รายงานวิจัย

# การพัฒนาผลิตภัณฑ์ทางเภสัชกรรมของสารสกัดจากเปลือกมังคุด

# (Formulation of Pharmaceutical Products of *Garcinia mangostana* Linn. Extracts)


โดย

คณะผู้วิจัย

รองศาสตราจารย์ ดร.สุชาดา ชุติมาวรพันธ์

รองศาสตราจารย์ ดร.ชัยโย ชัยชาญทิพยุทธ์

รองศาสตราจารย์อารีรัตน์ ลออปักษา

คณะเภสัชศาสตร์

จุฬาลงกรณ์มหาวิทยาลัย



งานวิจัยนี้ได้รับทุนอุดหนุนการวิจัยจากงบประมาณแผ่นดิน

ปีงบประมาณ 2546-2549

RESEARCH REPORT

# FORMULATION OF PHARMACEUTICAL PRODUCTS OF *GARCINA MANGOSTANA* LINN. EXTRACTS

BY

Assoc.Prof. Suchada Chutimaworapan. Ph.D.

Assoc.Prof. Chaiyo Chaichantippayuth, Ph.D.

Assoc.Prof. Areerat Laorpaksa

Faculty of Phamaceutical Sciences

Chulalongkorn University

# กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบพระคุณอาจารย์ ทันแพทย์ ดร.กิตติ ต.รุ่งเรือง ภาควิชาปริทันตวิทยา คณะทันตแพทย์ศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ที่อนุเคราะห์ให้เชื้อ *Streptococcus mutans* $KPSK_2$ ซึ่งใช้ในการวิจัย ขอขอบพระคุณภาควิชาเภสัชเวทและภาควิชาจุลชีววิทยาที่ให้ความสะดวกแก่ผู้วิจัยในการใช้เครื่องมืออุปกรณ์การวิจัยและสถานที่วิจัย ขอขอบพระคุณภาควิชาเภสัชกรรมในความเอื้อเฟื้อเครื่องมืออุปกรณ์สถานที่ และความช่วยเหลือและอำนวยความสะดวกของเจ้าหน้าที่ของภาควิชา และความช่วยเหลือในงานวิจัยของผู้ช่วยวิจัยซึ่งเป็นนิสิตปริญญาโทของภาควิชาเภสัชกรรม คือ นางสาววรทัย ดัน นางสาวนิตยา รวมกิจธรรม และนางสาวปิยภัค หิรัญัศมิ์ที่ได้ช่วยเหลือทำให้การวิจัยเป็นไปด้วยดี

คณะผู้วิจัยขอขอบพระคุณบริษัทฮงฮวด จำกัดที่ให้ความอนุเคราะห์บริจาคสารเคมีที่ใช้ในการวิจัย ได้แก่ glyceryl monooleate และขอขอบพระคุณบริษัทสยามหาร์มาซูติคอล จำกัดในความอนุเคราะห์บริจาคสาร Hydroxypropyl methylcellulose, low M.W.

ผู้วิจัยขอขอบพระคุณคณะกรรมการวิจัยแห่งชาติ ที่ได้อนุมัติเงินทุนวิจัยงบประมาณแผ่นดินประจำปีงบประมาณ 2546-2548 ทำให้การวิจัยเป็นผลสำเร็จลุล่วงเป็นอย่างดี

# ส่วนที่ 1 การสกัด การตรวจสอบเอกลักษณ์ การวิเคราะห์เชิงคุณภาพและเชิงปริมาณ และฤทธิ์ทางจุลชีววิทยาของสารสกัดเปลือกมังคุด

## บทคัดย่อ 1

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาวิธีการเตรียมสารสกัดเปลือกมังคุดด้วยวิธีที่ง่ายและสะดวก สามารถให้ผลผลิตที่สูง สามารถสกัดโดยการหมักแช่ผงเปลือกแห้งโดยใช้เอทิลอะซีเทตได้สารสกัดเป็นรูปผงผลึกสีเหลือง คาเปอร์เซ็นต์ผลผลิตเท่ากับ 7.47% ตรวจสอบเอกลักษณ์พบว่าสารสกัดมีปงค์ประกอบหลักคือ แมงโกสตินเมื่อศึกษาเทียบกับสารแมงโกสตินมาตรฐานโดยทินเลเยอร์โครมาโตกราฟีและดิฟเฟอเรนเชียลสแกนนิงคาลอรีเมตรี โดยการใช้แผ่นอะลูมินาและใช้สารละลายเอทิลอะซีเทต: เฮกเซน (3:1) ได้ค่าอาร์เอฟของสปอตที่เทียบเท่ากับสารแมงโกสตินมาตรฐานเท่ากับ 0.60 เทอโมแกรมจากดิฟเฟอเรนเชียลคาลอรีเมตรีแสดงว่าสารสกัดอยู่ในสภาวะรูปผลึกมีจุดหลอมเหลวกว้างมีค่าระหว่าง 165.04-166.80 องศาเซลเซียส การวิเคราะห์โดยไฮเพร์ฟอร์มานซ์ลิควิดโครมาโทกราฟีใช้ส่วนผสมเมทานอล:น้ำ (87:13) เป็นวัฏภาคเคลื่อนที่และใช้โคลไทรมาโซลเป็นสารมาตรฐานภายในและตรวจสอบวัดการดูดกลืนแสงที่ช่วงคลื่น 243 นาโนเมตร เมื่อตรวจสอบความถูกต้องของวิธีวิเคราะห์ พบว่าวิธีมีความจำเพาะ ความเป็นเส้นตรง ความถูกต้องและความแม่นยำสูง สำหรับการวิเคราะห์โดยวิธีอัลตราไวโอเลตสเปกโทรโฟโตเมตรีที่ความยาวคลื่น 243 นาโนเมตร เมื่อตรวจสอบความถูกต้องของวิธีวิเคราะห์ พบว่าวิธีมีความจำเพาะ ความเป็นเส้นตรง ความถูกต้องและความแม่นยำสูง การศึกษาคุณสมบัติของสารสกัดด้านการละลายพบว่า สารสกัดมีการละลายน้ำต่ำและการละลายจะเพิ่มมากขึ้นเมื่อมีสัดส่วนของเอทานอลในน้ำเพิ่มมากขึ้น
การศึกษาฤทธิ์ทางจุลชีววิทยาแบบนอกกายต่อเชื้อ *Staphylococcus aureus* ATCC 25923 และ *Streptococcus mutans* ATCC พบว่ามีฤทธิ์ในการต้านเชื้อแบคทีเรียทั้งสองได้ดี ได้ค่าความเข้มข้นต่ำสุดในการยับยั้งเชื้อเท่ากับ 3 และ 1.5 ไมโครกรัม/มิลลิลิตร ตามลำดับ ความเข้มข้นต่ำสุดในการฆ่าเชื้อเท่ากับ 4 และ 3 ไมโครกรัม/มิลลิลิตร ตามลำดับ

## PART I: Extraction, Identification, Quanlitative and Quantitative Analysis And Antibacterial Activity of *Garcinia mangostana* Husk Extract

### ABSTRACT 1

The purpose of the investigation was to develop the extraction process that was simple, practical and giving high yield. The maceration of dried powder of *Garcinia mangostana* fruit husk with ethyl acetate gave yellow crystalline powder of mangostin. The yield was calculated as 7.47%. The identification of the *Garcinia mangostanahusk* extract was carried out by thin-layer chromatography (TLC) and differential scanning calorimetry. The TLC of mangostin was done by using the alumina sheet and ethyl acetate: hexane (3:1) as mobile phase. The Rf value as compared with standard mangostin was 0.60. The DSC thermogram showed the board melting range of the crude extract at 165.04-166.80 $^{o}$C. The quantitative analyses of mangostin were developed using the high performance liquid chromatography (HPLC) and ultraviolet (UV) spectrophotometry. The HPLC system using methanol: water (87:13) as mobile phase, clotrimazole as internal standard and using UV detector at 243 nm. The UV spectrophotometric method was carried out using the UV spectrophotometer at 243 nm. The validation of both systems gave high specificity, linearity, accuracy and precision. The solubility study of mangostin showed the low water insolubility. The water solubility was improving with increasing ethanol content. The in vitro microbiological activity of mangostin to *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$was studied. The minimum inhibitory concentrations of the extract were 3 μg/ml and 1.5 μg/ml, respectively. The minimum bactericidal concentrations of the extract was 4 μg/ml and 3 μg/ml, respectively.

