at ambient condition

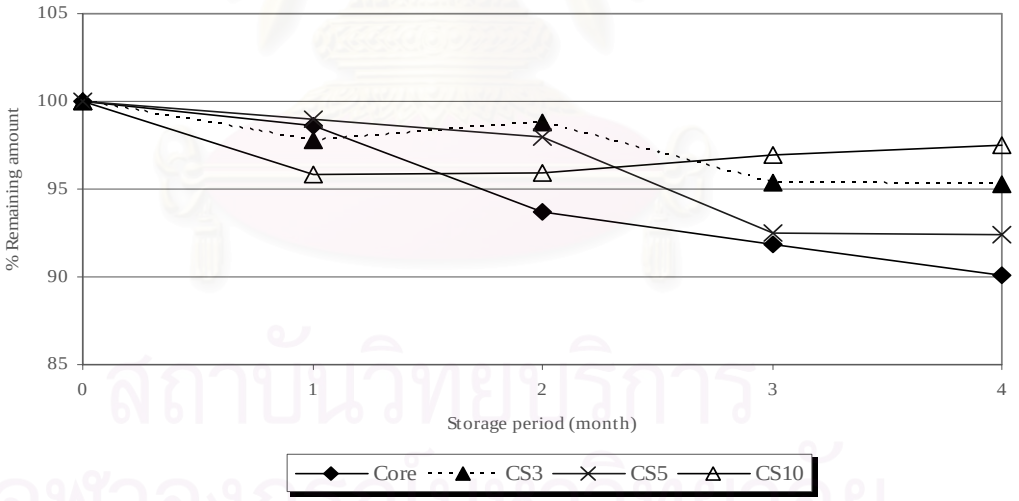



**Figure 48** Residual total percent in *Centella asiatica* tablets coated with chitosan after storage at accelerated condition

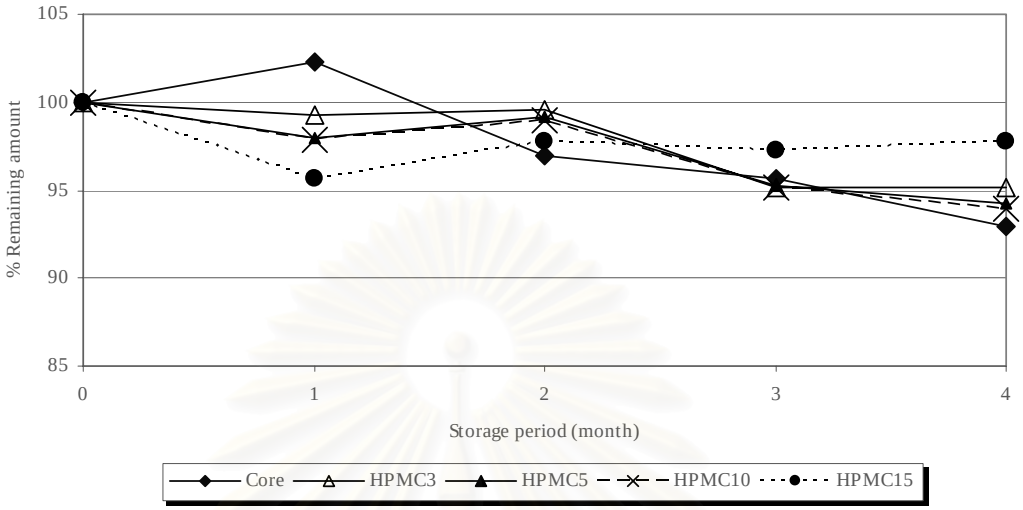



**Figure 49** Residual total percent content in *Centella asiatica* tablets coated with HPMC after storage at ambient condition

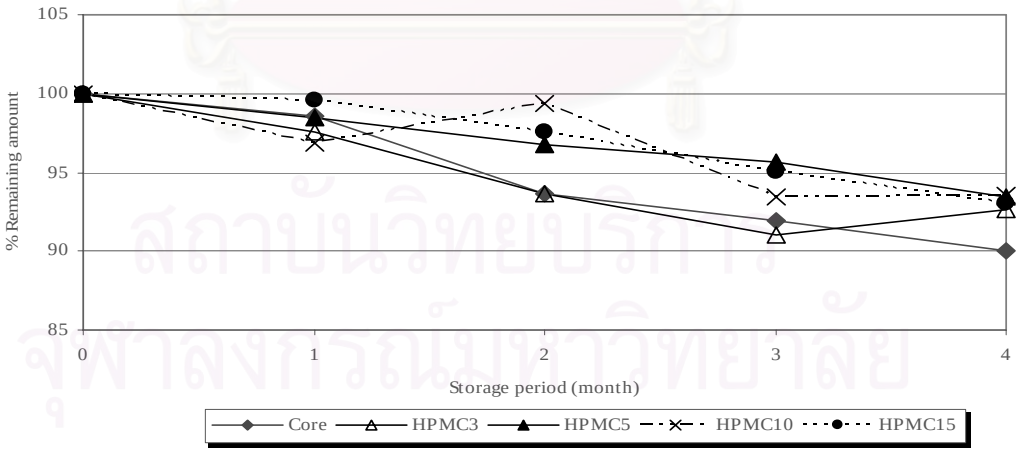



**Figure 50** Residual total percent content in *Centella asiatica* tablets coated with HPMC after storage at accelerated condition

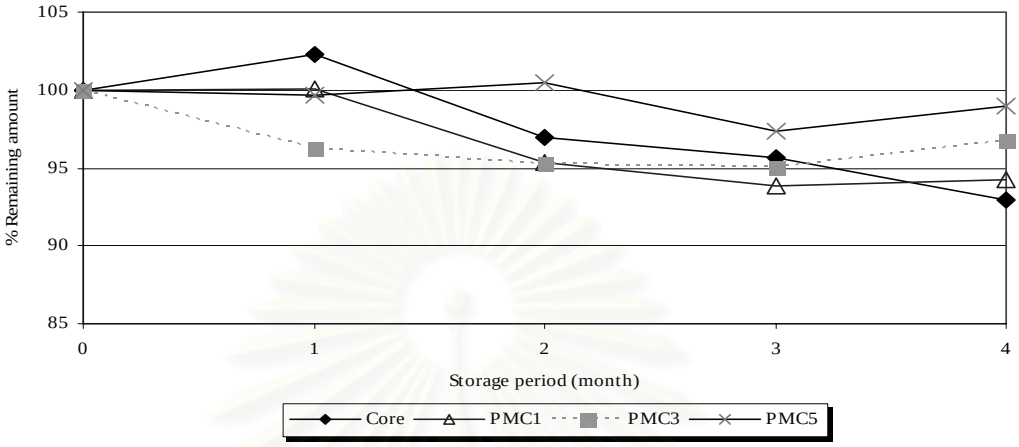



**Figure 51** Residual total percent content in *Centella asiatica* tablets coated with polymethacrylate after storage at ambient condition

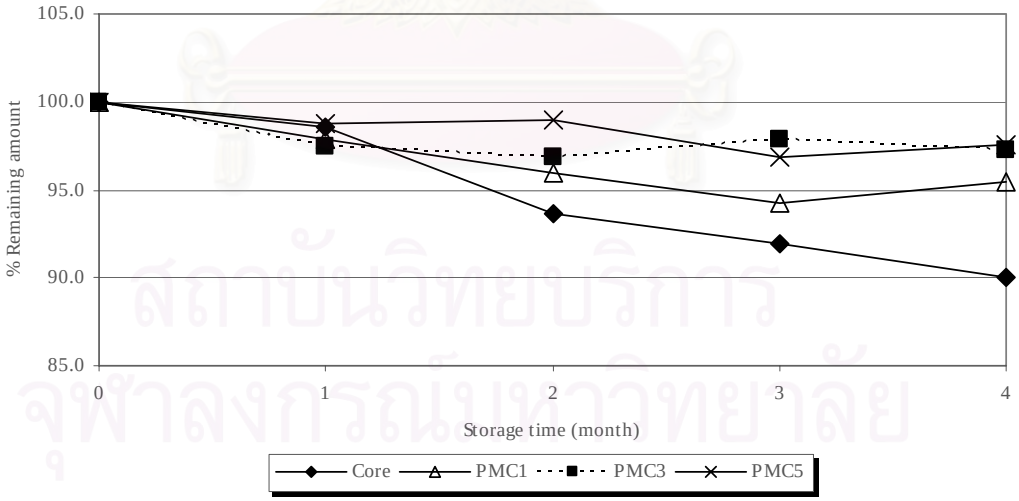



**Figure 52** Residual total percent content in *Centella asiatica* tablets coated with polymethacrylate after storage at accelerated condition

Zero-order kinetics was determined by using the correlation coefficient ($R^2$) of the relation between the storage periods versus the percent remaining amount of the total active constituents from CST. Whereas first-order kinetics was determined by using the correlation coefficient ($R^2$) of the relation between the storage periods versus the natural logarithm of the percent remaining amount of the total active constituents from CST. This study found that kinetics of deterioration of film coated tablet could not be designated due to the fact that there was a slight difference between the values of $R^2$ of both order. It must be underlined that the studied period was short and numbers of sample was small; this might affect the evaluation of data (Table 65 in Appendix C). However, the finding from a previous study by Inamdar et al. (1996) which reported that their creams and tablets deteriorating according to first-order kinetics. It was possible due to many different factors such as the quality of raw material, formulations, storage conditions and periods.

Comparison of the stability at accelerated condition of CST powder and its core tablets showed that the total percent remaining are similar (Figure 53). In this study, the coated films could improve the stability of *Centella asiatica* core tablet. At the same coating level, the stability of the coated tablets could be ranked: polymethacrylate > chitosan ≅ HPMC. As the coating level was increased, thicker film was obtained. Consequently, more stable was the tablet. Interestingly, Felton and McGinity (1999) reported that when tributyl citrate was incorporated into the coating formulation, no significant differences in the adhesion properties of the acrylic film were found while the film containing other hydrophobic plasticizer showed weaker adhesion. Loss of adhesion may lead to significant affect the stability of drug mechanism. For chitosan citrate formulations, although the highest coating levels resulted in the highest percent remaining of drug contents, whereas the contents were not clearly different at the lower coating level. Centellase® tablets were stable at both conditions as well. Because of the small amount of the commercial tablets, assay preparations were prepared from the pool of only two tablets so it may not be a good random representative and follow the general assay method. Moreover, the residual percent content of these results was not decreased as the function of time that may be from the experimental error.

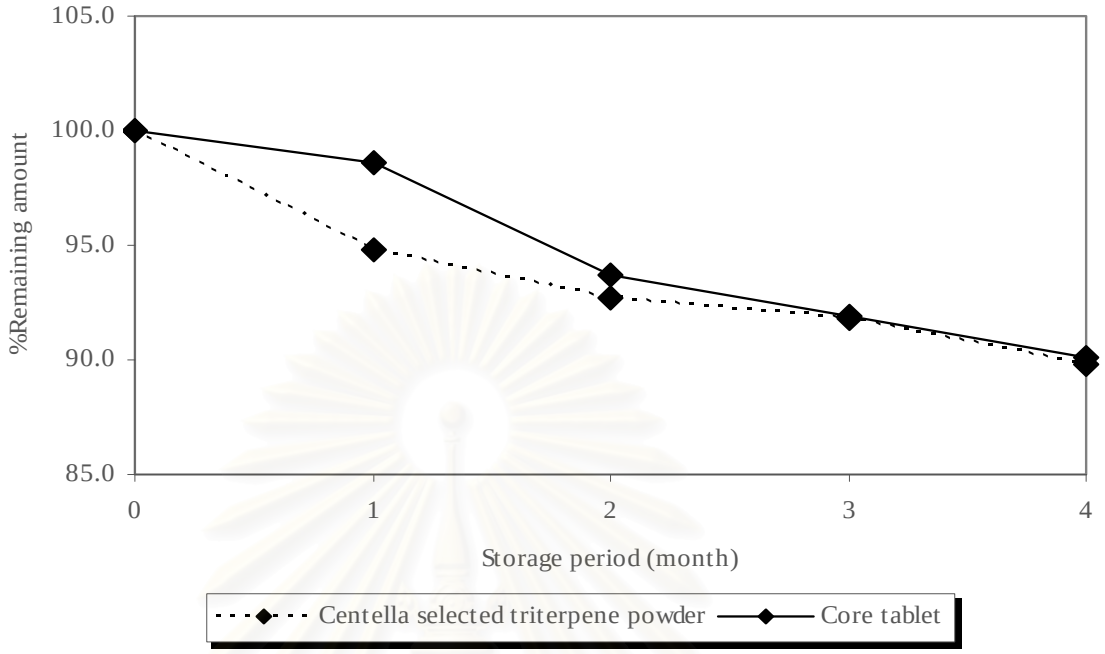



**Figure 53** Residual total percent content in *Centella asiatica* core tablets and CST powder after storage at accelerated condition

## 7. Scale-up

Due to the limitation of the instrument the scale-up process in this study may be not much difference in production volume from laboratory scale.

### 7.1 Scale-up production

Comparison of the coating process between a batch size of 1.5 and 3.0 kilograms of tablets, some parameters have to be adjusted. At 5% coating level of three types of polymer was chosen for the scale-up study of the coating process. The coating process of HPMC for a batch size of 3.0 kilograms was unchanged from a batch size of 1.5 kilograms of tablets. On the other hand, the pan speed should be decreasingly adjusted from 8 to 5 rpm for both chitosan citrate and polymethacrylate coating process for 3 kilograms batch size because the tablet could slide better than a

smaller batch size. Tackiness problem was still found, so the process had to be intermittent. Accordingly, it also consumed much time for a batch of coating process.

### 7.2 Evaluation of the tablets

From physical characteristics, the appearances of the tablets were still alike the small batch size (Figure 54). The core tablets and tablets coated with HPMC and polymethacrylate were white and glossy while the tablets coated with chitosan were slightly yellowish.

The evaluations of the tablets from scale-up production were shown in Figure 54-56. The friability, hardness, average weight and disintegration of all formulations were evaluated.

#### 7.2.1 Friability

The percentage of friability of core tablets from scale-up production was 0.13% while all of the coated tablet formulations were not friable. However, the friability value of tablet coated with chitosan citrate was not negative.

#### 7.2.2 Hardness

Figure 55 displays the hardness of the tablet formulations between small batch size and scale-up batch size. The mean and standard deviation of the hardness of core, tablet coated with chitosan, HPMC and polymethacrylate were 4.73 $\pm$ 0.9, 10.35 $\pm$ 1.05, 10.46 $\pm$ 1.03 and 5.14 $\pm$ 0.99, respectively. The statistical t-test values for the mean differences were determined at 95% confidences interval. No statistically significant difference in hardness between small scale batch and scale-up batch were determined ($P > 0.05$).