## ส่วนที่ 2 การตั้งตำรับแผ่นอมชนิดละลายเร็วในช่องปากของสารสกัดจากเปลือกผลมังคุด

### บทคัดย่อ 2

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาตำรับแผ่นอมชนิดละลายเร็วในช่องปากของสารสกัดจากเปลือกผลมังคุดเพื่อยับยั้งแบคทีเรียในช่องปาก โดยใช้สารก่อฟิล์มชนิดละลายน้ำที่มีความหนืดต่ำได้แก่ ไฮดรอกซิโพรพิลเมทิลเซลลูโลส (เอชพีเอ็มซี) และ ไฮดรอกซีโพรพิลเซลลูโลส (เอชพีซี) และประกอบด้วยสารปรุงแต่งอื่นๆในตำรับเช่น สารให้ความหวานคือ อะซิซัลเฟมโพแทสเซียม สารแต่งกลิ่นรส คือ เมนทอลและน้ำมันยูคาลิปตัส โดยศึกษาถึงผลของสารก่อฟิล์มต่อคุณสมบัติทางกายภาพ คุณสมบัติเชิงกลและเวลาในการละลายของแผ่นอม เปรียบเทียบกับผลิตภัณฑ์ทางการค้ารูปแบบฟิล์ม เอ ซึ่งเป็นแผ่นอมชนิดละลายเร็วที่มีขายในท้องตลาด จากการศึกษาเวลาในการละลายพบว่าแผ่นอมเปล่าที่มีสารก่อฟิล์มผสมระหว่างเอชพีเอ็มซี 3 ซีพีเอส และเอชพีซี แอลวี ในสัดส่วน 2:1 3:1 4:1 และ 5:1 มีค่าไม่แตกต่างกับผลิตภัณฑ์ทางการค้ารูปแบบฟิล์ม เอ อย่างมีนัยสำคัญทางสถิติ ($p$>0.05) จากการศึกษาสแกนนิงอิเล็กตรอนไมโครสโคปี พบว่าแผ่นอมที่มีสารสกัดจากเปลือกผลมังคุดมีพื้นผิวเป็นรูพรุน การปลดปล่อยยารวดเร็วมากกว่า 80 เปอร์เซ็นต์ ภายในเวลา 3-7 นาที โดยสูตรที่มีการปลดปล่อยยาเร็วที่สุดคือ สูตรผสมระหว่างเอชพีเอ็มซี 3 ซีพีเอส และเอชพีซี แอลวี ในสัดส่วน 5:1 ผลจากดิฟเฟอเรนเชียลสแกนนิงแคลอรีเมทรีแสดงให้เห็นว่าสารสกัดจากเปลือกผลมังคุดและสารปรุงแต่งอื่นๆในตำรับไม่อยู่ในรูปผลึก การศึกษาฤทธิ์ต้านจุลินทรีย์ในหลอดทดลองของแผ่นอมที่มีสารสกัดพบว่าสามารถต้านแบคทีเรียในช่องปากและฟัน ได้แก่ *สแตปฟิโลคอกคัส ออเรียส* เอทีซีซี 25923 และ *สเตรปโตคอกคัส มิวแตนส์* เอทีซีซี เคพีเอสเค$_2$ ในการศึกษาความคงตัวภายใต้สภาวะเค้นที่อุณหภูมิ 40 องศาเซลเซียส ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ พบว่าแผ่นอมมีความคงตัวดี

## PART II : FORMULATION OF FAST DISSOLVING ORAL STRIPS CONTAINING *GARCINIA MANGOSTANA* HUSK EXTRACT


### ABSTRACT 2

The purpose of this study was to develop fast dissolving oral strips containing *Garcinia mangostana* husk extract. The films consisted of low viscosity hydrophilic polymers such as hydroxypropyl methylcellulose and hydroxypropylcellulose, acesulfame potassium as sweetener, and menthol and eucalyptus oil as flavoring agents. The physical and mechanical properties and dissolution time of film bases were compared with commercial product strips A. From the dissolution time data, it was found that the film prepared from mixed polymer between HPMC 3 cps and HPC LV at ratios 2:1, 3:1, 4:1 and 5:1 were not significantly different from commercial product strips A ($p>0.05$). The films containing extract were light yellow and had porous surface based on observation from scanning electron microscopy. The dissolution profiles of all formulations showed the rapid release more than 80 percent of mangostin from films within 3-7 minutes and the fastest release was from formulation of HPMC 3 cps and HPC LV at ratio 5:1. Differential scanning calorimetry results exhibited that the *Garcinia mangostana* extract and additives were not in crystalline form in the films. The fast dissolving oral strips containing *Garcinia mangostana* husk extract showed *in vitro* antimicrobial activity against oro-dental bacteria, namely, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$. Under stress conditions at 40 degree Celcius and 75 percent relative humidity, the strips showed a good stability.

## ส่วนที่ 3 การพัฒนาระบบนำส่งยาที่ใช้โมโนกลีเซอไรด์ (ลิควิดคริสตัล) ที่ผสมสารสกัดเปลือมังคุด

### บทคัดย่อ 3

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาระบบนส่งยาที่ใช้โมโนกลเซอไรด์ผสมสารสกัดเปลือกมังคุด ระบบเตรียมจากส่วนผสมของโมโนกลิเซอไรด์ (กลิเซอริลโมโนโอลีเอต) และไดรกลิเซอไรด์ ทำให้เกิดลิควิดคริสตัลขึ้นเมื่อผสมกับน้ำ ระบบนำส่งยาสามารถให้แก่ผู้ป่วยโดยกาฉีดผ่านไซริงค์ โดยจะเปลี่ยนสภาพเป็นวัตภาคลิควิดคริสตัลที่หนืดข้นเมื่อฉีดเข้าที่บริเวณที่ต้องการ การสร้างแผนภูมิไดรภาคโดยใช้สารไดรกลิเซอไรด์ชนิดต่างๆ ได้แก่ น้ำมันงา น้ำมันถั่ว และน้ำมันมะกอก จากการศึกษานี้พบว่า ระบบนส่งยาที่ใช้มโนกลิเซอไรด์นี้สามารถเตรียมได้จากส่วนผสม ไดรกลิเซอไรด์: โมโนกลิเซอไรด์: น้ำ เท่ากับ 8:62:30 และ 12:58:30 ระบบนี้สามารถปลดปล่อยยาได้นาน 48 ชั่วโมงและเป็นไปตามจนศาสตร์การปลดปล่อยแบบรากที่สองของเวลาในช่วงการปลดปล่อยใน 24 ชัวโมแรก แสดงถึงการปลดปล่อยที่ถูกควบคุมโดยกระบวนการแพร่ออกของสาร ระบบที่ใช้น้ำมันงาแสกงการปลดปล่อยได้สูงสุด การเพิ่มปริมาณไดรกลิเซอไรด์ไม่มีผลต่อรูปแบบการปลดปล่อย ผลจากดิฟเฟอเรนเชียลสแกนนิงคาลอรีเมตรีแสดงว่าสามารถผสมสารสกัดเปลือกมังคุดเข้าในระบบนำส่งยาโดยไม่เกิดการแยก วัฏภาค ในการทดสอบฤทธิ์ทางจุลชีววิทยาแบบนอกกายของระบบนำส่งยาที่ใช้โมโนกลิเซอไรด์ที่ผสมสารสกัดเปลือกมังคุดโดยวิธีการแพร่ผ่านในอะการ์ ไม่แสดงฤทธิ์ในการด้านเชื้อแบคทีเรีย อาจจะเนื่องจากตัวยาไม่สามารถแพร่ผ่านอะการ์ที่มีความชอบน้ำแตกต่างจากสารสกัดเปลือกมังคุด การ ศึกษา ความคงตัวของระบบนำส่งยาที่ใช้โมโนกลิเซอไรด์ที่ผสมสารสกัดเปลือกมังคุดนี้มีพบว่ามีความคงตัวภายใต้สภาวะเค้น

## PART III FORMULATION OF MONOGLYCERIDE-BASED DRUG DELIVERY SYSTEMS (LIQUID CRYSTALLINE) CONTAINING *GARCINIA MANGOSTANA* HUSK EXTRACT

### ABSTRACT 3

The purpose of the study was to develop monoglyceride-based drug delivery systems containing *Garcinia Mangostana* extract. The system is based on the ability of mixtures of monoglyceride (glyceryl monooleate) and triglycerides to form liquid crystals upon contact with water. The drug delivery systems can be administered by syringe and transformed into high-viscous liquid crystalline phases at the injection site. Ternary phase diagrams were constructed from various triglycerides: sesame oil, soybean oil and olive oil. In this study, monoglyceride-based drug delivery systems were prepared in the ratio of triglycerides: monoglyceride: water as 8: 62: 30 and 12: 58: 30. These systems could sustain release of *Garcinia Mangostana* husk extract over a period of 48 hr and followed squared root of time kinetics during the initial 24 hr of the release phase, indicating that the rate of release was diffusion-controlled. The system containing sesame oil showed the highest drug release. The increasing triglyceride content did not affect the release profiles. Differential scanning calorimetry results demonstrated that *Garcinia Mangostana* husk extract could be incorporated into drug delivery systems without causing phase transition. In the *in vitro* test, monoglyceride-based drug delivery systems containing *Garcinia Mangostana* husk extract did not show the antimicrobial activity probably due to the high lipophilicity of the extract therefore it did not diffuse into the medium. Additionally, the drug delivery systems containing *Garcinia Mangostana* husk extract showed good stability under the stress condition.

# TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ANOVA | = | analysis of variance |
| $^{o}C$ | = | degree Celcius |
| cm | = | centimeter |
| $cm^2$ | = | square centimeter |
| conc | = | concentration |
| cps | = | centipoises |
| CV | = | coefficient of variation |
| df | = | degree of freedom |
| E | = | hydroxypropyl methylcellulose |
| et al. | = | and others |
| g | = | gram |
| hr | = | hour |
| HPLC | = | high performance liquid chromatography |
| HPMC | = | hydroxypropyl methylcellulose |
| HPC LV | = | hydroxypropyl cellulose low viscosity |
| k | = | release rate constant |
| MBC | = | minimal bactericidal concentration |
| mg | = | milligram |
| MIC | = | minimal inhibitory concentration |
| min | = | minute |
| ml | = | milliliter |
| mm | = | millimeter |
| $mm^2$ | = | square millimeter |
| mPas | = | millipascal.second |
| N | = | Newton |
| No. | = | number |
| nm | = | nanometer |
| $R^2$ | = | coefficient of determination |
| RH | = | relative humidity |
| rpm | = | revolution per minute |
| RSD | = | Relative standard deviation |
| sec | = | second |
| SD | = | standard deviation |
| SS | = | sum of square |
| TLC | = | thin layer chromatography |

| | | |
|---|---|---|
| UV | = | ultraviolet |
| VR | = | variance ratio |
| v/v | = | volume by volume |
| w/w | = | weight by weight |
| mcg, µg | = | microgram |
| µl | = | microliter |
| µm | = | micrometer |

# CHAPTER I

# INTRODUCTION

## Backgrounds and Rationale

Nowadays, active substances derived from herbal plants have a major role in both pharmaceutical and cosmetic industries. Many products from natural substances were developed under new drug delivery technologies for added valuable cost. Since the sources of the herbal plants are commonly found, it is possible to produce a high quantity with low cost.

*Garcinia mangostana* Linn. or mangosteen is one of the medicinal plants that have been used for a long time for treatment of both normal and infected wounds. It was also used for diarrhea and dysentery (Fransworth and Bunyapraphatsara, 1992). Phytochemical studies indicated that the fruit rinds contain various xanthones and mangostin is the most active component. These xanthones show many considerable pharmacological activities such as antimicrobial, anti-inflammatory, antioxidative, antihistamine, antiserotonin and antitumor activities (Wong, 2002). Previous studies showed that the extract from this plant exhibited significant antibacterial activities against *Streptococcus mutans*, bacteria causing dental caries and dental plaque and *Porphyromonas gingivalis*, bacteria that causes periodontitis and oral malodor (Tan, 2004).

Oral hygiene has primary purpose to reduce or counteract the effects of bacterial populations which cause caries, malodor, staining and periodontal diseases. Oral cleansing and breath freshening practices should be conducted repeatedly throughout the day. However, it may be inconvenient sometimes. Typical methods of oral cleansing and hygiene including brushing, flossing, gargling and tongue cleansing are well suited for the privacy of one's home (Leung, Leone, and Kumar, 2003).

As solution to these problems, a fast dissolving film with breath freshening and intraoral bacteria inhibitory benefits is a convenient delivery for oral cleansing and freshening breath. Fast dissolving films are the dosage forms that have been developed for providing pharmaceutical and/or cosmetic benefits. Fast dissolving films refer to the thin and flexible films that can rapidly dissolve in oral cavity within a few second or minutes, without the need to drink or chew (Xu et al., 2002; Kulkarni, Kumar, and Sorg, 2003). Additionally, there are many advantages for delivery of the medicines to pediatric and geriatric patients, who have difficulty in swallowing or chewing solid dosage forms.

Now, there are many successful reports in development of the fast dissolving films released into the market. One of them is film that claims for cosmetic benefit, breath freshening and inhibitory growth of bacteria in oral cavity such as Listerine® PocketPak. In another published research, the fast dissolving film containing salbutamol sulphate as pharmaceutical agent was developed for treatment of acute and chronic asthma (Mashru et al., 2005). Moreover, natural antiseptic agents such as magnolia bark extract and

cinnamaldehyde were delivered into the oral cavity using fast dissolving film preparation (Maxwell and Greenberg, 2004a, 2004b).

With applicable fast dissolving dosage form and application of mangosteen extract, orally fast dissolving film containing *Garcinia mangostana* extract was developed for oral hygiene products to provide antimicrobial and breath freshening effect. In this study, the investigation of appropriate polymers as film former was carried out for desirable dissolution time, mechanical properties, stability and inexpensiveness.

Liquid crystalline phases offer a number of useful properties for drug delivery. First, they allow drug solubilization; both water soluble and oil soluble drugs may be incorporated. Second, liquid crystalline phases display a high viscosity, which offer opportunities for drug localized such as in the oral cavity (Malmsten, 2002). Glyceryl monooleate or monoolein is a polar lipid which swells in water and gives rise to different kinds of liquid crystalline phases. The liquid crystalline phases formed by glyceryl monooleate have the potential of acting as an *in situ* forming drug delivery system. Moreover, glyceryl monooleate is a biodegradable and nontoxic material (Ganem-Quintanar, Quintanar-Guerrero and Buri, 2000). The glyceryl monooleate-water systems have been described as a sustained release carrier for both conventional and peptide or protein drugs. For periodontal delivery, there have been developed stable and sustained release formulations containing metronidazole and *Andrographis paniculata* extract for treatment of periodontal diseases (Norling et al., 1992; Komwatchara, 1996). The formulations were transformed to release-controlling and high-viscous liquid crystalline phases when contact with the gingival crevicular fluid. These studies suggested that addition of triglyceride into the glyceryl monooleate improved the flow properties of glyceryl monooleate and the formulations could be administered through a syringe into a periodontal pocket and was found to have the most favorable sustained release properties, compared to no addition.