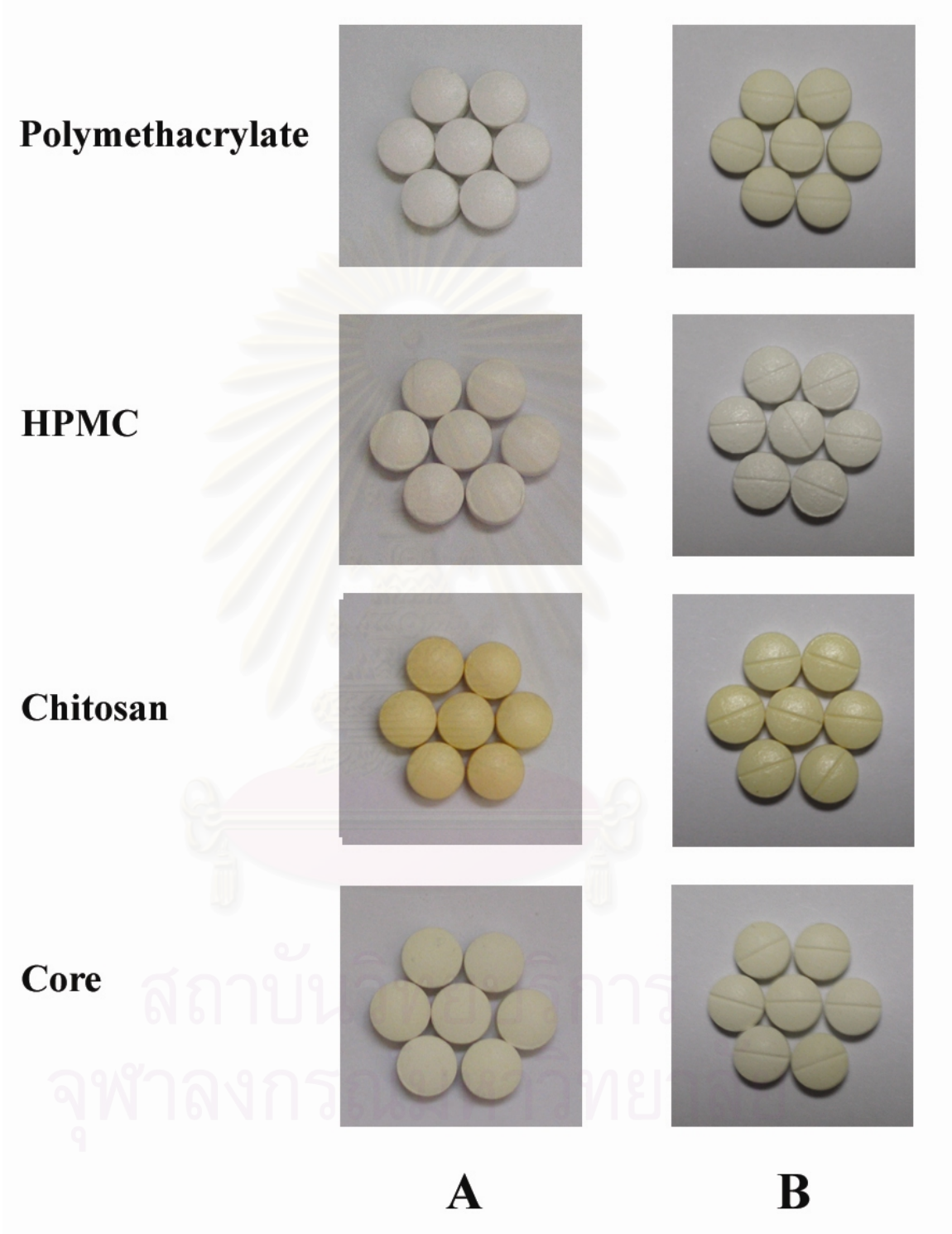



**Figure 54** The appearance of core and film coated tablets ( A = small batch size, B = scale-up batch size)

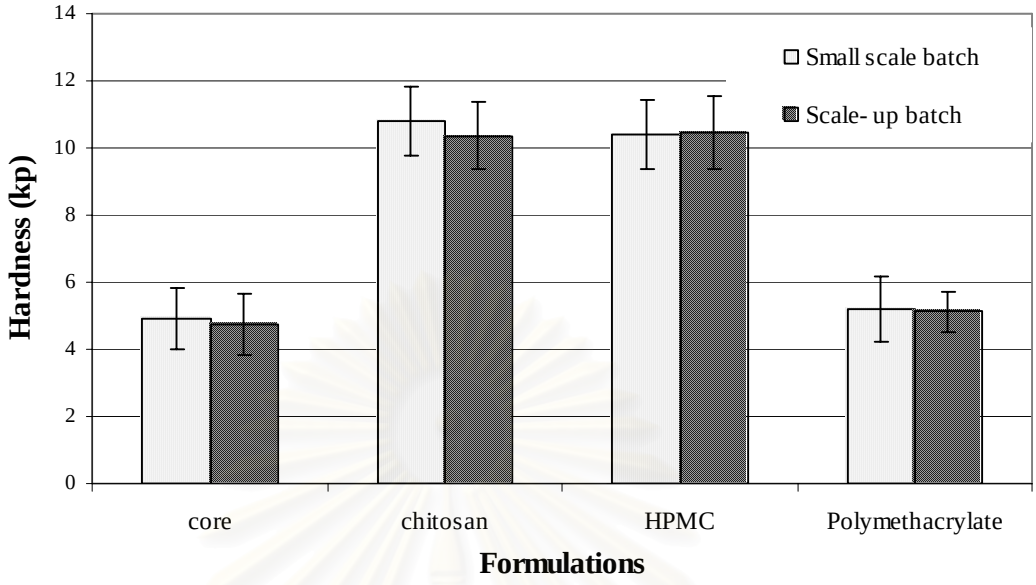



**Figure 55** The hardness of core tablets and film coated tablets formulations from small scale batch and scale-up batch

### 7.2.3 Weight variation

Figure 56 displays weight variation of the tablet formulations between small batch size and scale-up batch size. The mean and standard deviation of the average weight of core, tablet coated with chitosan, HPMC and polymethacrylate were 148.7 $\pm$ 3.3, 162.2 $\pm$ 2.7, 161.5 $\pm$ 4.1 and 160.4 $\pm$ 2.1, respectively. There was no significant difference between small scale batch and scale-up batch ($P > 0.05$).

### 7.2.4 Disintegration

The comparison of disintegration time of the tablet formulations between small batch size and scale-up batch size were showed in Figure 57. The disintegration of core, tablets coated with chitosan, HPMC and polymethacrylate were 0.50, 2.80, 2.83 and 3.17 minutes, respectively. It was showed that the disintegration time of the scale-up batch were nearly to the small scale batch.

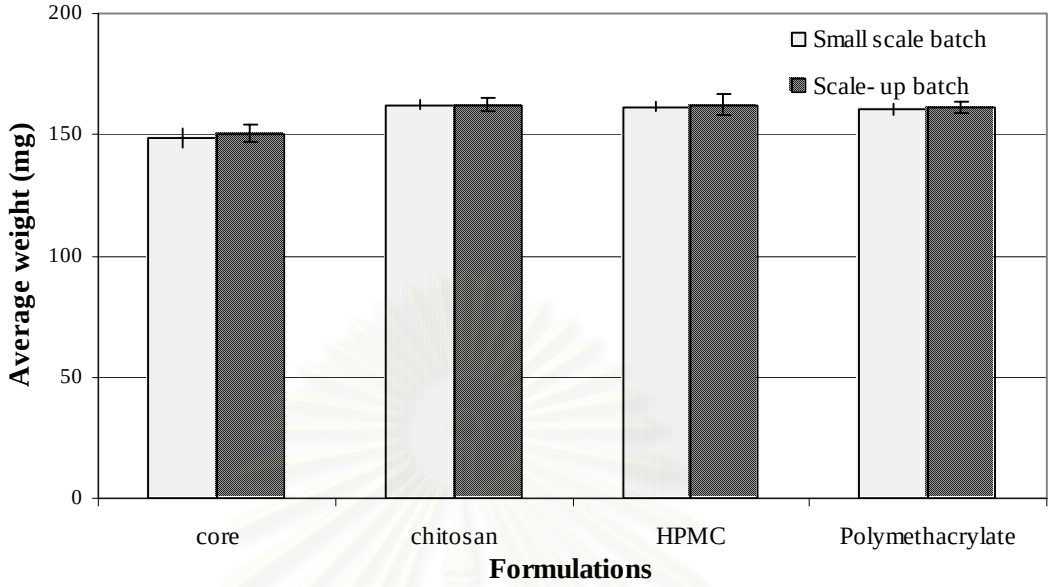



**Figure 56** The average weight of core tablets and film coated tablets formulations from small scale batch and scale-up batch

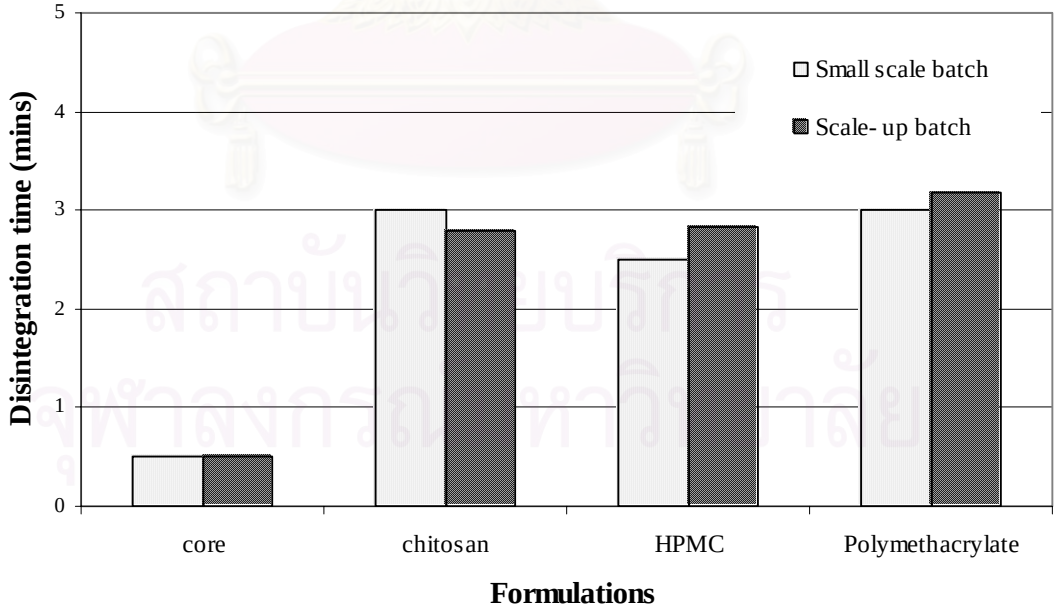



**Figure 57** The disintegration of core tablets and film coated tablets formulations from small scale batch and scale-up batch

In conclusion, the scale-up production of *Centella asiatica* tablets up to 3 kilograms of core tablets and 3 kilograms of coating tablet formulation were satisfactory. The evaluation results of the produced tablets as the physical appearance, friability, hardness, weight variation and disintegration were alike the tablets produced from smaller batch. However, this scale-up process had a limitation of the instrument so it could enlarge the batch size within the limitation of the instrument.

# CHAPTER V
# CONCLUSIONS

The results of this study were concluded as followed:

1. *Centella asiatica* extract particles were in various shapes with a wide size distribution. Thus, they showed the poor flow property.

2. *Centella asiatica* extract core tablets could be made by using direct compression method in the controlled humidity chamber. The core tablets had a good appearance and meet the requirement of the pharmacopoeia.

3. Regarding the coating process, the tackiness problem was found with chitosan and polymethacrylate film coating formulations. To overcome such the problem, the spraying rate was adjusted downward or even if the spaying 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 yellowish and glossy. After storage, the degree of yellow color of chitosan citrate film coated tablet 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 conditions of the storage.

5. Chitosan, HPMC and polymethacrylate solutions could be cast 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: polymathacrylate > chitosan > HPMC. The percent moisture sorption of cast films could be ranked as: chitosan > HPMC > polymethacrylate.

6. Evaluation data of core and coated tablets showed that

6.1 The coated tablets showed the improving of the friability of core tablets.

6.2 The hardness of coated tablets with chitosan and HPMC were higher than 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 agreed with the specification.

6.4 The assay contents were in the range of 86.13-95.03 %label amount.

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

6.6 The drug release (dissolution) of the prepared tablets could be test in the acid alcoholic medium (0.1N hydrochloric acid: isopropyl alcohol, 70: 30) for 30 minutes. The drug release from tablets coated with chitosan and high coating level of HPMC were significantly decreased after the storage. On the other hand, the commercial *Centella asiatica* tablet were slightly disintegrated and dissolved in this dissolution medium.

7. The film coated tablet formulations were stable under both ambient and accelerated condition for 4 months of storage.

8. The scale-up production of the direct compression tableting and coating process could be produced. For coating process the pan speed should be decreasingly adjusted to make satisfactory tablets.

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# APPENDICES

# APPENDICES

## APPENDIX A

**Table 12** Calibration data of AS in methanol solution at 210 nm.

| Concentration (µg/ml) | Area |
|---|---|
| 91.74 | 361956 |
| 183.48 | 699037 |
| 275.22 | 1049736 |
| 366.96 | 1370633 |
| 458.70 | 1698992 |
| 917.40 | 3394922 |

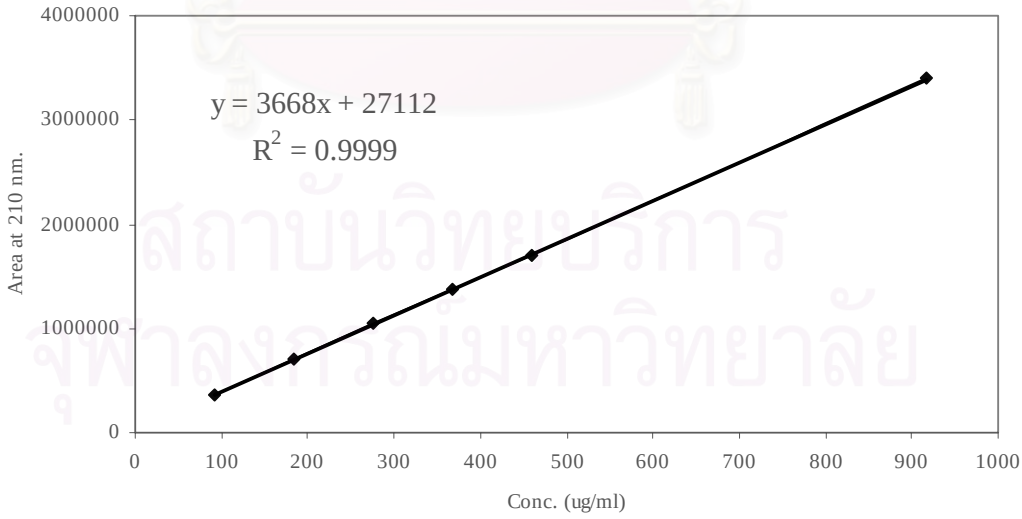


**Figure 58** Calibration curve of AS in methanol at 210 nm.

**Table 13** Calibration data of MA in methanol solution at 210 nm.

| Concentration (µg/ml) | Area |
|---|---|
| 85.62 | 708591 |
| 171.24 | 1430275 |
| 256.86 | 2169308 |
| 342.49 | 2831174 |
| 428.11 | 3508504 |
| 856.21 | 7029908 |

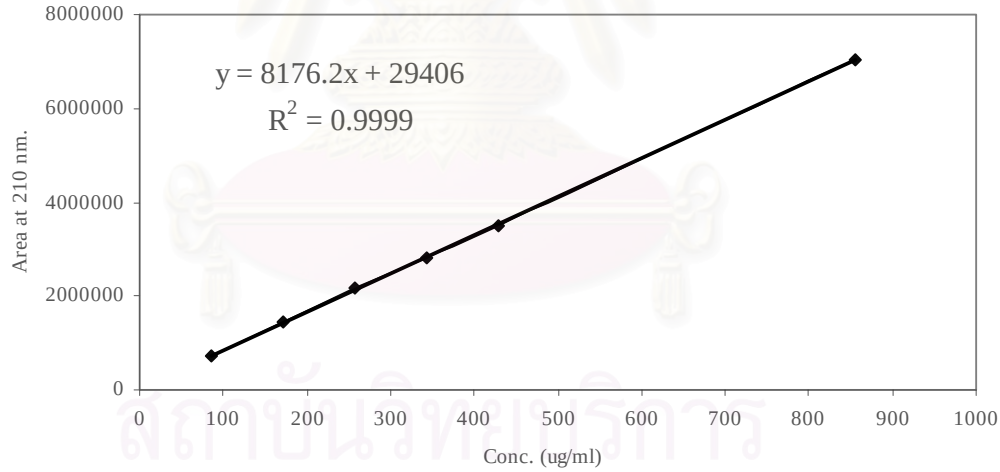


**Figure 59** Calibration curve of MA in methanol at 210 nm

**Table 14** Calibration data of AA in methanol solution at 210 nm.

| Concentration (µg/ml) | Area |
|---|---|
| 90.89 | 852594 |
| 181.79 | 1643434 |
| 272.68 | 2489892 |
| 363.58 | 3238914 |
| 454.47 | 4003718 |
| 908.94 | 7912346 |