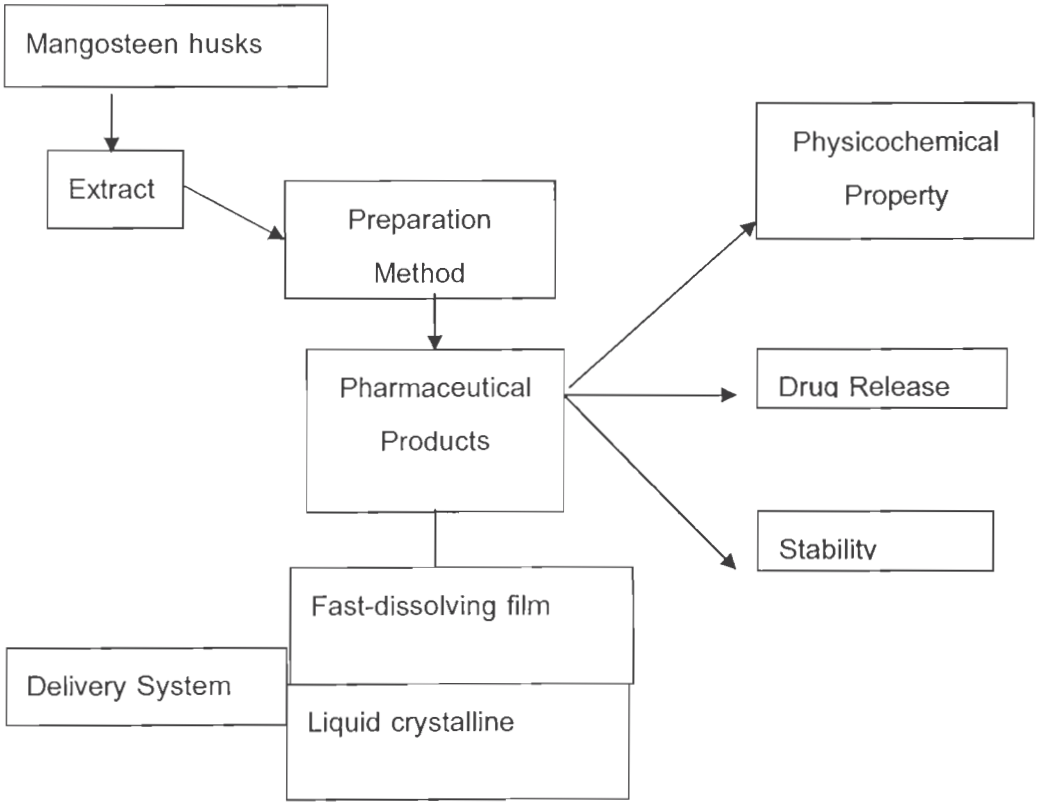
The successful results of the above study demonstrate an interesting application of glyceryl monooleate for periodontal drug delivery. In this study, a drug delivery system based on a mixture of monoglycerides and triglycerides containing *Garcinia mangostana* extract was developed. Triglycerides from vegetable oils such as sesame oil, soybean oil and olive oil were used in the study due to their desirable release, stability, inexpensiveness and easy availability.

**The purposes of this study were as follows:**

1. To develop orally fast dissolving films containing *Garcinia mangostana* extract.
2. To investigate the physicochemical properties of orally fast dissolving films with and without *Garcinia mangostana* extract.
3. To investigate the chemical stability of *Garcinia mangostana* extract in orally fast dissolving films.

4. To evaluate antimicrobial activity of orally fast dissolving films containing *Garcinia mangostana* extract.
5. To study the effects of different triglycerides on the ternary phase diagram.
6. To develop the formulation of monoglyceride-based drug delivery systems containing *Garcinia mangostana* extract.
7. To study the physicochemical properties and antimicrobial activity of *Garcinia mangostana* extract monoglyceride-based drug delivery systems

**Conceptual framework of the research project**



# LITERATURE REVIEW

## I Botanical, Chemical and Pharmacological Aspects of *Garcinia mangostana* Linn.

### 1. Botanical aspects of *Garcinia mangostana* Linn.

*Garcinia mangostana* Linn. is in the family of Guttiferae, its common name is mangosteen (Figure 1). It is a slow-growing tree usually progate by seeds and mostly proliferates in hot and humid climate, preferably with a short dry season such as in India, Thailand, Indonesia and Philippines.

*Garcinia mangostana* Linn. is a tree, 7-12 m high, having straight trunk, brown to blackish bark, young branch quadrangular, exuding yellow gum-resin. Inner bark exudes an opaque yellow gum resin. Leaves are simple without stipules and decussate, opposite, ovate or elliptic-oblong, 6-11 cm wide, 15-25 cm long, with dark green and grossy above, yellowish green below. Flowers are solitary or in pair near the twig ends, yellowish green with red edges or almost entirely red. Fruit is globose 4-7 cm in diameter, having short and thick stalk, dark purple with four persistent sepals at the base. Seed embedded into a thick, whitish, edible and juicy pulp (Fransworth, and Bunyapraphatsara, 1992; Wong, 2002).

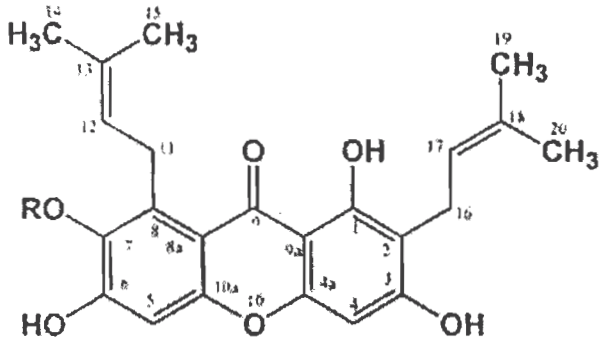
### 2. Chemical components of *Garcinia mangostana* Linn.

The chemical studies on the constituents of the fruit rind of *Garcinia mangostana* Linn. have revealed that the major substances are xanthones, the others component are cathecol triterpenoid, benzophenone (maclurin) and anthocyanin glycosides (Fransworth, and Bunyapraphatsara, 1992).

Figure 1 *Garcinia mangostana* fruit

Xanthones are highly active plant phenols found in a limited number of plants. They are formed by the condensation of a phenylpropanoid precursor. Hydroxyxanthones are mainly found in Guttiferae and Gentianaceae. Xanthones show considerable biological activities such as strong antimicrobial, anti-inflammatory and antitumor activities (Wong, 2002).

The major xanthones from fruit rind of *Garcinia mangostana* Linn. are α, β, and γ-mangostin, gartanin, 8-deoxygartanin and garcinones A, B, C and E, all of them have mono or diprenylated skeleton (Mahabusarakam, Wiriyachitra, and Tayler, 1987; Sen et al., 1980, 1981). Mangostin or α-mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis-(3-methyl-2-butenyl)-9*H*-xanthen-9-one; $C_{24}H_{26}O_6$), a prenylated xanthone is the most active component. It is isolated from various parts of *Garcinia mangostana*. Isolated from fruit rind by extracted with hexane or benzene, it appears as yellow crystals with melting point of 181.6 – 182.6 °C and molecular weight of 410.46. It is soluble in alcohol, ether, acetone, chloroform and ethyl acetate but practically insoluble in water. UV max in ethanol is 243, 259, 318 and 351 nm. (Mahabusarakam, Wiriyachitra, and Tayler, 1987; Budavari, 2001).




**R = $CH_3$** α-Mangostin
**R = H** γ-Mangostin

Figure 2 Chemical structure of α-mangostin and γ-mangostin

### 3. Pharmacological activities and toxicities of *Garcinia mangostana* Linn.

#### 3.1 Pharmacological activities

There are many investigations on pharmacological activities of xanthones from *Garcinia mangostana* Linn.

##### 3.1.1 Antimicrobial activity

There are many reports on the antimicrobial activity of extracts of fruit rind and other parts from *Garcinia mangostana.* Mangostin and its derivatives exhibited inhibitory effect against *Staphylococcus aureus*, bacteria causing wound infection (Mahabusarakam et al., 1983; Sundaram et al., 1983). It was found that the extracts have an intense antimicrobial activity against both methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) nearly equal to vancomycin (Fransworth, and Bunyapraphatsara, 1992; Iinuma et al., 1996; Voravuthikunchai and Kitpipat, 2005). Other studies showed that α-mangostin was found to be active against vancomycin resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), two leading causes of nosocomial infections (Phongpaichit et al., 1994; Sakagami et al., 2005).

The extract from *Garcinia mangostana* was tested for the activity against *Streptococcus mutans*, bacteria causing dental caries, and *Streptococcus sanguis* (a clinical isolate). The results showed that the extract exhibited significant antibacterial activity against both bacteria. (Hiranras, 2001; Tan, 2004).

The ethyl acetate extract from fruit hulls was determined antimicrobial effect against *Porphyromonas gingivalis* W50 with the MIC value of 20 μg/ml and the MBC value of 40 μg/ml. *Porphyromonas gingivalis*, which are gram-negative anaerobic bacteria generated volatile sulfur compounds caused oral malodor. Moreover, this bacteria has important role in periodontal diseases (Tan, 2004).