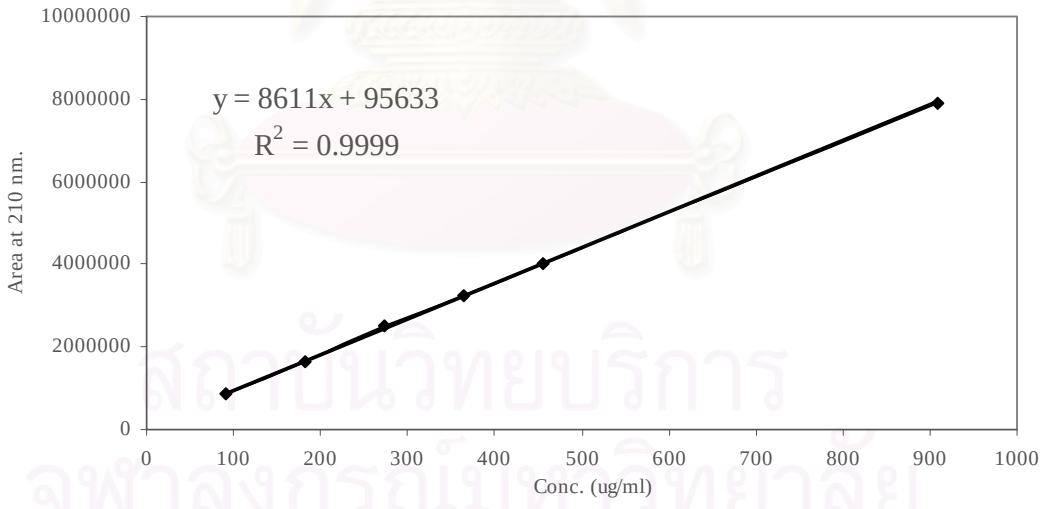


**Figure 60** Calibration curve of AA in methanol at 210 nm

**Table 15** Data of precision of AS

| Number | Area at 210 nm. | | |
|---|---|---|---|
| | 1st day | 2nd day | 3rd day |
| 1 | 1392967 | 1388666 | 1507518 |
| 2 | 1398169 | 1423734 | 1505417 |
| 3 | 1406243 | 1407506 | 1500812 |
| 4 | 1414163 | 1425119 | 1498274 |
| 5 | 1458465 | 1420729 | 1496381 |
| 6 | 1434096 | 1418914 | 1498448 |
| Average | 1417351 | 1414111 | 1501142 |
| %CV | 1.75 | 0.99 | 0.29 |

**Table 16** Data of precision of MA

| Number | Area at 210 nm. | | |
|---|---|---|---|
| | 1st day | 2nd day | 3rd day |
| 1 | 3122957 | 2856499 | 3376184 |
| 2 | 3137698 | 2936913 | 3376339 |
| 3 | 3146183 | 2930789 | 3376484 |
| 4 | 3129579 | 2930794 | 3366908 |
| 5 | 3196003 | 2919977 | 3360022 |
| 6 | 3119598 | 2916799 | 3363288 |
| Average | 3142003 | 2915295 | 3369871 |
| %CV | 0.9 | 1.02 | 0.20 |

**Table 17** Data of precision of AA

| Number | Area at 210 nm. | | |
|---|---|---|---|
| | 1st day | 2nd day | 3rd day |
| 1 | 3454671 | 3309067 | 3581290 |
| 2 | 3458011 | 3401417 | 3559107 |
| 3 | 3480108 | 3397859 | 3587210 |
| 4 | 3457816 | 3394156 | 3625690 |
| 5 | 3519736 | 3380455 | 3625410 |
| 6 | 3435431 | 3375028 | 3630671 |
| Average | 3467629 | 3376330 | 3601563 |
| %CV | 0.84 | 1.02 | 0.75 |

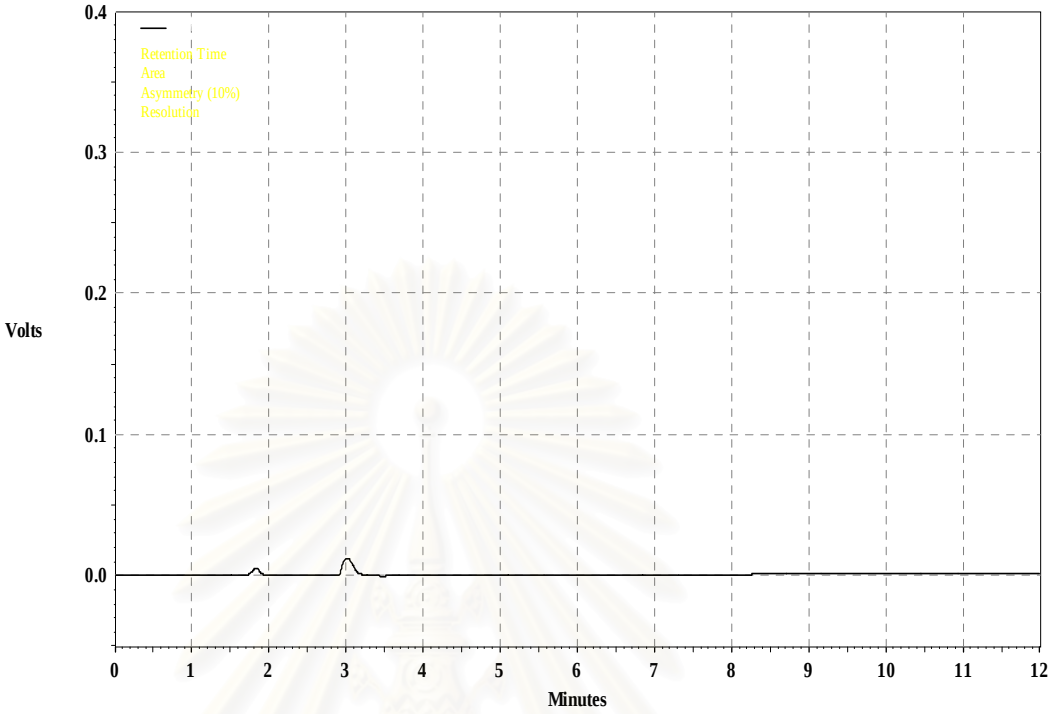


**Figure 61** HPLC chromatogram of placebo solution (sample bank solution) in methanol

**Table 18** The percentage of recovery of AS

| Analytical concentration (mg/ml) | % Recovery of AS | | | Mean | SD |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 200 | 102.8 | 98.4 | 99.3 | 100.2 | 2.3 |
| 300 | 98.3 | 99.0 | 98.9 | 98.7 | 0.4 |
| 400 | 101.3 | 99.1 | 99.3 | 99.9 | 1.2 |
| 500 | 99.8 | 100.1 | 99.4 | 99.8 | 0.4 |
| 600 | 99.0 | 99.5 | 100.3 | 99.6 | 0.7 |

**Table 19** The percentage of recovery of MA

| Analytical concentration (mg/ml) | %Recovery of MA | | | Mean | SD |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 200 | 101.9 | 98.2 | 98.3 | 99.5 | 2.1 |
| 300 | 98.2 | 98.9 | 98.3 | 98.5 | 0.4 |
| 400 | 100.7 | 98.8 | 98.4 | 99.3 | 1.2 |
| 500 | 99.7 | 99.3 | 98.1 | 99.0 | 0.8 |
| 600 | 98.2 | 98.3 | 98.7 | 98.4 | 0.3 |

**Table 20** The percentage of recovery of AA

| Analytical concentration (mg/ml) | %Recovery of AA | | | Mean | SD |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 200 | 102.3 | 98.0 | 98.2 | 99.5 | 2.4 |
| 300 | 98.0 | 98.8 | 97.9 | 98.2 | 0.5 |
| 400 | 101.3 | 98.9 | 98.3 | 99.5 | 1.6 |
| 500 | 100.1 | 99.9 | 98.4 | 99.5 | 0.9 |
| 600 | 98.8 | 99.3 | 99.5 | 99.2 | 0.4 |

# APPENDIX B

**Table 21** Bulk density, tap density and Carr's compressibility index

| Sample No. | Mearsurement values | | |
|---|---|---|---|
| | Bulk Density | Tap Density | Carr's Index |
| 1 | 0.4 | 0.55 | 27.27 |
| 2 | 0.39 | 0.56 | 30.35 |
| 3 | 0.4 | 0.55 | 27.27 |
| Average | 0.4 | 0.55 | 28.74 |
| SD | 0.01 | 0.01 | 1.55 |

**Table 22** Particle size distribution

| Mesh No. | Aperture (mm) | Particle size (mm) | Particle size distribution (%) |
|---|---|---|---|
| - | - | <0.063 | 2.20 |
| 230 | 0.063 | 0.063-0.075 | 9.99 |
| 200 | 0.075 | 0.075-0.090 | 13.37 |
| 170 | 0.090 | 0.090-0.106 | 11.58 |
| 140 | 0.100 | 0.106-0.125 | 22.04 |
| 120 | 0.125 | 0.125-0.150 | 12.98 |
| 100 | 0.150 | 0.150-0.180 | 10.5 |
| 80 | 0.180 | 0.180-0.212 | 3.83 |
| 70 | 0.212 | 0.212-0.250 | 3.99 |
| 60 | 0.250 | 0.250-0.300 | 3.91 |
| 50 | 0.300 | 0.300-0.425 | 1.74 |
| 40 | 0.425 | 0.425-0.500 | 1.75 |
| 35 | 0.500 | 0.500-0.600 | 1.11 |
| 30 | 0.600 | >0.600 | 1.01 |

**Table 23** Solubility data of AS from CST in water

| Time (hrs.) | Solubility (mg/ml) | | | Average | SD | %CV |
|---|---|---|---|---|---|---|
| | A | B | C | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.3 | 0.018 | 0.013 | 0.017 | 0.016 | 0.002 | 15.002 |
| 1 | 0.030 | 0.027 | 0.030 | 0.029 | 0.002 | 6.494 |
| 2 | 0.037 | 0.036 | 0.039 | 0.037 | 0.001 | 3.809 |
| 4 | 0.039 | 0.039 | 0.041 | 0.040 | 0.001 | 2.750 |
| 6 | 0.040 | 0.040 | 0.041 | 0.040 | 0.001 | 1.310 |
| 8 | 0.040 | 0.040 | 0.042 | 0.041 | 0.001 | 1.898 |
| 12 | 0.039 | 0.040 | 0.041 | 0.040 | 0.001 | 2.241 |
| 24 | 0.039 | 0.040 | 0.040 | 0.040 | 0.000 | 1.063 |

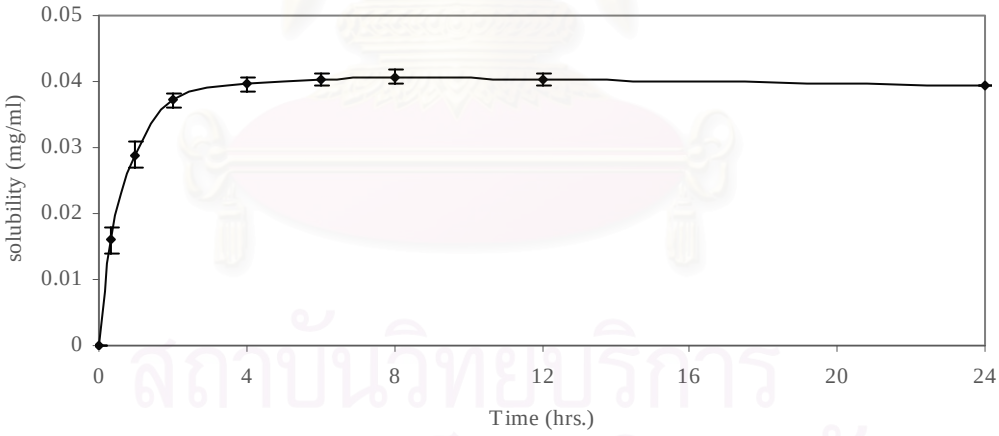


**Figure 62** Solubility profile of AS from CST in water

**Table 24** Solubility data of AS from CST in the mixture of 0.1N hydrochloric acid : isopropyl alcohol (70:30)

| Time (hrs.) | Solubility (mg/ml) | | | Average | SD | %CV |
|---|---|---|---|---|---|---|
| | A | B | C | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0.236 | 0.235 | 0.233 | 0.235 | 0.002 | 0.651 |
| 2 | 0.234 | 0.242 | 0.239 | 0.238 | 0.004 | 1.696 |
| 4 | 0.242 | 0.243 | 0.241 | 0.242 | 0.001 | 0.413 |
| 6 | 0.242 | 0.245 | 0.243 | 0.243 | 0.002 | 0.628 |
| 12 | 0.249 | 0.249 | 0.247 | 0.248 | 0.001 | 0.465 |
| 24 | 0.242 | 0.243 | 0.242 | 0.242 | 0.001 | 0.238 |

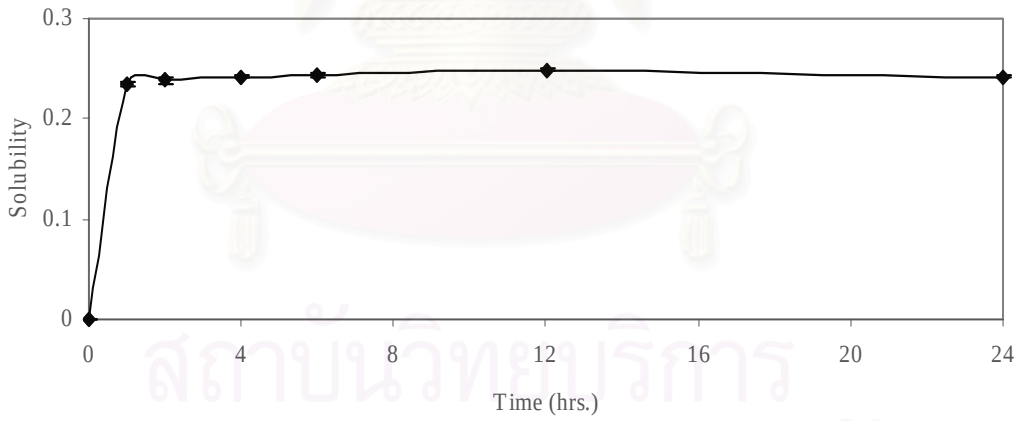


**Figure 63** Solubility profile of AS from CST in the mixture of 0.1N hydrochloric acid : isopropyl alcohol (70:30)

**Table 25** Solubility data of MA from CST in the mixture of 0.1N hydrochloric acid : isopropyl alcohol (70:30)

| Time (hrs.) | Solubility (mg/ml) | | | Average | SD | %CV |
|---|---|---|---|---|---|---|
| | A | B | C | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0.032 | 0.032 | 0.033 | 0.032 | 0.001 | 1.786 |
| 2 | 0.033 | 0.035 | 0.033 | 0.034 | 0.001 | 3.430 |
| 4 | 0.033 | 0.033 | 0.032 | 0.033 | 0.001 | 1.767 |
| 6 | 0.034 | 0.033 | 0.034 | 0.034 | 0.001 | 1.715 |
| 12 | 0.032 | 0.031 | 0.031 | 0.031 | 0.001 | 1.843 |
| 24 | 0.031 | 0.029 | 0.029 | 0.030 | 0.001 | 3.892 |

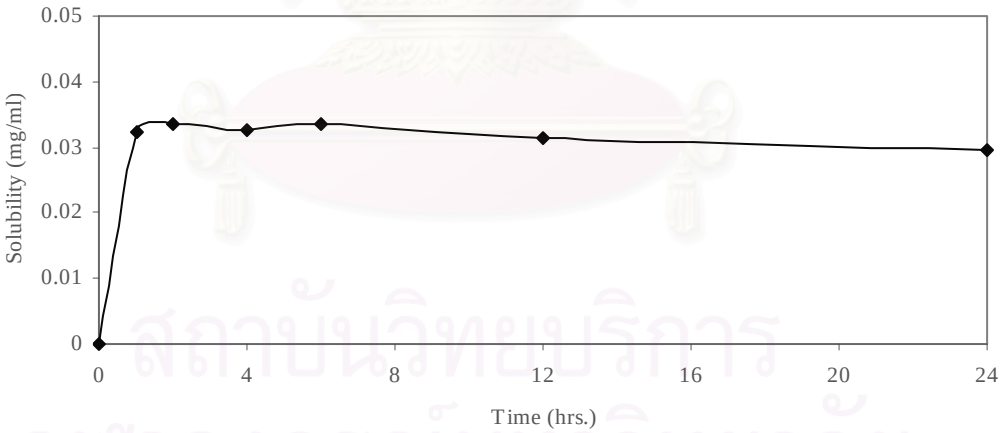


**Figure 64** Solubility profile of MA from CST in the mixture of 0.1N hydrochloric acid : isopropyl alcohol (70:30)

**Table 26** Solubility data of AA from CST in the mixture of 0.1N hydrochloric acid : isopropyl alcohol (70:30)

| Time (hrs.) | Solubility (mg/ml) | | | Average | SD | %CV |
|---|---|---|---|---|---|---|
| | A | B | C | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0.012 | 0.013 | 0.013 | 0.013 | 0.000 | 2.008 |
| 2 | 0.013 | 0.014 | 0.013 | 0.013 | 0.001 | 3.907 |
| 4 | 0.013 | 0.013 | 0.013 | 0.013 | 0.000 | 2.460 |
| 6 | 0.014 | 0.013 | 0.014 | 0.014 | 0.000 | 1.534 |
| 12 | 0.013 | 0.012 | 0.011 | 0.012 | 0.001 | 5.911 |
| 24 | 0.013 | 0.012 | 0.012 | 0.012 | 0.000 | 3.828 |

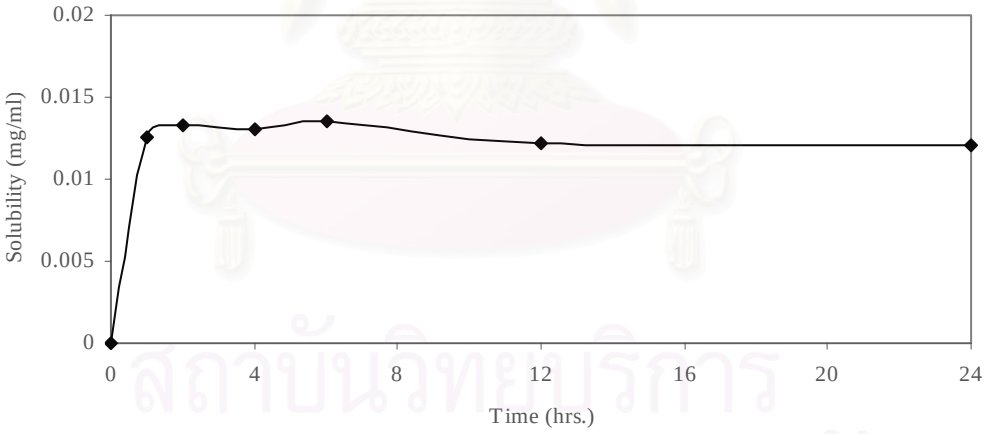


**Figure 65** Solubility profile of AA from CST in the mixture of 0.1N hydrochloric acid : isopropyl alcohol (70:30)

**Table 27** The data of remaining amount (%w/w) of active constituents from CST after exposure to the stress conditions

| Stress tests | Remaining amount (%w/w) compared with the initial amount | | | | | |
|---|---|---|---|---|---|---|
| | AS | MA | AA | CST | | |
| | | | | AS | MA | AA |
| Moisture hydrolysis | | | | | | |
| method I | 98.3 | 99.6 | 101.8 | 100.2 | 100.2 | 98.6 |
| method II | 106.9 | 99.3 | 96.1 | 96.1 | 96.0 | 96.7 |
| Acid hydrolysis | 0.0 | 95.5 | 99.3 | 0.0 | 81.5 | 216.8 |
| Alkaline hydrolysis | 0.0 | 75.9 | 102.0 | 0.0 | 87.8 | 208.9 |
| Temperature | 96.7 | 102.0 | 101.6 | 102.8 | 103.2 | 101.9 |
| Oxidation | 78.1 | 70.9 | 63.8 | 56.5 | 72.2 | 69.2 |
| Photolysis | | | | | | |
| light | 99.9 | 96.1 | 100.3 | 96.2 | 94.6 | 94.5 |
| dark | 98.9 | 98.1 | 100.8 | 95.9 | 95.0 | 94.8 |

**Table 28** The % degradation of AS, MA and AA after storage CST with additives for 4 months at 45°C, 75% RH

| Storage period (month) | %recovery of AS (%w/w) | % degradation of AS | %recovery of MA (%w/w) | % degradation of MA | %recovery of AA (%w/w) | % degradation of AA | %recovery Total (%w/w) | % degradation Total | Total %remaining amount |
|---|---|---|---|---|---|---|---|---|---|
| CST | | | | | | | | | |
| Initial | 37.33 | 0.00 | 33.72 | 0.00 | 18.52 | 0.00 | 89.57 | 0.00 | 100.00 |
| 1 | 37.20 | 0.35 | 31.14 | 7.65 | 16.53 | 10.75 | 84.87 | 5.25 | 94.75 |
| 2 | 36.75 | 1.55 | 30.06 | 10.85 | 16.18 | 12.63 | 82.99 | 7.35 | 92.65 |
| 4 | 36.39 | 2.52 | 29.85 | 11.48 | 15.98 | 13.71 | 82.22 | 8.21 | 91.79 |
| 3 | 35.68 | 4.42 | 29.08 | 13.76 | 15.71 | 15.17 | 80.47 | 10.16 | 89.84 |
| CST + Supertab® | | | | | | | | | |
| Initial | 1.61 | 0.00 | 1.40 | 0.00 | 0.74 | 0.00 | 3.75 | 0.00 | 100.00 |
| 1 | 1.61 | 0.25 | 1.38 | 1.71 | 0.75 | -1.09 | 3.73 | 0.53 | 99.47 |
| 2 | 1.52 | 5.34 | 1.32 | 5.50 | 0.72 | 1.76 | 3.57 | 4.70 | 95.81 |
| 3 | 1.56 | 3.24 | 1.37 | 2.09 | 0.72 | 1.72 | 3.65 | 2.51 | 97.49 |
| 4 | 1.51 | 6.50 | 1.30 | 7.06 | 0.70 | 4.40 | 3.51 | 6.29 | 93.71 |
| CST + Starch 1500® | | | | | | | | | |
| Initial | 1.95 | 0.00 | 1.68 | 0.00 | 0.92 | 0.00 | 4.55 | 0.00 | 100.00 |
| 1 | 1.81 | 7.32 | 1.54 | 8.28 | 0.85 | 7.31 | 4.20 | 7.67 | 92.33 |
| 2 | 1.69 | 13.47 | 1.44 | 14.23 | 0.78 | 14.94 | 3.91 | 14.05 | 85.95 |
| 3 | 1.76 | 9.88 | 1.49 | 11.26 | 0.80 | 12.76 | 4.05 | 10.97 | 89.03 |
| 4 | 1.76 | 9.88 | 1.50 | 10.66 | 0.80 | 12.76 | 4.06 | 10.75 | 89.25 |

**Table 28** The % degradation of AS, MA and AA after storage CST with additives for 4 months at 45°C, 75% RH (cont.)

| Storage period (month) | %recovery of AS (%w/w) | % degradation of AS | %recovery of MA (%w/w) | % degradation of MA | %recovery of AA (%w/w) | % degradation of AA | %recovery Total (%w/w) | % degradation Total | Total %remaining amount |
|---|---|---|---|---|---|---|---|---|---|
| CST + Magnesium stearate | | | | | | | | | |
| Initial | 18.63 | 0.00 | 16.1 | 0.00 | 8.698 | 0.00 | 43.43 | 0.00 | 100.00 |
| 1 | 18.31 | 1.72 | 15.6 | 2.98 | 8.72 | -0.25 | 42.65 | 1.79 | 98.21 |
| 2 | 18.46 | 0.91 | 15.5 | 3.60 | 8.17 | 6.07 | 42.15 | 2.94 | 97.06 |
| 3 | 18.3 | 1.77 | 15.4 | 4.41 | 8.23 | 5.38 | 41.92 | 3.47 | 96.53 |
| 4 | 17.49 | 6.12 | 14.8 | 7.89 | 8.02 | 7.79 | 40.34 | 7.11 | 92.89 |
| CST + Aerosil® | | | | | | | | | |
| Initial | 18.02 | 0.00 | 15.51 | 0.00 | 8.5 | 0.00 | 42.03 | 0.00 | 100.00 |
| 1 | 17.03 | 5.49 | 14.84 | 4.32 | 7.86 | 7.53 | 39.73 | 5.47 | 94.53 |
| 2 | 16.62 | 7.77 | 14.44 | 6.90 | 7.79 | 8.35 | 38.85 | 7.57 | 92.43 |
| 3 | 16.38 | 9.10 | 14.15 | 8.77 | 7.65 | 10.00 | 38.18 | 9.16 | 90.84 |
| 4 | 16.35 | 9.27 | 13.95 | 10.06 | 7.49 | 11.88 | 37.79 | 10.09 | 89.91 |
| CST + Explotab® | | | | | | | | | |
| Initial | 17.86 | 0.00 | 15.55 | 0.00 | 8.51 | 0.00 | 41.91 | 0.00 | 100.00 |
| 1 | 16.10 | 9.84 | 13.60 | 12.51 | 7.17 | 15.74 | 36.87 | 12.03 | 87.97 |
| 2 | 15.60 | 12.64 | 13.10 | 15.73 | 7.12 | 16.32 | 35.82 | 14.53 | 85.47 |
| 3 | 15.40 | 13.76 | 13.60 | 12.53 | 7.11 | 16.44 | 36.11 | 13.85 | 86.15 |
| 4 | 16.25 | 9.01 | 13.57 | 12.72 | 7.26 | 14.65 | 37.08 | 11.53 | 88.47 |

**Table 28** The % degradation of AS, MA and AA after storage CST with additives for 4 months at 45°C, 75% RH (cont.)

| Storage period (month) | %recovery of AS (%w/w) | % degradation of AS | %recovery MA (%w/w) | % degradation of MA | %recovery AA (%w/w) | % degradation of AA | %recovery Total (%w/w) | % degradation Total | Total %remaining amount |
|---|---|---|---|---|---|---|---|---|---|
| CST + talcum | | | | | | | | | |
| Initial | 18.50 | 0.00 | 16.23 | 0.00 | 8.89 | 0.00 | 43.61 | 0.00 | 100.00 |
| 1 | 18.01 | 2.65 | 15.44 | 4.86 | 8.15 | 8.25 | 41.60 | 4.61 | 95.39 |
| 2 | 18.41 | 0.48 | 15.46 | 4.71 | 8.26 | 7.03 | 42.13 | 3.39 | 96.61 |
| 3 | 17.61 | 4.81 | 15.19 | 6.38 | 7.87 | 11.42 | 40.67 | 6.74 | 93.26 |
| 4 | 17.63 | 4.70 | 15.27 | 5.89 | 8.18 | 7.93 | 41.08 | 5.80 | 94.20 |

# APPENDIX C

**Table 29** Flow rate and angle of repose of the core tablet formulations

| Formulations | Flow rate (g/sec) [a] | Angle of repose (°) [a] |
|---|---|---|
| F1 | 1.06 ± 2.16 | 23.3 ± 12.0 |
| F2 | 1.79 ± 1.35 | 25.0 ± 12.5 |
| F3 | 2.11 ± 0.90 | 20.0 ± 5.0 |
| F4 | 2.19 ± 0.40 | 19.3 ± 1.2 |
| F5 | 2.01 ± 0.67 | 20.0 ± 0.0 |
| F6 | 4.28 ± 0.17 | 16.7 ± 2.9 |
| F7 | 4.17 ± 0.23 | 18.3 ± 2.9 |

[a] All values were mean ± SD of three samples.

**Table 30** Tensile strength and elongation at break of cast films

| Film Formulations | Tensile Strength ($N/mm^2$) [a] | Elongation at Break (%) [a] |
|---|---|---|
| HPMC | 17.41 ± 2.89 | 6.33 ± 1.92 |
| Chitosan | 6.86 ± 0.87 | 170.09 ± 14.16 |
| Polymethacrylate | 0.78 ± 0.39 | 179.38 ± 58.73 |

[a] All values were mean ± SD of five samples

**Table 31** The percentage of moisture sorption of the cast film

| Number of test | % Moisture sorption | | |
|---|---|---|---|
| | Chitosan | HPMC | Polymethacrylate |
| 1 | 8.5003 | 2.1794 | 0.2530 |
| 2 | 10.3020 | 1.4787 | 0.3422 |
| 3 | 9.6921 | 3.9477 | 0.6783 |
| Average | 9.5172 | 2.5454 | 0.4244 |
| S.D. | 0.9164 | 1.2724 | 0.2243 |

**Table 32** The percentage of friability of core tablet and tablet coated with chitosan citrate 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 | 2.976 | 2.962 | 0.470 |
| | 3 | 3.086 | 3.086 | 0.000 |
| | 5 | 3.230 | 3.232 | -0.060 |
| | 10 | 3.336 | 3.337 | -0.030 |
| B | core tablet | 3.012 | 2.992 | 0.664 |
| | 3 | 3.076 | 3.076 | 0.000 |
| | 5 | 3.222 | 3.222 | 0.000 |
| | 10 | 3.340 | 3.340 | 0.000 |

**Table 33** The percentage of friability of 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 | 3 | 3.068 | 3.068 | 0.000 |
| | 5 | 3.210 | 3.210 | 0.000 |
| | 10 | 3.290 | 3.290 | 0.000 |
| | 15 | 3.528 | 3.528 | 0.000 |
| B | 3 | 3.060 | 3.060 | 0.000 |
| | 5 | 3.222 | 3.222 | 0.000 |
| | 10 | 3.304 | 3.304 | 0.000 |
| | 15 | 3.526 | 3.526 | 0.000 |

**Table 34** 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 | 1 | 3.014 | 3.014 | 0.000 |
| | 3 | 3.066 | 3.066 | 0.000 |
| | 5 | 3.224 | 3.224 | 0.000 |
| B | 1 | 2.978 | 2.978 | 0.000 |
| | 3 | 3.084 | 3.084 | 0.000 |
| | 5 | 3.198 | 3.198 | 0.000 |

**Table 35** Hardness of core tablet and film coated tablet formulations after preparation, storage at ambient and accelerated condition

| Formulations | Hardness (kp) Mean (SD) (n=10) | | |
|---|---|---|---|
| | Initial | 4th month, RT | 4th month, AC |
| Core | 4.9 (0.9) | 3.9 (0.4) | 3.8 (0.8) |
| CS3 | 7.2 (1.5) | 8.0 (1.0) | 6.6 (1.2) |
| CS5 | 10.4 (1.1) | 11.0 (1.2) | 8.4 (1.3) |
| CS10 | >20 | >20 | 13.3 (2.3) |
| HPMC3 | 7.8 (1.4) | 7.2 (1.2) | 7.0 (0.7) |
| HPMC5 | 10.8 (1.0) | 10.5 (1.8) | 10.3 (1.1) |
| HPMC10 | 11.4 (2.5) | 10.8 (1.0) | 10.2 (1.3) |
| HPMC15 | >20 | >20 | >20 |
| PMC1 | 5.0 (0.6) | 4.8 (0.7) | 4.7 (0.6) |
| PMC3 | 5.2 (0.8) | 5.0 (0.9) | 5.2 (1.0) |
| PMC5 | 5.2 (0.6) | 5.1 (1.0) | 5.1 (1.0) |