Moreover, the activity against dysenteric bacteria and diarrheal infecting bacteria such as *Shigella dysenteriae, Shigella sonnei, Escherichia coli, Salmonella typhimurium, Vibrio cholera, Pseudomonas aeruginosa* and *Bacillus subtilis* have been reported. (Sundaram et al., 1983; Sindermsuk and Deekijsermphong, 1989; Fransworth, and Bunyapraphatsara, 1992).

**3.1.2 Antimycobacterial activity**

Prenylated xanthones isolated from the fruit hulls, edible arils and seeds of *Garcinia mangostana* were tested for their antituberculosis potential, α-mangostin, β-mangostin and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the MIC value of 6.25 μg/ml (Suksamrarn et al., 2003).

**3.1.3 Antifungal activity**

Mangostin and its derivatives showed the activity against *Trichophyton mentagrophytes, Microsporum gypseum, Alternaria solani, Cunninghamella echinulata* and *Epidermophyton floccosum* but all test compounds had no effect on *Candida albicans*

(Fransworth, and Bunyapraphatsara, 1992; Mahabusarakam, Phongpaichit and Wiriyachitra, 1983; Sundaram et al., 1983). Moreover, the activity against three phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenuis* and *Dreschlera oryzae*, have been reported (Gopalakrishnan, Banumathi and Suresh, 1997).

**3.1.4 Anti-inflammatory activity**

Mangostin and its derivatives exhibited anti-inflammatory activity both by intraperitoneal and oral administration in normal and bilaterally adrenalectomized rats when tested by the carageenan-induced pedal edema, cotton pellet implantation and granuloma pouch technique (Fransworth, and Bunyapraphatsara, 1992).

Mangostin, 1-isomangostin and mangostin triacetate produced anti-inflammatory activity both by intraperitoneal and oral routes in rats when tested by carrageenin-induced hind paw edema, cotton pellet implantation and granuloma pouch techniques. These compounds did not produce any mast cell membrane stabilizing effect and the degranulation effect of polymixin B, diazoxide and Triton X-100 on rat peritoneal mast cells in vitro was not prevented. These compounds did not alter the prothrombin time of albino rats. Only mangostin produced significant anti-ulcer activity in rats (Shankaranarayan, Gopalakrishnan and Kasemswaran, 1979).

The crude extracts from fruit hulls were examined the effect on prostaglandin $E_2$ ($PGE_2$) synthesis. The study found that the 40% ethanol extracts potently inhibited A23187-induced prostaglandin $E_2$ synthesis in C6 rat glioma cells in a concentration-dependent manner (Nakatani, Atsumi et al., 2002). In further study, γ-mangostin was isolated and examined the effect on arachidonic acid cascade in C6 rat glioma cells. The results indicated that γ-mangostin exhibited potent inhibitory activity of prostaglandin E2 release induces by A23187, a $Ca^{2+}$ ionophore and competitively inhibited the activities of both constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2) in concentration-dependent manner (Nakatani, Nakahata et al., 2002). These results suggest that γ-mangostin is new useful lead compound for anti-inflammatory drug development.

**3.1.5 Antihistamine and antiserotonin activities**

A crude methanolic extract inhibited the contractions of isolated thoracic rabbit aortas induced by histamine and serotonin. The extract has been fractionated by siliga gel chromatography. The active compounds were α-mangostin and γ-mangostin. The results suggested that α-mangostin and γ-mangostin are a histaminergic and a

serotonergic receptor blocking agent, respectively. A further study revealed that α-mangostin completely inhibited not only the histamine $H_1$ receptor-mediated smooth muscle contraction but also the [$^3$H]mepyramine blocking directly to histamine $H_1$ receptor sites on intact smooth muscle cells. It is suggested that α-mangostin is novel type of histamine $H_1$ receptor antagonist and may become a valuable leading compound for the development of antihistamines (Chirungsrilerd, Furukawa et al., 1996a, 1996b).

The 40% ethanol extract of mangosteen fruit hull was examined the inhibitory effect on histamine release. The study found that the extract inhibited IgE-mediated histamine release from rat basophilic leukemia (RBL-2H3) cells displayed properties of mucosal-type mast cells. This result suggests that the mangosteen extract may be useful crude drug for treatment of allergy (Nakatani, Atsumi et al., 2002).

**3.1.6 Antioxidant activity**

The methanol extract of the fruit hulls was found to exhibit a potent radical scavenging effect. By monitoring the radical scavenging effect, two xanthones, α -mangostin and γ-mangostin were isolated. The antioxidative activity of these two xanthones was measured by the ferric thiocyanate method. It was found that γ-mangostin showed more potent antioxidative activity than BHA and α -tocopherol (Yoshikawa et al., 1994).

Mangostin was investigated the antioxidant effects on metal ion dependent ($Cu^{2+}$) and independent (aqueous peroxyl radicals) oxidation of human low density lipoprotein (LDL). Mangostin prolonged the lag time to both metal ion dependent and independent oxidation of LDL in a dose dependent manner. Formation of thiobarbituric reactive substances (TBARS), generated in LDL after oxidation, was inhibited with 100 microM of mangostin. Mangostin (100 μM) significantly inhibited the consumption of α-tocopherol in the LDL during $Cu^{2+}$ initiated oxidation. From these results, conclude that mangostin is acting as a free radical scavenger to protect the LDL from oxidative damage which is a critical role in cardiovascular and other chronic diseases (William et al., 1995). In further study, mangostin and structural modification of mangostin were tested the antioxidant activity on an isolates LDL and plasma assay. The results of this study show that structural modification of mangostin can have a profound effect on antioxidant activity. Derivatisation of C-3 and C-6 with aminoethyl derivatives enhances antioxidant activity, which may be related to change in solubility (Mahabusarakam, Proudfoot, and Croft, 2000).

### 3.2 Toxicities of *Garcinia mangostana* Linn.

Toxicological study of mangostin was performed by Sornprasit et al. (1987). The effect of mangostin on the activities of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) enzymes were tested by treated the rats with a high dose of mangostin (200 mg/kg body weight) by intraperitoneal injection. The activities of these two enzymes were increased and and reached the maximal level after 12 hr injection. The activities of enzymes were dose dependent. Another experiment in this study, the effect of mangostin which were forced fed to rats was compared with paracetamol in the same dose (1.5 g/kg body weight). It was found that paracetamol increased the activities of SGOT and SGPT much more than mangostin and the amounts of total liver protein of paracetamol treated rats decreased significantly, whereas mangostin treated rats did not change total liver protein. The results indicate that mangostin has lower hepatotoxicity than paracetamol.

Hepatotoxic effects of xanthones extracted from fruit rind of *Garcinia mangostana* were studied in isolated rat hepatocytes using the release of cellular transaminase (SGOT and SGPT) as the criteria for loss of cell membrane integrity. Malondialdehyde (MDA) formation, glutathione (GSH) content and aminopyrine N-demethylase activity were investigated for the preliminary information of xanthones hepatotoxic mechanism. Carbon tetrachloride ($CCl_4$) was selected as the reference hepatotoxin. Xanthones demonstrated the dose-related hepatotoxic effect, at 200 μg/ml, by increasing the release of cellular transaminases, decreasing MDA formation and GSH content with no change in aminopyrine N-demethylase activity. $CCl_4$ (0.08 mM) showed different result, especially the increase in MDA formation, illustrating its different hepatotoxic mechanism. Coadministration of xanthones and $CCl_4$ exhibited no additive cytotoxic effects. Pretreatment of xanthones (100 mg/kg/day, po., 5 days) prior to isolation of hepatocytes, caused no changes in transaminase activities and MDA formation with the increase in GSH content. In the presence of $CCl_4$, hepatocytes from xanthone and Tween (as the solvent used to dissolve xanthones) treated rats demonstrated higher production of MDA than the control hepatocytes. Lowering of GSH content by $CCl_4$ was also observed, with similar reduction in hepatocytes from the control and Tween treated groups. In xanthone treated hepatocytes, the GSH content was similar to control level after incubation with $CCl_4$. in conclusion, the hepatotoxic mechanism of xanthones may be different from $CCl_4$ and may involve cellular GSH level (Sapwarobol, 1997; Pramyothin, Sapwarobol and Ruangrungsi, 2003).