**Table 36** 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 | | | |
| | core | 3% | 5% | 10% | core | 3% | 5% | 10% | core | 3% | 5% | 10% |
| 1 | 4.2 | 5.0 | 11.2 | 14.7 | 4.2 | 8.8 | 13.0 | 17.1 | 3.8 | 5.0 | 10.2 | 17.0 |
| 2 | 5.2 | 8.2 | 9.0 | 16.8 | 4.2 | 7.6 | 10.0 | 11.0 | 3.5 | 6.8 | 7.4 | 11.8 |
| 3 | 5.8 | 9.0 | 9.5 | >20 | 3.5 | 9.0 | 10.6 | 14.8 | 4.0 | 8.4 | 9.7 | 12.6 |
| 4 | 3.8 | 7.6 | 10.7 | >20 | 4.1 | 7.4 | 12.4 | >20 | 3.5 | 6.2 | 6.9 | 17.0 |
| 5 | 5.5 | 5.2 | 12.6 | 17.0 | 4.2 | 6.4 | 10.4 | 19.6 | 3.6 | 5.8 | 6.7 | 14.0 |
| 6 | 5.3 | 7.0 | 9.8 | 14.2 | 3.5 | 9.8 | 10.6 | 13.0 | 3.3 | 8.4 | 7.2 | 11.0 |
| 7 | 5.1 | 7.4 | 11.0 | 18.6 | 4.4 | 7.4 | 9.8 | >20 | 5.9 | 6.2 | 8.1 | 11.2 |
| 8 | 6.2 | 9.0 | 9.8 | 17.8 | 3.5 | 8.4 | 10.6 | 19.8 | 3.7 | 7.8 | 10.2 | 15.0 |
| 9 | 3.4 | 8.2 | 9.3 | >20 | 3.5 | 7.8 | 12.4 | 12.5 | 3.4 | 5.8 | 8.9 | 12.0 |
| 10 | 4.5 | 5.2 | 11.4 | 14.0 | 3.6 | 7.1 | 9.8 | >20 | 3.5 | 5.8 | 8.2 | 11.2 |
| Average | 4.9 | 7.2 | 10.4 | >20.0 | 3.9 | 8.0 | 11.0 | >20.0 | 3.8 | 6.6 | 8.4 | 13.3 |
| SD | 0.9 | 1.5 | 1.1 | - | 0.4 | 1.0 | 1.2 | - | 0.8 | 1.2 | 1.3 | 2.3 |
| %CV | 18.3 | 21.6 | 10.9 | - | 9.7 | 12.8 | 10.8 | - | 19.9 | 18.0 | 16.0 | 17.6 |

**Table 37** Hardness of tablet coated with HPMC 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% | 10% | 15% | 3% | 5% | 10% | 15% | 3% | 5% | 10% | 15% |
| 1 | 6.4 | 11.0 | 14.5 | 20.0 | 5.8 | 8.0 | 10.2 | 14.2 | 7.0 | 10.8 | 9.8 | >20 |
| 2 | 8.2 | 10.8 | 15.4 | >20 | 6.6 | 7.8 | 11.4 | 13.6 | 6.6 | 11.0 | 9.5 | 14.0 |
| 3 | 5.3 | 9.4 | 10.8 | >20 | 7.3 | 12.0 | 9.7 | 14.5 | 7.6 | 11.8 | 9.6 | 18.4 |
| 4 | 8.8 | 9.8 | 8.8 | 16.8 | 9.8 | 8.0 | 11.1 | 17.8 | 7.2 | 10.3 | 11.7 | 18.4 |
| 5 | 6.2 | 11.4 | 12.0 | 19.4 | 7.2 | 11.0 | 9.8 | >20 | 8.2 | 10.2 | 9.3 | 17.6 |
| 6 | 7.8 | 10.0 | 9.0 | >20 | 8.0 | 12.3 | 10.0 | 19.2 | 6.4 | 9.4 | 12.8 | 19.6 |
| 7 | 9.8 | 11.4 | 10.4 | 18.4 | 5.5 | 12.0 | 10.8 | 14.2 | 6.7 | 8.8 | 8.3 | 19.6 |
| 8 | 7.2 | 12.8 | 10.0 | 14.4 | 7.3 | 10.3 | 10.4 | 16.0 | 7.2 | 10.3 | 10.7 | 18.0 |
| 9 | 8.8 | 10.4 | 14.2 | 15.0 | 7.5 | 11.5 | 11.2 | 15.4 | 5.6 | 8.6 | 10.2 | 14.2 |
| 10 | 9.0 | 11.0 | 9.0 | 15.0 | 6.8 | 11.6 | 13.1 | >20 | 7.6 | 11.3 | 10.5 | 16.8 |
| Average | 7.8 | 10.8 | 11.4 | >20 | 7.2 | 10.5 | 10.8 | >20 | 7.0 | 10.3 | 10.2 | >20 |
| SD | 1.4 | 1.0 | 2.5 | - | 1.2 | 1.8 | 1.0 | - | 0.7 | 1.1 | 1.3 | - |
| %CV | 18.6 | 9.0 | 21.7 | - | 16.7 | 17.5 | 9.4 | - | 10.4 | 10.2 | 12.5 | - |

**Table 38** Hardness of 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% | 3% | 5% | 1% | 3% | 5% | 1% | 3% | 5% |
| 1 | 6.2 | 4.7 | 5.3 | 5.4 | 4.4 | 5.2 | 4.6 | 6.0 | 4.4 |
| 2 | 5.0 | 5.3 | 5.2 | 4.6 | 5.0 | 6.4 | 4.4 | 4.5 | 4.4 |
| 3 | 4.0 | 5.6 | 5.2 | 6.3 | 4.6 | 4.0 | 6.3 | 4.5 | 4.8 |
| 4 | 5.5 | 4.4 | 5.3 | 4.4 | 4.2 | 6.2 | 4.8 | 6.4 | 5.4 |
| 5 | 4.6 | 7.0 | 4.8 | 4.3 | 4.6 | 4.3 | 4.6 | 4.2 | 4.8 |
| 6 | 5.4 | 5.2 | 4.2 | 5.5 | 4.5 | 5.4 | 4.2 | 6.0 | 4.6 |
| 7 | 4.8 | 4.5 | 6.3 | 4.4 | 5.2 | 4.4 | 4.2 | 4.4 | 6.2 |
| 8 | 4.6 | 4.3 | 6.2 | 4.4 | 4.6 | 4.2 | 5.0 | 4.5 | 4.2 |
| 9 | 5.2 | 5.6 | 4.6 | 4.6 | 6.2 | 4.4 | 4.4 | 7.0 | 7.2 |
| 10 | 4.8 | 5.5 | 5.2 | 4.4 | 7.0 | 6.4 | 4.8 | 4.4 | 4.5 |
| Average | 5.0 | 5.2 | 5.2 | 4.8 | 5.0 | 5.1 | 4.7 | 5.2 | 5.1 |
| SD | 0.6 | 0.8 | 0.6 | 0.7 | 0.9 | 1.0 | 0.6 | 1.0 | 1.0 |
| %CV | 12.1 | 15.5 | 12.3 | 13.9 | 17.8 | 18.9 | 12.9 | 20.0 | 19.0 |

**Table 39** Weight variation of core tablet and tablet coated with chitosan citrate film with different coating level (A) freshly prepared and (B) after exposure to the accelerated condition for 4 months

| No. | Weight (mg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | | | B | | | |
| | Core | 3% | 5% | 10% | Core | 3% | 5% | 10% |
| 1 | 143.0 | 153.2 | 163.1 | 163.5 | 156.0 | 152.0 | 160.0 | 175.2 |
| 2 | 149.2 | 154.6 | 160.1 | 175.9 | 148.9 | 157.8 | 160.1 | 170.2 |
| 3 | 152.3 | 148.9 | 165.0 | 169.2 | 151.1 | 156.2 | 162.2 | 167.8 |
| 4 | 148.0 | 153.2 | 166.7 | 165.9 | 151.5 | 153.1 | 160.2 | 170.1 |
| 5 | 146.3 | 151.5 | 157.0 | 173.2 | 148.6 | 149.8 | 163.5 | 164.0 |
| 6 | 145.4 | 154.6 | 165.3 | 158.1 | 152.9 | 152.3 | 161.2 | 177.0 |
| 7 | 150.0 | 153.2 | 162.8 | 155.2 | 147.7 | 150.4 | 159.6 | 166.3 |
| 8 | 149.7 | 153.2 | 161.6 | 166.9 | 149.0 | 152.3 | 161.5 | 165.2 |
| 9 | 147.0 | 152.5 | 164.9 | 166.6 | 149.5 | 154.3 | 163.7 | 162.5 |
| 10 | 151.0 | 151.7 | 158.2 | 156.3 | 147.9 | 152.6 | 159.8 | 162.2 |
| 11 | 150.9 | 155.7 | 159.2 | 171.8 | 151.7 | 153.6 | 166.0 | 170.0 |
| 12 | 144.4 | 153.0 | 163.4 | 161.7 | 150.8 | 153.2 | 160.1 | 172.3 |
| 13 | 155.0 | 149.9 | 164.0 | 167.8 | 153.7 | 155.1 | 160.0 | 167.7 |
| 14 | 152.4 | 151.3 | 165.7 | 167.7 | 150.2 | 152.2 | 161.3 | 164.2 |
| 15 | 150.0 | 152.4 | 161.4 | 167.8 | 146.8 | 152.4 | 159.9 | 166.7 |
| 16 | 147.4 | 157.0 | 160.7 | 166.5 | 150.4 | 154.7 | 160.7 | 165.3 |
| 17 | 147.0 | 153.1 | 160.0 | 162.6 | 147.9 | 148.7 | 162.6 | 163.7 |
| 18 | 145.4 | 150.2 | 163.8 | 170.6 | 146.0 | 152.1 | 163.1 | 169.8 |
| 19 | 146.0 | 155.5 | 161.4 | 160.1 | 150.2 | 152.0 | 164.1 | 171.0 |
| 20 | 154.1 | 149.9 | 160.0 | 176.7 | 145.7 | 153.0 | 162.9 | 163.4 |
| Average | 148.7 | 152.7 | 162.2 | 166.2 | 149.8 | 152.9 | 161.6 | 167.7 |
| SD | 3.3 | 2.1 | 2.7 | 6.0 | 2.6 | 2.1 | 1.8 | 4.2 |
| %CV | 2.0 | 1.4 | 1.6 | 3.6 | 1.7 | 1.4 | 1.1 | 2.5 |

**Table 40** Weight variation of tablet coated with HPMC film with different coating level (A) freshly prepared and (B) after exposure to the accelerated condition for 4 months

| No. | Weight (mg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | | | B | | | |
| | 3% | 5% | 10% | 15% | 3% | 5% | 10% | 15% |
| 1 | 156.1 | 165.1 | 165.8 | 183.0 | 155.0 | 158.5 | 168.0 | 180.3 |
| 2 | 153.9 | 161.1 | 165.3 | 187.0 | 154.2 | 157.4 | 165.1 | 178.8 |
| 3 | 150.0 | 157.0 | 166.2 | 187.0 | 153.0 | 159.8 | 168.9 | 176.8 |
| 4 | 151.7 | 167.0 | 166.3 | 183.2 | 154.1 | 155.5 | 167.2 | 181.5 |
| 5 | 152.9 | 164.0 | 161.4 | 175.4 | 154.0 | 159.6 | 166.1 | 176.0 |
| 6 | 152.4 | 167.0 | 164.0 | 179.2 | 151.4 | 158.0 | 160.9 | 181.4 |
| 7 | 156.5 | 160.8 | 169.2 | 173.3 | 158.0 | 155.0 | 169.0 | 178.6 |
| 8 | 155.0 | 159.8 | 165.4 | 168.0 | 150.0 | 158.3 | 167.2 | 175.5 |
| 9 | 152.0 | 163.9 | 162.8 | 172.1 | 152.6 | 159.8 | 167.8 | 185.0 |
| 10 | 151.5 | 163.8 | 166.8 | 177.6 | 152.2 | 156.7 | 165.0 | 183.5 |
| 11 | 154.4 | 165.7 | 167.2 | 172.3 | 151.9 | 158.6 | 165.1 | 179.6 |
| 12 | 154.0 | 156.0 | 164.8 | 185.0 | 151.0 | 160.5 | 168.8 | 174.6 |
| 13 | 151.2 | 163.1 | 168.6 | 181.2 | 150.0 | 160.2 | 167.0 | 176.6 |
| 14 | 153.7 | 164.0 | 166.1 | 174.3 | 151.2 | 159.8 | 168.9 | 184.4 |
| 15 | 151.0 | 158.1 | 163.7 | 164.1 | 153.2 | 158.6 | 168.0 | 179.3 |
| 16 | 155.0 | 161.0 | 168.5 | 182.8 | 152.8 | 166.0 | 165.9 | 177.2 |
| 17 | 153.4 | 161.7 | 164.1 | 180.0 | 154.2 | 157.7 | 161.9 | 174.3 |
| 18 | 155.4 | 152.3 | 169.3 | 182.9 | 152.2 | 160.7 | 166.2 | 176.5 |
| 19 | 153.4 | 164.5 | 162.0 | 182.8 | 153.2 | 160.6 | 163.2 | 181.4 |
| 20 | 156.7 | 155.0 | 166.7 | 185.0 | 149.9 | 161.3 | 166.7 | 182.2 |
| Average | 153.5 | 161.5 | 165.7 | 178.8 | 152.7 | 159.1 | 166.3 | 179.2 |
| SD | 1.9 | 4.1 | 2.3 | 6.4 | 2.0 | 2.4 | 2.3 | 3.2 |
| %CV | 1.3 | 2.5 | 1.4 | 3.6 | 1.3 | 1.5 | 1.4 | 1.8 |

**Table 41** Weight variation of tablet coated with polymethacrylate film with different coating level (A) freshly prepared and (B) after exposure to the accelerated condition for 4 months

| No. | Weight (mg) | | | | | |
|---|---|---|---|---|---|---|
| | A | | | B | | |
| | 1% | 3% | 5% | 1% | 3% | 5% |
| 1 | 150.7 | 151.7 | 161.7 | 154.0 | 156.1 | 160.0 |
| 2 | 148.0 | 154.9 | 163.2 | 151.1 | 157.8 | 161.1 |
| 3 | 147.9 | 154.6 | 159.9 | 151.9 | 153.1 | 160.3 |
| 4 | 147.9 | 151.6 | 159.3 | 149.2 | 153.7 | 157.9 |
| 5 | 149.3 | 150.1 | 158.6 | 147.2 | 155.5 | 161.3 |
| 6 | 148.3 | 151.6 | 161.5 | 146.0 | 154.2 | 161.5 |
| 7 | 146.6 | 154.2 | 164.9 | 147.1 | 154.7 | 161.7 |
| 8 | 150.0 | 149.3 | 162.4 | 147.0 | 154.0 | 162.3 |
| 9 | 151.6 | 154.2 | 158.0 | 152.1 | 153.2 | 164.0 |
| 10 | 148.8 | 150.8 | 160.6 | 153.2 | 151.2 | 161.0 |
| 11 | 149.8 | 151.2 | 158.2 | 151.1 | 154.3 | 160.7 |
| 12 | 152.2 | 155.4 | 159.7 | 149.1 | 154.6 | 158.7 |
| 13 | 149.7 | 154.3 | 157.2 | 149.0 | 154.2 | 160.2 |
| 14 | 148.2 | 154.4 | 164.1 | 150.8 | 155.2 | 159.2 |
| 15 | 147.2 | 149.9 | 157.9 | 150.2 | 153.2 | 159.3 |
| 16 | 150.3 | 149.0 | 159.0 | 148.8 | 152.2 | 163.4 |
| 17 | 145.5 | 150.9 | 160.5 | 149.5 | 151.2 | 157.5 |
| 18 | 150.3 | 154.1 | 159.8 | 154.0 | 154.1 | 161.1 |
| 19 | 149.0 | 152.2 | 160.4 | 151.1 | 155.2 | 160.5 |
| 20 | 150.5 | 151.3 | 162.0 | 151.0 | 153.6 | 160.3 |
| Average | 149.1 | 152.3 | 160.4 | 150.1 | 154.1 | 160.6 |
| SD | 1.7 | 2.0 | 2.1 | 2.3 | 1.6 | 1.6 |
| %CV | 1.1 | 1.3 | 1.3 | 1.5 | 1.0 | 1.0 |

**Table 42** The disintegration time of core tablets and tablets coated with different coating level (A) freshly prepared or as received and (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 | - | 0.50 | 0.52 | 0.50 |
| Chitosan citrate | 3 | 2.87 | 4.67 | 8.00 |
| | 5 | 3.00 | 4.50 | 20.00 |
| | 10 | 4.55 | 8.00 | >30.00 |
| HPMC | 3 | 1.12 | 1.42 | 2.00 |
| | 5 | 2.50 | 3.23 | 3.33 |
| | 10 | 3.33 | 3.15 | 4.25 |
| | 15 | 3.50 | 4.17 | 5.75 |
| Polymethacrylate | 1 | 1.17 | 1.08 | 1.25 |
| | 3 | 1.33 | 1.50 | 1.50 |
| | 5 | 3.00 | 3.63 | 3.50 |
| Centellase® tablet (n=1) | - | 9.33 | - | - |

**Table 43** Dissolution data of *Centella asiatica* tablets at initial period (n=3)

| Formulations | Sample 1 | | Sample 2 | | Sample 3 | | Average | | SD |
|---|---|---|---|---|---|---|---|---|---|
| | (mg) | %disso | (mg) | %disso | (mg) | %disso | (mg) | %disso | |
| CORE | 24.8944 | 83.0 | 26.0241 | 86.7 | 24.5204 | 81.7 | 25.1463 | 83.8 | 2.61 |
| CS3 | 22.5653 | 75.2 | 25.7725 | 85.9 | 26.1034 | 87.0 | 24.8137 | 82.7 | 6.51 |
| CS5 | 23.9541 | 79.8 | 25.0245 | 83.4 | 21.1389 | 70.5 | 23.3725 | 77.9 | 6.69 |
| CS10 | 26.1355 | 87.1 | 25.9001 | 86.3 | 24.3320 | 81.1 | 25.4559 | 84.9 | 3.27 |
| HPMC3 | 22.2926 | 74.3 | 21.6681 | 72.2 | 24.5276 | 81.8 | 22.8294 | 76.1 | 5.01 |
| HPMC5 | 25.5135 | 85.0 | 20.2804 | 67.6 | 23.5657 | 78.6 | 23.1198 | 77.1 | 8.82 |
| HPMC10 | 23.3486 | 77.8 | 23.7095 | 79.0 | 22.5476 | 75.2 | 23.2019 | 77.3 | 1.98 |
| HPMC15 | 25.8596 | 86.2 | 27.6881 | 92.3 | 26.0144 | 86.7 | 26.5207 | 88.4 | 3.38 |
| PMC1 | 22.0145 | 73.4 | 23.7163 | 79.1 | 23.2107 | 77.4 | 22.9805 | 76.6 | 2.91 |
| PMC3 | 23.0569 | 76.9 | 22.3787 | 74.6 | 24.2280 | 80.8 | 23.2212 | 77.4 | 3.12 |
| PMC5 | 23.3104 | 77.7 | 23.8886 | 79.6 | 24.4705 | 81.6 | 23.8898 | 79.6 | 1.93 |
| Centellase®30 | 4.2216 | 14.1 | 4.9065 | 16.4 | 4.5541 | 15.2 | 4.5607 | 15.2 | 1.14 |
| Centellase®45 | 6.1674 | 20.6 | 7.4996 | 25.0 | 6.8106 | 22.7 | 6.8259 | 22.8 | 2.22 |

* Centellase®30 and 45 = the dissolution data of the commercial tablets, Centellase® tablets, at 30 and 45 minutes, %disso. = %dissolution.

**Table 44** Dissolution data of *Centella asiatica* tablets after exposure to the ambient condition for 4 months (n=3)

| Formulations | Sample 1 | | Sample 2 | | Sample 3 | | Average | | SD |
|---|---|---|---|---|---|---|---|---|---|
| | (mg) | %disso | (mg) | %disso | (mg) | %disso | (mg) | %disso | |
| CORE | 23.7260 | 79.1 | 24.8796 | 82.9 | 24.1777 | 80.6 | 24.2611 | 80.9 | 1.94 |
| CS3 | 22.1454 | 73.8 | 23.3001 | 77.7 | 23.7429 | 79.1 | 23.0628 | 76.9 | 2.75 |
| CS5 | 21.9173 | 73.1 | 21.9605 | 73.2 | 19.3257 | 64.4 | 21.0678 | 70.2 | 5.00 |
| CS10 | 25.8315 | 86.1 | 24.1824 | 80.6 | 22.8082 | 76.0 | 24.2740 | 80.9 | 1.51 |
| HPMC3 | 23.9116 | 79.7 | 22.9581 | 76.5 | 23.5279 | 78.4 | 23.4658 | 78.2 | 1.60 |
| HPMC5 | 21.0444 | 70.1 | 24.2427 | 80.8 | 22.8455 | 76.2 | 22.7108 | 75.7 | 5.34 |
| HPMC10 | 21.9798 | 73.3 | 21.5462 | 71.8 | 22.3983 | 74.7 | 21.9748 | 73.2 | 1.42 |
| HPMC15 | 24.5554 | 81.9 | 24.7751 | 82.6 | 23.7489 | 79.2 | 24.3598 | 81.2 | 1.80 |
| PMC1 | 23.2609 | 77.5 | 22.6565 | 75.5 | 21.7704 | 72.6 | 22.5626 | 75.2 | 2.50 |
| PMC3 | 22.8969 | 76.3 | 22.7513 | 75.8 | 22.8860 | 76.3 | 22.8447 | 76.1 | 0.27 |
| PMC5 | 21.6187 | 72.1 | 23.0343 | 76.8 | 22.5286 | 75.1 | 22.3939 | 74.6 | 2.39 |
| Centellase®30 | 2.8096 | 9.4 | 2.9246 | 9.7 | 2.8703 | 9.6 | 2.8682 | 9.6 | 0.19 |
| Centellase®45 | 3.9734 | 13.2 | 4.2171 | 14.1 | 4.1000 | 13.7 | 4.0969 | 13.7 | 0.41 |

* Centellase®30 and 45 = the dissolution data of the commercial tablets, Centellase® tablets, at 30 and 45 minutes
%disso. = %dissolution.

**Table 45** Dissolution data of *Centella asiatica* tablets after exposure to the accelerated condition for 4 months (n=3)

| Formulations | Sample 1 | | Sample 2 | | Sample 3 | | Average | | SD |
|---|---|---|---|---|---|---|---|---|---|
| | (mg) | %disso | (mg) | %disso | (mg) | %disso | (mg) | %disso | |
| CORE | 22.9482 | 76.5 | 22.1492 | 73.8 | 22.6258 | 75.4 | 22.5744 | 75.2 | 1.34 |
| CS3 | 18.4034 | 61.3 | 20.4599 | 68.2 | 21.6696 | 72.2 | 20.1776 | 67.3 | 5.50 |
| CS5 | 18.8634 | 62.9 | 17.4293 | 58.1 | 21.9429 | 73.1 | 19.4119 | 64.7 | 7.69 |
| CS10 | 21.8515 | 72.8 | 23.7356 | 79.1 | 21.8759 | 72.9 | 22.4877 | 75.00 | 3.60 |
| HPMC3 | 22.5431 | 75.1 | 20.6552 | 68.9 | 22.4180 | 74.7 | 21.8721 | 72.9 | 3.50 |
| HPMC5 | 21.4152 | 78.1 | 23.1379 | 77.1 | 23.7289 | 79.1 | 23.4273 | 78.1 | 1.00 |
| HPMC10 | 21.3901 | 71.3 | 20.4061 | 68.00 | 21.9526 | 73.2 | 21.2496 | 70.8 | 2.60 |
| HPMC15 | 22.1886 | 74.0 | 23.3426 | 77.8 | 23.4752 | 78.3 | 23.0021 | 76.7 | 2.36 |
| PMC1 | 21.4231 | 71.4 | 21.4045 | 71.3 | 23.3687 | 77.9 | 22.0654 | 73.6 | 3.76 |
| PMC3 | 22.4538 | 74.8 | 21.9728 | 73.2 | 23.7982 | 76.3 | 22.7416 | 75.8 | 3.20 |
| PMC5 | 21.8242 | 72.7 | 22.4538 | 74.8 | 23.7982 | 79.3 | 22.6921 | 75.6 | 3.40 |
| Centellase®30 | 3.5076 | 11.7 | 2.7409 | 9.1 | 3.1401 | 10.5 | 3.1295 | 10.4 | 1.28 |
| Centellase®45 | 5.4966 | 18.3 | 4.1859 | 14.0 | 4.7549 | 15.8 | 4.8125 | 16.0 | 2.19 |

* Centellase®30 and 45 = the dissolution data of the commercial tablets, Centellase® tablets, at 30 and 45 minutes.
%disso. = %dissolution.

**Table 46** Dissolution data of *Centella asiatica* tablets at initial (n=3)

| Formulations | AS | | MA | | AA | | Total | | % |
|---|---|---|---|---|---|---|---|---|---|
| | mg | SD | mg | SD | mg | SD | mg | SD | dissolution |
| CORE | 13.0703 | 0.20 | 7.8313 | 0.32 | 4.2267 | 0.29 | 25.1283 | 0.63 | 83.8 |
| CS3 | 11.9823 | 0.77 | 8.2051 | 0.89 | 4.6263 | 0.50 | 24.8137 | 1.95 | 82.7 |
| CS5 | 11.5583 | 1.05 | 7.6906 | 0.55 | 4.1236 | 0.43 | 23.3725 | 2.01 | 77.9 |
| CS10 | 12.2790 | 0.64 | 8.4369 | 0.29 | 4.7399 | 0.15 | 25.4558 | 0.98 | 84.9 |
| HPMC3 | 11.6499 | 0.01 | 7.1582 | 0.97 | 4.0213 | 0.53 | 22.8294 | 1.50 | 76.1 |
| HPMC5 | 11.9161 | 1.70 | 7.2651 | 0.88 | 3.9387 | 0.46 | 23.1198 | 2.64 | 77.1 |
| HPMC10 | 12.6569 | 0.24 | 6.7532 | 0.30 | 3.7917 | 0.16 | 23.2019 | 0.59 | 77.3 |
| HPMC15 | 12.4356 | 0.52 | 9.0380 | 0.33 | 5.0471 | 0.18 | 26.5207 | 1.01 | 88.4 |
| PMC1 | 11.8818 | 0.09 | 7.1059 | 0.61 | 3.9928 | 0.28 | 22.9805 | 0.87 | 76.6 |
| PMC3 | 11.7586 | 0.36 | 7.3586 | 0.39 | 4.1040 | 0.21 | 23.2212 | 0.94 | 77.4 |
| PMC5 | 13.9751 | 0.61 | 6.2850 | 0.50 | 3.6297 | 0.23 | 23.8898 | 0.58 | 79.6 |
| Centellase30 | 3.3920 | 1.96 | 0.8640 | 0.52 | 0.3081 | 0.20 | 4.5640 | 2.66 | 15.2 |
| Centellase45 | 4.6777 | 2.70 | 1.6005 | 1.02 | 0.5554 | 0.37 | 6.8336 | 4.00 | 22.78 |

* Centellase®30 and 45 = the dissolution data of the commercial tablets, Centellase® tablets, at 30 and 45 minutes.

**Table 47** Dissolution data of *Centella asiatica* tablets after exposure to the ambient condition (n=3)

| Formulations | AS | | MA | | AA | | Total | | % |
|---|---|---|---|---|---|---|---|---|---|
| | mg | SD | mg | SD | mg | SD | mg | SD | dissolution |
| CORE | 11.8922 | 0.38 | 8.0533 | 0.13 | 4.3156 | 0.08 | 24.2611 | 0.58 | 80.9 |
| CS3 | 11.6893 | 0.42 | 7.3080 | 0.27 | 4.0656 | 0.14 | 23.0628 | 0.82 | 76.9 |
| CS5 | 11.8093 | 0.17 | 6.7632 | 0.51 | 3.7541 | 0.23 | 22.3265 | 0.67 | 74.4 |
| CS10 | 12.0505 | 1.12 | 7.4743 | 0.44 | 4.1132 | 0.16 | 23.6381 | 0.73 | 78.8 |
| HPMC3 | 11.2061 | 0.64 | 7.5566 | 0.88 | 4.0645 | 0.41 | 22.8271 | 1.56 | 76.1 |
| HPMC5 | 11.3153 | 0.81 | 7.2922 | 0.50 | 4.1034 | 0.30 | 22.7108 | 1.60 | 75.7 |
| HPMC10 | 11.2480 | 0.23 | 6.9749 | 0.38 | 3.7518 | 0.18 | 21.9748 | 0.43 | 73.2 |
| HPMC15 | 12.4968 | 0.12 | 7.5862 | 0.44 | 4.2768 | 0.22 | 24.3598 | 0.54 | 81.2 |
| PMC1 | 11.3878 | 0.71 | 7.2289 | 0.22 | 3.9459 | 0.10 | 22.5626 | 0.75 | 75.2 |
| PMC3 | 11.8803 | 0.95 | 7.1345 | 0.65 | 3.8299 | 0.31 | 22.8447 | 0.08 | 76.1 |
| PMC5 | 10.7959 | 0.76 | 7.5358 | 0.33 | 4.0621 | 0.16 | 22.3939 | 0.72 | 74.6 |
| Centellase30 | 2.0614 | 1.20 | 0.5136 | 0.30 | 0.2921 | 0.19 | 2.8671 | 1.66 | 9.6 |
| Centellase45 | 2.9311 | 1.69 | 0.8627 | 0.50 | 0.3015 | 0.17 | 4.0953 | 2.37 | 13.7 |

* Centellase®30 and 45 = the dissolution data of the commercial tablets, Centellase® tablets, at 30 and 45 minutes.