Additionally, It has been reported that the application of 1.5% mangostin cream in the patients with chronic ulcer, produced no harmful side effects, allergic condition and irritation (Panchinda, 1992).

**4. The medicinal uses of *Garcinia mangostana* Linn.**

*Garcinia mangostana* Linn. has traditionally been used for a long time for the treatment of wounds and diarrhoea. The medicinal uses are as follows:

1. Root may be treated for irregular menstruation.
2. Bark has been used for washing and healing wounds, treatment of aphthous ulcer and diarrhea.
3. Leaves are useful for treatment of dysentery.
4. Fruit rind is the astringent and has been used for treatment of both normal and infectious wounds including aphthous ulcer, it was also used for treatment of diarrhea and dysentery.

As the traditional recipes of *Garcinia mangostana* Linn. in treatment of diarrhea, the dried fruit rind was boiled with water or saturated calcium hydroxide solution and the extract is taken. For treatment of wounds, the dried fruit rind was rubbed with saturated calcium hydroxide solution as a solvent the suspension was applied over the wound area (Fransworth, and Bunyapraphatsara, 1992).

Considering pharmacological activities of *Garcinia mangostana*, this medicinal plant exhibits many interesting activities. The extracts from this plant can be developed into the pharmaceutical and cosmetic products. However, only few studies have been developed from this plant such as Hiranras (2001) designed the buccal mucoadhesive films for aphthous ulcer treatment. Panchinda (1992) evaluated the efficacy of 1.5% mangostin cream for chronic wound treatment and reported that it was effective for wound healing and no serious side effect. Kumjorn (2003) compared the healing of foot ulcers in diabetic patients by dressing with mangostin cream and normal saline wet dressing. The results showed that patients who received mangostin cream dressing treatment earned higher healing tares when compared with normal saline dressing treatment and recommended that the mangostin cream is new alternative dressing for promoting wound healing.

Table 1 Predominant cultivatable bacteria from various sites of the oral cavity.

| Type | Predominant Genus or Family | Total viable count (mean %) | | | |
|---|---|---|---|---|---|
| | | Gingival crevice | Dental plaque | Tongue | Saliva |
| **Facultative** | | | | | |
| Gram-positive cocci | Streptococcus | 28.8 | 28.2 | 44.8 | 46.2 |
| | *S. mutans* | (0-30) | (0-50) | (0-1) | (0-1) |
| | *S. sanguis* | (10-20) | (40-60) | (10-20) | (10-30) |
| | *S. mitior* | (10-30) | (20-40) | (10-30) | (30-50) |
| | *S. salivarius* | (0-1) | (0-1) | (40-60) | (40-60) |
| Gram-positive rods | *Lactobacillus* | 15.3 | 23.8 | 13.0 | 11.8 |
| | *Corynebacterium* | | | | |
| Gram-negative cocci | *Moraxella* | 0.4 | 0.4 | 3.4 | 1.2 |
| Gram-negative rods | *Enterobacteriaceae* | 1.2 | ND | 3.2 | 2.3 |
| **Anaerobic** | | | | | |
| Gram-positive cocci | *Peptostreptococcus* | 7.4 | 12.6 | 4.2 | 13.0 |
| Gram-positive rods | *Actinomyces* | 20.2 | 18.4 | 8.2 | 4.8 |
| | *Eubacterium* | | | | |
| | *Lactobacillus* | | | | |
| | *Leptotrichia* | | | | |
| Gram-negative cocci | *Veillonella* | 10.7 | 6.4 | 16.0 | 15.9 |
| Gram-negative rods | | 16.1 | 10.4 | 8.2 | 4.8 |
| | *Fusobacterium* | 1.9 | 4.1 | 0.7 | 0.3 |
| | Porphyromonas *(or* Prevotella*)* | 4.7 | ND | 0.2 | ND |
| | *Bacteroides* | 5.6 | 4.8 | 5.1 | 2.4 |
| | *Campylobacter* | 3.8 | 1.3 | 2.2 | 2.1 |
| **Spirochetes** | *Treponema* | 1.0 | ND | ND | ND |

Abbreviation: ND, Not detected.

**II Oral Conditions**

The oral cavity is an extremely complicated system contained within a complicated organism. Bacteria are the most numerous, but yeasts (*Candida albicans*) and protozoa (*Entamoeba gingivalis*, *Trichomonas tenax*) occur in many individuals. Oral bacteria include streptococci, micrococci and diphtheroids, together with *Actinomyces israeli* and other anaerobic bacteria. Some of these are able to make very firm attachments to mucosal surfaces and others to teeth which provide a long term, non- desquamating surface (Mims et al., 1995; Mandell, Bennett, and Dolin, 2000).

Essential influences upon oral condition such as oral cavity temperature, salivation, buffer capacity of the saliva, food intake, air flow and microbiological environment conditions. The oral conditions were as follows:

**1. Dental pellicle**

Within minutes to hours after a tooth has been thoroughly cleaned, dental pellicle, a film that deposits selectively from saliva, covers the tooth. It is this film to which the bacterial mass (known as dental plaque) adheres, and which stains when exposed to chromogenic materials. Dental pellicle consists of glycoproteins selectively absorbed from saliva. Toothbrushing alone is inadequate to remove pellicle and must be used in conjunction with an abrasive. Chemical removal is possible, but only at the risk of damaging the underlying enamel.

**2. Dental plaque**

Dental plaque is primarily a bacterial accumulation or complex microbial mass containing about $10^9$ bacteria per gram. It occurs supragingivally (above the gum line) and subgingivally (below the gum line). Dental plaque generates acid from carbohydrate. And this acid attacks the tooth enamel and initiates formation of the carious lesion. The actions of self-administered oral hygiene procedures are usually limited to the supragingival plaque. Subgingival plaque is best cared by the dental professional (Mims et al., 1995).

**3. Periodontal disease**

Periodontal disease is the general description applied to the inflammatory response of the gingiva and surrounding connective tissue to the bacterial of plaque accumulations on the teeth. These inflammatory responses are divided into two general groupings: gingivitis and periodontitis. Gingivitis is extremely common, and is manifested clinically as bleeding of the gingival or gum tissues without evidence of bone loss or deep periodontal pockets. Pocketing is the term given to the pathologic loss of tissue between the tooth and the gingival, creating

spaces that are filled by dental plaque. Periodontitis occurs when the plaque-induced inflammatory response in the tissue results in actual loss of collagen attachment of the tooth to the bone, to loss of bone and to deep periodontal pockets.

Table 2 Selected bacterial species found in dental plaque

| Types | Bacterias | |
|---|---|---|
| | Facultative | anaerobic |
| Gram positive | *Sreptococcus mutans* | |
| | *Streptococcus sanguis* | |
| | *Actinomyces viscosus* | |
| Gram negative | *Actinobacillus actinomycetemcomitans* | *Porphyromonas gingivalis* |
| | *Capnocytophaga species* | *Fusobacterium nucleatum* |
| | *Eikenella corrodens* | *Prevotella intermedia* |
| | | *Bacteroids forsythus* |
| | | *Campylobacter species* |
| Spirochetes | | *Treponema denticola* |

The microorganisms involved in periodontal disease are largely gram-negative anaerobic bacilli with some anaerobic cocci and a largely quantity of anaerobic spirochetes. The main organisms linked with deep destructive periodontal lesions are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Actinomyces viscosis* and *Actinobacillus actinomycetemcomitans*. *Porphyromonas gingivalis* is more frequency detected in severe adult periodontitis.

**4. Dental caries**

Dental caries, which is also called tooth decay, is the gradual destruction of enamel of tooth and thus open a path for bacteria to reach the pulp. *Streptococcus mutans* is important gram positive bacteria that cause dental caries. It is able to form acids by fermenting dietary sugars and maintain sugar metabolism under extreme acidic environment such as carious lesion. The pH in an active caries lesion may be as low as 4.0. Unless the bacteria, the sugar are present, dental caries does not develop (Mims et al., 1995).