**Table 48** Dissolution data of *Centella asiatica* tablets after exposure to the accelerated condition (n=3)

| Formulations | AS | | MA | | AA | | Total | | % |
|---|---|---|---|---|---|---|---|---|---|
| | mg | SD | mg | SD | mg | SD | mg | SD | dissolution |
| CORE | 12.0232 | 0.55 | 6.7416 | 0.24 | 3.8096 | 0.10 | 22.5744 | 1.08 | 75.2 |
| CS3 | 10.9793 | 1.02 | 5.9441 | 0.82 | 3.2542 | 0.43 | 20.1776 | 1.65 | 67.3 |
| CS5 | 11.4455 | 1.12 | 5.1436 | 0.76 | 2.8228 | 0.43 | 19.4119 | 2.31 | 64.7 |
| CS10 | 10.7052 | 1.58 | 6.3293 | 0.46 | 3.5331 | 0.30 | 20.5676 | 1.08 | 68.6 |
| HPMC3 | 11.4593 | 0.53 | 7.5270 | 0.42 | 4.0696 | 0.17 | 23.0559 | 0.84 | 76.9 |
| HPMC5 | 11.9816 | 0.28 | 7.1044 | 0.49 | 4.0211 | 0.33 | 23.1071 | 1.10 | 77.0 |
| HPMC10 | 11.3629 | 0.37 | 6.9475 | 0.26 | 3.8163 | 0.21 | 22.1267 | 0.84 | 73.8 |
| HPMC15 | 11.8364 | 0.38 | 7.1385 | 0.20 | 4.0273 | 0.14 | 23.0021 | 0.71 | 76.7 |
| PMC1 | 11.2411 | 0.58 | 7.0040 | 0.39 | 3.8204 | 0.17 | 22.0654 | 1.13 | 73.6 |
| PMC3 | 11.4275 | 0.96 | 6.9325 | 0.54 | 3.7459 | 0.29 | 22.1060 | 1.78 | 73.7 |
| PMC5 | 11.2550 | 0.32 | 6.7906 | 0.71 | 3.8618 | 0.25 | 21.9074 | 0.64 | 73.0 |
| Centellase30 | 2.3237 | 1.36 | 0.5973 | 0.36 | 0.2030 | 0.13 | 3.1240 | 1.84 | 10.4 |
| Centellase45 | 3.5075 | 2.06 | 0.9849 | 0.61 | 0.3489 | 0.21 | 4.8413 | 2.87 | 16.1 |

* Centellase®30 and 45 = the dissolution data of the commercial tablets, Centellase® tablets, at 30 and 45 minutes.

**Table 49** The assay content of the tablet formulations

| Formulations | Amount (mg) | Label amount (%) |
|---|---|---|
| Core | 28.38 | 94.60 |
| CS3 | 28.15 | 93.83 |
| CS5 | 27.43 | 91.43 |
| CS10 | 27.55 | 91.83 |
| HPMC3 | 26.53 | 88.43 |
| HPMC5 | 27.40 | 91.33 |
| HPMC10 | 25.84 | 86.13 |
| HPMC15 | 27.46 | 91.53 |
| PMC1 | 28.17 | 93.90 |
| PMC3 | 28.51 | 95.03 |
| PMC5 | 28.20 | 94.00 |
| Centellase® | 24.86 | 82.87 |

**Table 50** The stability data of *Centella asiatica* core tablets and tablets coated with chitosan citrate after exposure to the ambient and accelerated conditions

| Storage condition | Storage period | % Residual amount | | | |
|---|---|---|---|---|---|
| | | Core | CS3 | CS5 | CS10 |
| Ambient | Initial | 100.0 | 100.0 | 100.0 | 100.0 |
| | 1 month | 102.3 | 99.4 | 100.1 | 99.2 |
| | 2 months | 97.0 | 96.8 | 98.9 | 99.0 |
| | 3 months | 95.7 | 97.7 | 95.0 | 96.6 |
| | 4 months | 93.0 | 96.9 | 93.6 | 97.3 |
| Accelerated | Initial | 100.0 | 100.0 | 100.0 | 100.0 |
| | 1 month | 100.0 | 97.8 | 99.0 | 95.8 |
| | 2 months | 93.7 | 98.8 | 98.0 | 96.0 |
| | 3 months | 91.9 | 95.4 | 92.5 | 97.0 |
| | 4 months | 90.1 | 95.3 | 92.5 | 97.5 |

**Table 51** The stability data of *Centella asiatica* tablets coated with HPMC after exposure to the ambient and accelerated conditions

| Storage condition | Storage period | % Residual amount | | | |
|---|---|---|---|---|---|
| | | HPMC3 | HPMC5 | HPMC10 | HPMC15 |
| Ambient | Initial | 100.0 | 100.0 | 100.0 | 100.0 |
| | 1 month | 99.3 | 98.0 | 97.9 | 95.7 |
| | 2 months | 99.6 | 99.2 | 99.0 | 97.7 |
| | 3 months | 95.2 | 95.3 | 95.2 | 97.3 |
| | 4 months | 95.1 | 94.2 | 94.0 | 97.8 |
| Accelerated | Initial | 100.0 | 100.0 | 100.0 | 100.0 |
| | 1 month | 97.5 | 98.4 | 96.9 | 93.6 |
| | 2 months | 93.6 | 96.8 | 99.4 | 99.6 |
| | 3 months | 91.0 | 95.7 | 93.4 | 97.5 |
| | 4 months | 92.6 | 93.4 | 93.5 | 95.0 |

**Table 52** The stability data of *Centella asiatica* tablets coated with polymethacrylate after exposure to the ambient and accelerated conditions

| Storage condition | Storage period | % Residual amount | | | |
|---|---|---|---|---|---|
| | | PMC1 | PMC3 | PMC5 | Centellase |
| Ambient | Initial | 100.0 | 100.0 | 100.0 | 100.0 |
| | 1 month | 100.1 | 96.3 | 99.6 | 100.7 |
| | 2 months | 95.3 | 95.3 | 100.5 | 100.5 |
| | 3 months | 93.9 | 95.1 | 97.3 | 98.9 |
| | 4 months | 94.2 | 96.7 | 99.0 | 98.1 |
| Accelerated | Initial | 100.0 | 100.0 | 100.0 | 100.0 |
| | 1 month | 97.9 | 97.5 | 98.8 | 104.9 |
| | 2 months | 96.0 | 96.9 | 99.0 | 96.5 |
| | 3 months | 94.2 | 97.8 | 96.8 | 92.8 |
| | 4 months | 95.5 | 97.2 | 97.6 | 99.7 |

**Table 53** The stability data of *Centella asiatica* core tablets after exposure to the ambient and accelerated conditions

| Storage period (months) | Core tablet | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 12.16 | 100.0 | 10.33 | 100.0 | 5.89 | 100.0 | 28.38 | 100.0 |
| 1 | 12.35 | 101.6 | 10.75 | 104.1 | 5.94 | 100.8 | 29.04 | 102.3 |
| 2 | 11.52 | 94.7 | 10.4 | 100.7 | 5.60 | 95.1 | 27.52 | 97.0 |
| 3 | 11.51 | 94.7 | 10.15 | 98.3 | 5.49 | 93.2 | 27.15 | 95.7 |
| 4 | 11.78 | 96.9 | 9.57 | 92.6 | 5.03 | 85.4 | 26.38 | 93.0 |
| Accelerated condition | | | | | | | | |
| Initial | 12.16 | 100.0 | 10.33 | 100.0 | 5.89 | 100.0 | 28.38 | 100.0 |
| 1 | 11.76 | 96.7 | 10.46 | 101.2 | 5.75 | 97.7 | 27.97 | 98.6 |
| 2 | 11.49 | 94.5 | 9.58 | 92.7 | 5.51 | 93.5 | 26.58 | 93.7 |
| 3 | 11.46 | 94.2 | 9.80 | 94.9 | 4.82 | 81.8 | 26.08 | 91.9 |
| 4 | 11.75 | 96.6 | 8.99 | 87.0 | 4.82 | 81.8 | 25.56 | 90.1 |

**Table 54** The stability data of *Centella asiatica* tablets coated with chitosan citrate at 3% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with chitosan citrate at coating level 3% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 12.28 | 100.0 | 10.39 | 100.0 | 5.48 | 100.0 | 28.15 | 100.0 |
| 1 | 12.23 | 99.6 | 10.28 | 98.9 | 5.48 | 100.0 | 27.99 | 99.4 |
| 2 | 11.85 | 96.5 | 10.00 | 96.2 | 5.41 | 98.7 | 27.26 | 96.8 |
| 3 | 11.87 | 96.7 | 9.98 | 96.1 | 5.66 | 103.3 | 27.51 | 97.7 |
| 4 | 11.51 | 93.7 | 10.43 | 100.4 | 5.33 | 97.3 | 27.27 | 96.9 |
| Accelerated condition | | | | | | | | |
| Initial | 12.28 | 100.0 | 10.39 | 100.0 | 5.48 | 100.0 | 28.15 | 100.0 |
| 1 | 11.94 | 97.2 | 10.09 | 97.1 | 5.49 | 100.2 | 27.52 | 97.8 |
| 2 | 12.14 | 98.9 | 10.22 | 98.4 | 5.44 | 99.3 | 27.80 | 98.8 |
| 3 | 11.65 | 94.9 | 10.06 | 96.8 | 5.14 | 93.8 | 26.85 | 95.4 |
| 4 | 11.44 | 93.2 | 9.98 | 96.1 | 5.40 | 98.5 | 26.82 | 95.3 |

**Table 55** The stability data of *Centella asiatica* tablets coated with chitosan citrate at 5% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with chitosan citrate at coating level 5% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 11.79 | 100.0 | 10.01 | 100.0 | 5.63 | 100.0 | 27.43 | 100.0 |
| 1 | 11.56 | 98.0 | 10.20 | 101.9 | 5.70 | 101.2 | 27.46 | 100.1 |
| 2 | 11.26 | 95.5 | 10.50 | 104.9 | 5.37 | 95.4 | 27.13 | 98.9 |
| 3 | 11.26 | 95.5 | 9.61 | 96.0 | 5.18 | 92.0 | 26.05 | 95.0 |
| 4 | 11.60 | 98.4 | 9.29 | 92.8 | 4.77 | 84.7 | 25.66 | 93.6 |
| Accelerated condition | | | | | | | | |
| Initial | 11.79 | 100.0 | 10.01 | 100.0 | 5.63 | 100.0 | 27.43 | 100.0 |
| 1 | 11.66 | 98.9 | 10.09 | 100.8 | 5.66 | 100.5 | 27.41 | 99.9 |
| 2 | 11.12 | 94.3 | 10.34 | 103.3 | 5.42 | 96.3 | 26.88 | 98.0 |
| 3 | 11.59 | 98.3 | 9.43 | 94.2 | 4.93 | 87.5 | 25.94 | 94.6 |
| 4 | 11.57 | 98.1 | 9.06 | 90.5 | 4.74 | 84.2 | 25.37 | 92.5 |

**Table 56** The stability data of *Centella asiatica* tablets coated with chitosan citrate at 10% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with chitosan citrate at coating level 10% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 12.21 | 100.0 | 10.04 | 100.0 | 5.30 | 100.0 | 27.55 | 100.0 |
| 1 | 11.89 | 97.4 | 10.01 | 99.7 | 5.41 | 102.1 | 27.31 | 99.2 |
| 2 | 11.94 | 97.8 | 9.99 | 99.5 | 5.34 | 100.8 | 27.27 | 99.0 |
| 3 | 11.53 | 94.4 | 9.77 | 97.3 | 5.31 | 100.2 | 26.61 | 96.6 |
| 4 | 11.62 | 95.2 | 9.88 | 98.4 | 5.29 | 99.8 | 26.80 | 97.3 |
| Accelerated condition | | | | | | | | |
| Initial | 12.21 | 100.0 | 10.04 | 100.0 | 5.30 | 100.0 | 27.55 | 100.0 |
| 1 | 11.52 | 94.3 | 9.66 | 96.2 | 5.22 | 98.5 | 26.40 | 95.8 |
| 2 | 11.54 | 94.5 | 9.72 | 96.8 | 5.18 | 97.7 | 26.44 | 96.0 |
| 3 | 11.62 | 95.2 | 9.82 | 97.8 | 5.28 | 99.6 | 26.72 | 97.0 |
| 4 | 11.56 | 94.7 | 9.99 | 99.5 | 5.30 | 100.0 | 26.85 | 97.5 |

**Table 57** The stability data of *Centella asiatica* tablets coated with HPMC at 3% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with HPMC at coating level 3% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 11.37 | 100.0 | 9.92 | 100.0 | 5.24 | 100.0 | 26.53 | 100.0 |
| 1 | 11.34 | 99.7 | 9.75 | 98.3 | 5.25 | 100.2 | 26.34 | 99.3 |
| 2 | 11.42 | 100.4 | 9.60 | 96.8 | 5.41 | 103.2 | 26.43 | 99.6 |
| 3 | 10.82 | 95.2 | 9.44 | 95.2 | 4.99 | 95.2 | 25.25 | 95.2 |
| 4 | 10.86 | 95.5 | 9.29 | 93.6 | 5.09 | 97.1 | 25.24 | 95.1 |
| Accelerated condition | | | | | | | | |
| Initial | 11.37 | 100.0 | 9.92 | 100.0 | 5.24 | 100.0 | 26.53 | 100.0 |
| 1 | 11.16 | 98.2 | 9.55 | 96.3 | 5.17 | 98.7 | 25.88 | 97.5 |
| 2 | 10.72 | 94.3 | 9.07 | 91.4 | 5.05 | 96.4 | 24.84 | 93.6 |
| 3 | 10.37 | 91.2 | 9.04 | 91.1 | 4.73 | 90.3 | 24.14 | 91.0 |
| 4 | 10.64 | 93.6 | 8.99 | 90.6 | 4.94 | 94.3 | 24.57 | 92.6 |

**Table 58** The stability data of *Centella asiatica* tablets coated with HPMC at 5% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with HPMC at coating level 5% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 11.82 | 100.0 | 9.94 | 100.0 | 5.64 | 100.0 | 27.40 | 100.0 |
| 1 | 11.42 | 96.6 | 9.85 | 99.1 | 5.58 | 98.9 | 26.85 | 98.0 |
| 2 | 12.08 | 102.2 | 9.93 | 99.9 | 5.16 | 91.5 | 27.17 | 99.2 |
| 3 | 11.45 | 96.9 | 9.63 | 96.9 | 5.03 | 89.2 | 26.11 | 95.3 |
| 4 | 11.14 | 94.2 | 9.41 | 94.7 | 5.27 | 93.4 | 25.82 | 94.2 |
| Accelerated condition | | | | | | | | |
| Initial | 11.82 | 100.0 | 9.94 | 100.0 | 5.64 | 100.0 | 27.40 | 100.0 |
| 1 | 11.55 | 97.7 | 9.83 | 98.9 | 5.59 | 99.1 | 26.97 | 98.4 |
| 2 | 11.37 | 96.2 | 9.73 | 97.9 | 5.42 | 96.1 | 26.52 | 96.8 |
| 3 | 11.28 | 95.4 | 9.57 | 96.3 | 5.36 | 95.0 | 26.21 | 95.7 |
| 4 | 11.03 | 93.3 | 9.32 | 93.8 | 5.24 | 92.9 | 25.59 | 93.4 |

**Table 59** The stability data of *Centella asiatica* tablets coated with HPMC at 10% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with HPMC at coating level 10% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 11.09 | 100.0 | 9.57 | 100.0 | 5.18 | 100.0 | 25.84 | 100.0 |
| 1 | 10.9 | 98.3 | 9.36 | 97.8 | 5.03 | 97.1 | 25.29 | 97.9 |
| 2 | 11.02 | 99.4 | 9.30 | 97.2 | 5.25 | 101.4 | 25.57 | 99.0 |
| 3 | 10.53 | 95.0 | 9.10 | 95.1 | 4.96 | 95.8 | 24.59 | 95.2 |
| 4 | 10.69 | 96.4 | 8.86 | 92.6 | 4.73 | 91.3 | 24.28 | 94.0 |
| Accelerated condition | | | | | | | | |
| Initial | 11.09 | 100.0 | 9.57 | 100.0 | 5.18 | 100.0 | 25.84 | 100.0 |
| 1 | 10.76 | 97.0 | 9.28 | 97.0 | 4.99 | 96.3 | 25.03 | 96.9 |
| 2 | 11.4 | 102.8 | 9.56 | 99.9 | 4.73 | 91.3 | 25.69 | 99.4 |
| 3 | 10.17 | 91.7 | 9.17 | 95.8 | 4.80 | 92.7 | 24.14 | 93.4 |
| 4 | 10.72 | 96.7 | 8.87 | 92.6 | 4.86 | 93.8 | 24.45 | 94.6 |