Table 3 Relationship between clinical forms of periodontal disease and various bacterial species

| Clinical entity | Bacterial factor |
|---|---|
| **Gingivitis** | |
| Experimantal | Plaque accumulation, streptococci, actinomycetes |
| Pregnancy | *Prevotella intermedia* |
| Puberty | *Prevotella intermedia* |
| Stress (acute necrotizing ulcerative gingivitis) | *Prevotella intermedia*, Spirochetes |
| Simple | Plaque accumulation, Spirochetes |
| Generalized severe | Spirochetes |
| **Periodontitis** | |
| Prepuberty | Spirochetes, black-pigmented species |
| Localized juvenile | *Actinobacillus actinomycetemcomitans*, Spirochetes |
| Early onset, adult and progressive | Spirochetes, *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola* |

Both dental caries and periodontal disease are infectious diseases, caused by dental plaque. Therefore, elimination of bacteria in dental plaque is an essential factor in prevention and treatment of the diseases. Clinical study indicated that scaling and root planning, in combination with optimal oral hygiene, results in an alteration of the subgingival plaque which is sufficient to stop periodontal destruction. Thus oral hygiene is important for the clinical outcome of the treatment.

**5. Oral malodor**

Oral malodor is also commonly known as "bad breath," "foul breath," or "halitosis." It has been estimated that 9 of 10 persons exhibit oral malodor on arising in the morning. In healthy persons, over 90% of the malodors are produced by local oral conditions. "Morning mouth" has been attributed to reduced activity of the tongue and cheeks during sleep, along with reduced salivary flow, all of which enhance activity of the oral bacterial flora, some of which generate volatile, odoriferous sulfur compounds. These compounds consist primarily of hydrogen sulfide, methyl mercaptan, and dimethyl sulfide (Pader, 1988; Williams and Schmitt, 1996).

Oral microorganisms that play an important role in the production of malodor were *Peptostreptococcus, Eubacterium, Selenomonas Bacteroides*, and *Fusobacterium*. From these species, specific microorganisms such as *Porphyromonas gingivalis, Treponema Denticola,* and *Porphyromonas endodontalis* tend to be associated with periodontitis infections and are rarely found in a healthy mouth.

Mouth malodor can be controlled by vigorous oral hygiene practice. Most of the hydrogen sulfide and methyl mercaptan in periodontitis-free individuals derive from the dorsoposterior surface of the tongue and can be considerably reduced by tongue brushing, tooth brushing, food ingestion and use of an oral rinse that reduces oral bacterial populations.

## III Drug Selivery Systens

### III.A Fast Dissolving Drug Delivery

Many pharmaceutical dosage forms are administered in the form of tablets and capsules that designed for swallowing or chewing to deliver a precise dosage of medication to patients. However, some patients, particularly pediatric and geriatric patients, have difficulty in swallowing or chewing solid dosage forms. In order to assist these patients, several fast dissolving drug delivery systems have been developed. Fast dissolving drug delivery systems are dosage forms which dissolve or disintegrate rapidly upon contact with the moist mucosal surfaces of the oral cavity without the need for water or chewing.

**1. Types of fast dissolving dosage forms**

Fast dissolving dosage forms were divided in 2 major units as fast dissolving tablet and fast dissolving films/strips.

**1.1 Fast dissolving tablet (Rathbone, Hadgraft, and Roberts, 2003)**

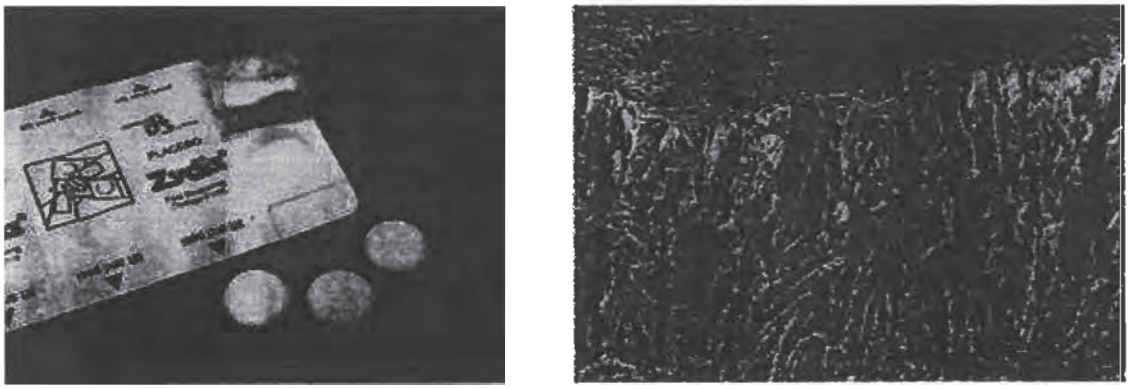
The oral fast dissolving tablets are also known as fast dispersing and quick disintegrating tablets, however the function and concept of all these dosage forms are similar. The original "fast dissolving tablets" are the molded tablets for sublingual use. These tablets generally consist of active drug and lactose moistened with an alcohol-water mixture to form a paste. The tablets were then molded, dried and packaged.

There currently are no standards that define a "rapidly dissolving tablet" but one that could be considered would be a tablet that disintegrates/dissolves within approximately 15-30 seconds in the mouth; anything slower than about 15-30 seconds would not really be categorized as "rapidly" dissolving (Ansel, Allen, and Popovich, 1999).

Zydis is the first fast dissolving dosage form in the market. This technology is based on the concept of forming an open matrix network containing active ingredient. These are freeze-dried products containing water soluble matrix material and drug, which is performed in blister pockets and freeze dried to remove the water by sublimation. The resultant structures are very porous in nature and rapidly disintegrate or dissolve upon contact with saliva (Kuchekar, Bhise and Arumugam, 2001).

There are a number of disadvantages and difficulties associated with formulating rapidly dissolving tablets, including drug loading, taste masking, manufacturing costs and stability of the product. In addition, many fast dissolving tablets are soft, friable, and/or brittle (such as the lyophilized dosage forms) so they often required specialized and individually expensive packaging and processing due to lacking the mechanical strength common to traditional tablets and more susceptible to degradation via temperature and humidity. These products can be manufactured by a variety of technologies, including direct compression, wet granulation, and freeze drying.

Currently, four fast-dissolving/disintegrating technologies have reached the U.S. market. Each technology has a different mechanism and listed in Table 4.



(a) (b)

Figure 3 (a) Zydis dosage form and blister pack. (b) Scanning electron micrograph of cross section through a Zydis formulation.

Table 4 Comparison of fast dissolving/disintegrating technologies

| Technologies | Manufacturing | Handling/stroage | Drug release /bioavailability |
|---|---|---|---|
| Zydis (R.P. Scherer, Inc.) | Freeze dried | - Packaged in foil<br>- Sensitive to degradation at humidities > 65% | - Dissolves in 2 to 10 seconds<br>- May allow for pregastric absorption leading to enhanced bioavailability |
| Wowtab (Yamanouchi pharma technologies, Inc.) | Compressed | - Packaged in bottles<br>- Avoid exposure to moisture or humidity | - Disintegrates in 5 to 45 seconds depending upon the size of the tablet<br>-No significant change in drug bioavailability |
| Orasolv (Cima labs, Inc.) | Lightly compressed | packaged in patented foil packs | -Disintegrates in 5 to 45 seconds depending upon the size of the tablet<br>-No significant change in drug bioavailability |
| Durasolv (Cima labs, Inc.) | Similar to orasolv | - Packaged in foil or bottles<br>- If packaged in bottles | - Disintegrates in 5 to 45 seconds depending upon the size of the tablet<br>-No significant change in drug bioavailability |


Figure 4 Fast dissolving tablet containing acetaminophen and caffeine (http://www.excedrin.com)

### 1.2 Fast dissolving films/strips

Fast dissolving film is a thin, flexible, and quick dissolving film in oral cavity. The film is placed on the top or the floor of the tongue and is retained at the site of application. It is rapidly release the active agent for local and/or systemic absorption. The fast dissolving film is the system that can deliver both pharmaceutical and cosmetic substances. Therapeutic effect of film is to deliver either water soluble or insoluble drug in the oral cavity without the need for water or chewing. Breath freshening films delivering volatile oils have antimicrobial effect by inhibiting microorganisms in oral cavity and enhanced breath freshening during day (Kulkarni, Kumar and Sorg, 2003).