**Table 60** The stability data of *Centella asiatica* tablets coated with HPMC at 15% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with HPMC at coating level 15% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 11.93 | 100.0 | 10.17 | 100.0 | 5.36 | 100.0 | 27.46 | 100.0 |
| 1 | 11.61 | 97.3 | 9.52 | 93.6 | 5.14 | 95.9 | 26.27 | 95.7 |
| 2 | 11.78 | 98.7 | 9.81 | 96.5 | 5.25 | 97.9 | 26.84 | 97.7 |
| 3 | 11.58 | 97.1 | 9.84 | 96.8 | 5.29 | 98.7 | 26.71 | 97.3 |
| 4 | 11.88 | 99.6 | 9.94 | 97.7 | 5.04 | 94.0 | 26.86 | 97.8 |
| Accelerated condition | | | | | | | | |
| Initial | 11.93 | 100.0 | 10.17 | 100.0 | 5.36 | 100.0 | 27.46 | 100.0 |
| 1 | 11.15 | 93.5 | 9.45 | 92.9 | 5.10 | 95.1 | 25.70 | 93.6 |
| 2 | 11.99 | 100.5 | 10.01 | 98.4 | 5.34 | 99.6 | 27.34 | 99.6 |
| 3 | 11.54 | 96.7 | 10.03 | 98.6 | 5.21 | 97.2 | 26.78 | 97.5 |
| 4 | 11.26 | 94.4 | 9.50 | 93.4 | 5.33 | 99.4 | 26.09 | 95.0 |

**Table 61** The stability data of *Centella asiatica* tablets coated with polymethacrylate at 1% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with polymethacrylate at coating level 1% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 12.09 | 100.0 | 10.44 | 100.0 | 5.64 | 100.0 | 28.17 | 100.0 |
| 1 | 12.16 | 100.6 | 10.39 | 99.5 | 5.65 | 100.2 | 28.20 | 100.1 |
| 2 | 11.62 | 96.1 | 9.75 | 93.4 | 5.49 | 97.3 | 26.86 | 95.3 |
| 3 | 11.47 | 94.9 | 9.79 | 93.8 | 5.18 | 91.8 | 26.44 | 93.9 |
| 4 | 11.49 | 95.0 | 9.77 | 93.6 | 5.29 | 93.8 | 26.55 | 94.2 |
| Accelerated condition | | | | | | | | |
| Initial | 12.09 | 100.0 | 10.44 | 100.0 | 5.64 | 100.0 | 28.17 | 100.0 |
| 1 | 11.92 | 98.6 | 10.13 | 97.0 | 5.52 | 97.9 | 27.57 | 97.9 |
| 2 | 11.73 | 97.0 | 9.80 | 93.9 | 5.50 | 97.5 | 27.03 | 96.0 |
| 3 | 11.45 | 94.7 | 9.88 | 94.6 | 5.22 | 92.6 | 26.55 | 94.2 |
| 4 | 11.56 | 95.6 | 10.05 | 96.3 | 5.29 | 93.8 | 26.90 | 95.5 |

**Table 62** The stability data of *Centella asiatica* tablets coated with polymethacrylate at 3% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with polymethacrylate at coating level 3% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 12.13 | 100.0 | 10.63 | 100.0 | 5.75 | 100.0 | 28.51 | 100.0 |
| 1 | 11.88 | 98.0 | 10.06 | 94.6 | 5.51 | 95.8 | 27.45 | 96.3 |
| 2 | 11.81 | 97.4 | 9.84 | 92.6 | 5.52 | 95.9 | 27.17 | 95.3 |
| 3 | 11.68 | 96.3 | 10.08 | 94.9 | 5.34 | 92.8 | 27.10 | 95.1 |
| 4 | 11.89 | 98.1 | 10.15 | 95.5 | 5.53 | 96.2 | 27.58 | 96.7 |
| Accelerated condition | | | | | | | | |
| Initial | 12.13 | 100.0 | 10.63 | 100.0 | 5.75 | 100.0 | 28.51 | 100.0 |
| 1 | 12.01 | 99.0 | 10.21 | 96.1 | 5.58 | 96.9 | 27.80 | 97.5 |
| 2 | 11.91 | 98.3 | 10.04 | 94.4 | 5.68 | 98.6 | 27.63 | 96.9 |
| 3 | 12.00 | 99.0 | 10.36 | 97.5 | 5.53 | 96.1 | 27.89 | 97.8 |
| 4 | 11.87 | 97.9 | 10.39 | 97.8 | 5.46 | 94.8 | 27.72 | 97.2 |

**Table 63** The stability data of *Centella asiatica* tablets coated with polymethacrylate at 5% coating level after exposure to the ambient and accelerated conditions

| Storage period (months) | Tablet coated with polymethacrylate at coating level 5% increased weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 11.66 | 100.0 | 10.07 | 100.0 | 5.47 | 100.0 | 27.20 | 100.0 |
| 1 | 11.67 | 100.1 | 10.01 | 99.4 | 5.42 | 99.1 | 27.10 | 99.6 |
| 2 | 11.85 | 101.6 | 9.90 | 98.3 | 5.58 | 102.0 | 27.33 | 100.5 |
| 3 | 11.45 | 98.2 | 9.83 | 97.6 | 5.19 | 94.9 | 26.47 | 97.3 |
| 4 | 11.66 | 100.0 | 9.92 | 98.5 | 5.35 | 97.8 | 26.93 | 99.0 |
| Accelerated condition | | | | | | | | |
| Initial | 11.66 | 100.0 | 10.07 | 100.0 | 5.47 | 100.0 | 27.20 | 100.0 |
| 1 | 11.63 | 99.7 | 9.88 | 98.1 | 5.36 | 98.0 | 26.87 | 98.8 |
| 2 | 11.57 | 99.2 | 9.79 | 97.2 | 5.56 | 101.6 | 26.92 | 99.0 |
| 3 | 11.36 | 97.4 | 9.80 | 97.3 | 5.18 | 94.7 | 26.34 | 96.8 |
| 4 | 11.47 | 98.4 | 9.76 | 96.9 | 5.31 | 97.1 | 26.54 | 97.6 |

**Table 64** The stability data of Centellase® tablet after exposure to the ambient and accelerated conditions

| Storage period (months) | Centellase® tablet | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AS (mg) | %AS | MA (mg) | %MA | AA (mg) | %AA | Total (mg) | %Total content |
| Ambient condition | | | | | | | | |
| Initial | 10.92 | 100.0 | 9.82 | 100.0 | 4.12 | 100.0 | 24.86 | 100.0 |
| 1 | 10.72 | 98.2 | 10.26 | 104.5 | 4.05 | 98.4 | 25.04 | 100.7 |
| 2 | 10.82 | 99.0 | 9.99 | 101.8 | 4.18 | 101.6 | 24.99 | 100.5 |
| 3 | 10.85 | 99.4 | 9.99 | 101.8 | 3.75 | 91.1 | 24.59 | 98.9 |
| 4 | 10.71 | 98.1 | 9.57 | 97.4 | 4.23 | 102.7 | 24.51 | 98.6 |
| Accelerated condition | | | | | | | | |
| Initial | 10.92 | 100.0 | 9.82 | 100.0 | 4.12 | 100.0 | 24.86 | 100.0 |
| 1 | 11.23 | 102.8 | 10.60 | 108.0 | 4.24 | 103.0 | 26.08 | 104.9 |
| 2 | 10.20 | 93.4 | 9.87 | 100.5 | 3.92 | 95.0 | 23.98 | 96.5 |
| 3 | 10.24 | 93.7 | 9.33 | 95.0 | 3.51 | 85.2 | 23.07 | 92.8 |
| 4 | 10.96 | 100.4 | 9.56 | 97.4 | 4.27 | 103.6 | 24.79 | 99.7 |

**Table 65** The correlation coefficient ($R^2$) of the relation between the storage periods versus the percent remaining amount of the total active constituents from CST for testing the order kinetics

| Formulations | Conditions | $R^2$ | |
|---|---|---|---|
| | | zero order kinetics | first order kinetics |
| CST | ambient | | |
| | accelerated | 0.8975 | 0.9063 |
| Centellase® | ambient | 0.5924 | 0.5941 |
| | accelerated | 0.1997 | 0.1994 |
| Core tablet | ambient | 0.8003 | 0.8070 |
| | accelerated | 0.9591 | 0.9615 |
| CS3 | ambient | 0.7371 | 0.7357 |
| | accelerated | 0.8083 | 0.8087 |
| CS5 | ambient | 0.8786 | 0.8775 |
| | accelerated | 0.8786 | 0.8754 |
| CS10 | ambient | 0.8891 | 0.8883 |
| | accelerated | 0.1355 | 0.1335 |
| HPMC3 | ambient | 0.7864 | 0.7858 |
| | accelerated | 0.8254 | 0.8234 |
| HPMC5 | ambient | 0.8215 | 0.8223 |
| | accelerated | 0.9908 | 0.9894 |
| HPMC10 | ambient | 0.8405 | 0.8409 |
| | accelerated | 0.6862 | 0.6884 |
| HPMC15 | ambient | 0.5640 | 0.5590 |
| | accelerated | 0.9621 | 0.9603 |
| PMC1 | ambient | 0.8205 | 0.8218 |
| | accelerated | 0.7874 | 0.7863 |
| PMC3 | ambient | 0.3827 | 0.3802 |
| | accelerated | 0.4475 | 0.4464 |
| PMC5 | ambient | 0.3080 | 0.3071 |
| | accelerated | 0.7520 | 0.7500 |

# APPENDIX D

**Table 66** Weight variation of *Centella asiatica* core tablet and tablets coated with chitosan, HPMC and polymethacrylate at 5% coating level of scale-up production

| Tablet No. | Core tablet (mg) | Chitosan 3% (mg) | HPMC 3% (mg) | Polymethacrylate3% (mg) |
|---|---|---|---|---|
| 1 | 145.0 | 159.4 | 161.5 | 158.0 |
| 2 | 145.0 | 163.2 | 165.3 | 161.1 |
| 3 | 145.0 | 165.6 | 159.9 | 163.2 |
| 4 | 150.2 | 159.0 | 161.4 | 161.2 |
| 5 | 152.1 | 161.4 | 162.4 | 160.9 |
| 6 | 148.7 | 159.0 | 165.3 | 160.8 |
| 7 | 151.6 | 159.8 | 161.1 | 161.0 |
| 8 | 155.0 | 162.2 | 162.1 | 160.3 |
| 9 | 153.9 | 163.0 | 159.2 | 159.6 |
| 10 | 154.0 | 163.4 | 162.4 | 160.5 |
| 11 | 154.0 | 165.3 | 164.0 | 160.8 |
| 12 | 155.0 | 162.1 | 164.0 | 163.2 |
| 13 | 155.2 | 159.6 | 163.3 | 163.2 |
| 14 | 150.0 | 162.1 | 163.0 | 164.8 |
| 15 | 150.2 | 163.4 | 162.2 | 162.4 |
| 16 | 147.8 | 163.2 | 161.1 | 160.9 |
| 17 | 150.2 | 164.6 | 161.1 | 163.2 |
| 18 | 154.0 | 162.1 | 160.0 | 161.0 |
| 19 | 148.8 | 162.1 | 162.0 | 160.3 |
| 20 | 143.8 | 162.0 | 166.0 | 162.1 |
| Average | 150.5 | 162.1 | 162.4 | 161.4 |
| SD | 3.7 | 2.0 | 1.9 | 1.6 |
| %CV | 2.5 | 1.2 | 1.1 | 1.0 |

**Table 67** Hardness of core tablet and film coated tablet formulations at 5% coating level from scale-up production

| Tablet No. | Hardness (kp) | | | |
|---|---|---|---|---|
| | Core tablet | Chitosan | HPMC | Polymethacrylate |
| 1 | 4.5 | 11.80 | 10.30 | 4.70 |
| 2 | 4.2 | 10.80 | 10.20 | 7.10 |
| 3 | 5.2 | 9.70 | 12.00 | 5.20 |
| 4 | 4.3 | 9.60 | 8.90 | 5.30 |
| 5 | 5.3 | 10.20 | 11.00 | 5.40 |
| 6 | 3.8 | 10.30 | 10.00 | 4.20 |
| 7 | 4.6 | 12.00 | 9.60 | 3.80 |
| 8 | 7.0 | 11.00 | 9.60 | 5.30 |
| 9 | 4.3 | 8.90 | 12.00 | 6.20 |
| 10 | 4.1 | 9.20 | 11.00 | 4.20 |
| Average | 4.73 | 10.35 | 10.46 | 5.14 |
| SD | 0.92 | 1.05 | 1.03 | 0.99 |

**Table 68** Friability and disintegration of core tablets and film coated tablets at 5% coating level from scale-up production

| Test | Core tablet | Chitosan | HPMC | Polymethacrylate |
|---|---|---|---|---|
| Friability (%) | 0.13 | 0.00 | 0.00 | 0.00 |
| Disintegration (minutes) | 0.50 | 2.80 | 2.83 | 3.17 |

**Table 69** Statistical independent samples t-test for test of hardness and weight variation between small scale batch and scale-up batch

| Formulations | *P*-values | |
|---|---|---|
| | Hardness | Weight variation |
| Core | 0.681 | 0.123 |
| CS5 | 0.951 | 0.904 |
| HPMC5 | 0.333 | 0.420 |
| PMC5 | 0.813 | 0.286 |

# APPENDIX E

**Table 70** Statistical test for dissolution of film coated tablets among after freshly prepared, exposure to ambient condition and accelerated condition.

**Analysis of variance (ANOVA) and Scheffe test for post-hoc comparisons**

Dependent Variable: VAR00002
Scheffe

| (I) VAR00001 | (J) VAR00001 | Core | CS3 | CS5 | CS10 |
|---|---|---|---|---|---|
| | | Sig. | Sig. | Sig. | Sig. |
| Initial | Ambient | .281 | .306 | .413 | .530 |
| Initial | Accelerated | .006* | .032* | .122 | .064* |
| Ambient | Accelerated | .039* | .247 | .611 | .272 |

* The mean difference is significant at the .05 level.

| (I) VAR00001 | (J) VAR00001 | HPMC3 | HPMC5 | HPMC10 | HPMC15 |
|---|---|---|---|---|---|
| | | Sig. | Sig. | Sig. | Sig. |
| Initial | Ambient | .788 | .962 | .131 | .042* |
| Initial | Accelerated | .591 | .978 | .024* | .005* |
| Ambient | Accelerated | .282 | .888 | .409 | .187 |

* The mean difference is significant at the .05 level.

| (I) VAR00001 | (J) VAR00001 | PMC1 | PMC3 | PMC5 |
|---|---|---|---|---|
| | | Sig. | Sig. | Sig. |
| Initial | Ambient | .856 | .745 | .149 |
| Initial | Accelerated | .513 | .340 | .251 |
| Ambient | Accelerated | .812 | .724 | .912 |

**Table 71** Statistical test for dissolution of tablets coated with 3 and 5% coating level among chitosan, HPMC and polymethacrylate after freshly prepared, exposure to ambient condition and accelerated condition.

**Analysis of variance (ANOVA) and Scheffe test for post-hoc comparisons**

Dependent Variable: VAR00002
Scheffe

| (I) VAR00001 | (J) VAR00001 | Initial | | Ambient | | Accelerated | |
|---|---|---|---|---|---|---|---|
| | | 3% | 5% | 3% | 5% | 3% | 5% |
| | | Sig. | Sig. | Sig. | Sig. | Sig. | Sig. |
| Chitosan | HPMC | .344 | .988 | .692 | .386 | .275 | .041* |
| Chitosan | Polymethacrylate | .397 | .948 | .890 | .517 | .135 | .087 |
| HPMC | Polymethacrylate | .992 | .891 | .441 | .961 | .844 | .826 |

* The mean difference is significant at the .05 level.

# VITA

Soraya Hengsawas was born in Bangkok, Thailand, on May 12th, 1977. She received the degree of Bachelor of Science in Pharmacy in 1999 from Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. She has worked for the Department of Medical Sciences, Ministry of Public Health, Thailand.