The first step in the development of fast dissolving films is the selection and characterization of the appropriate film forming polymers that give the rapidly dissolving or disintegrating characteristic. The polymers that are commonly used for the development of fast dissolving films including low viscosity grade of cellulose derivatives (e.g. Sodium carboxymethylcellulose, hydroxypropylcellulose, and hydroxypropyl methylcellulose), polysaccharides from bacteria as pullulan, and synthetic polymers such as polyvinyl alcohol (Zerbe and Al-Khalil. 2003).

The fast dissolving films can be manufactured by one or a combination of following processes; hot melt extrusion, solid dispersion extrusion, rolling, semisolid casting, and solvent casting. These fast dissolving oral delivery systems can be packed using various options, such as single pouch, blister card with multiple units, multiple unit dispenser, and continuous roll dispenser, depending on the application and marketing objectives (Figures 8-12) (Borsadia, Halloran and Osborne, 2004).

Drug release from fast dissolving film (Rapifilm®) with the various drug substances such as ambroxol HCl, dextromethorphan HBr, metoclopramide HCl, ketotifen hydrogenfumarate and loperamide HCl were investigated by Mertin (1998). *In vitro* dissolution study showed that the drug was released completely within 2 to 3 minutes

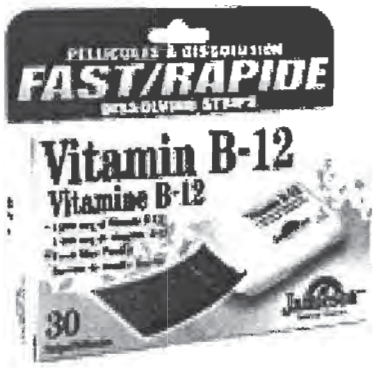



Figure 5 Fast dissolving films containing vitamin $B_{12}$ (http://www.jamiesonvitamins.com)

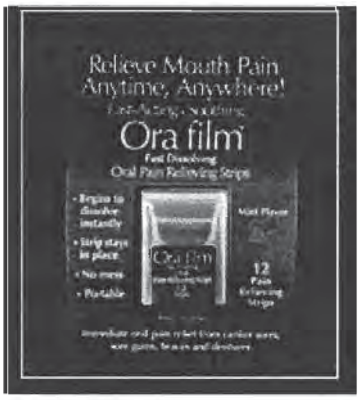



Figure 6 Fast dissolving films for relieve oral pain (http://www.apothecus.com)


(a)


(b)

Figure 7 Fast dissolving films containing medicine; (a) dextromethophan HBr; (b) diphenhydramine HCl (http://www.triaminic.com)

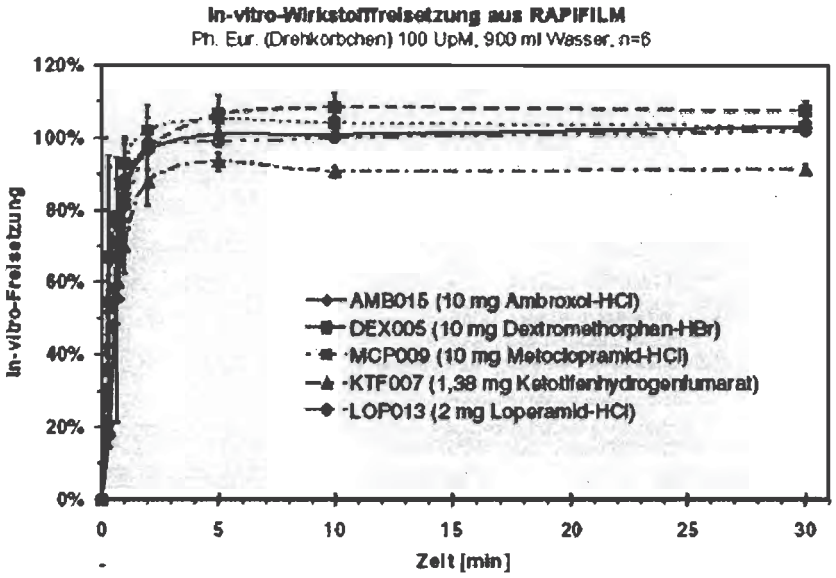



Figure 8 *In vitro* drug dissolution from Rapifilm® containing various drugs

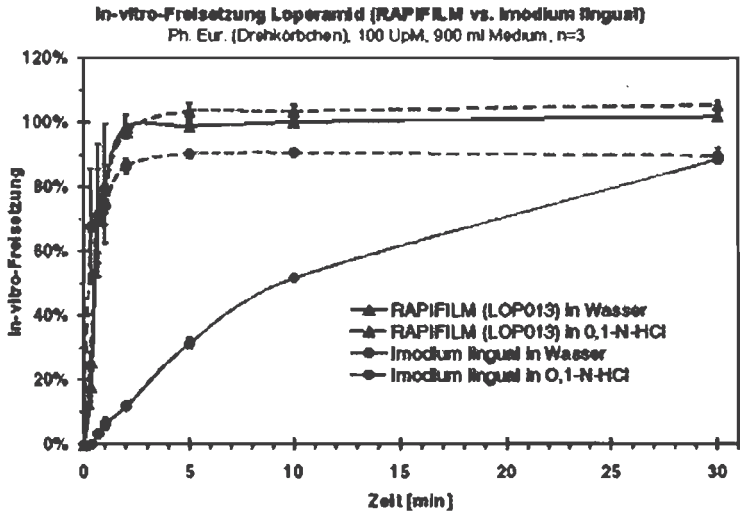



Figure 9 *In vitro* dissolution of loperamide from Rapifilm® and Imodium® lingual in water and 0.1 N HCl

### III B Liquid Crystalline Phases as Drug Delivery Systems

Liquid crystals, also called mesomorphs or mesophases, are states of matter intermediate between crystalline solids and isotropic liquids (Figure 10). Many amphiphilic compounds, such as soaps and surfactants, exhibit a tendency to form lyotropic mesophases on the addition of water. These structures result from amphiphilic nature of the molecule with

both a hydrophobic and a hydrophilic end. There are many types of liquid crystalline phases such as lamellar, hexagonal and cubic phases.

Solid Crystal Liquid crystal Liquid

Figure 10 Arrangement of molecules in solid crystal, liquid crystal and liquid

Liquid crystalline phases offer a number of useful properties for drug delivery. First, they allow drug solubilization and with a proper choice of self-association structure both water soluble and oil soluble drugs may be incorporated also in rather high concentrations. This offers possibilities to increase the drug solubility, decrease drug degradation and control and sustain the drug release rate. Second, liquid crystalline phases frequently display a rather high viscosity, which may also offer opportunities when the drug formulation needs to be localized such as intramuscular injection or application in the oral cavity (Malmsten, 2002).

Due to their frequently high viscosity and stiffness, liquid crystalline phases are often difficult to prepare and handle from a practical perspective. For example, mixing is difficult and administration is complicated, of limited patient compliance or inefficient. Therefore, the *in situ* transition from a low-viscous state to the required high-viscous liquid crystalline phase after administration is of major importance for the use of liquid crystalline phases for drug delivery. There are several parameters which may be used for triggering such a transition *in situ* after administration (Malmsten, 2002), including

1. Temperature (The body temperature is higher than the storage temperature)
2. Dilution (The formulation is often in contact with excess water after administration)
3. Salt (The physiological electrolyte concentration may be used to screen electrostatic interactions in the formulation)
4. pH (The physiological pH at the administration site may be used to either reduce or increase electrostatic interactions in the formulation)

5. Calcium ion concentration (Strong binding of $Ca^{2+}$ to carboxyl groups may be used to change the electrostatic interactions in the formulation after administration)

Polar amphiphilic lipids such as glyceryl monooleate (GMO) or monoolein is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. The acyl chain (oleic acid) is attached to the glycerol backbone by an ester bond (Figure 11). The two remaining carbons of the glycerol have active hydroxyl groups, giving polar characteristics to this portion of the molecule. The glycerol moiety may form hydrogen bonds with water in an aqueous environment and is commonly referred to as the head group. The hydrocarbon chain gives hydrophobic characteristics to glyceryl monooleate and is often termed the tail (Ganem-Quintanar, Quintanar-Guerrero and Buri, 2000).

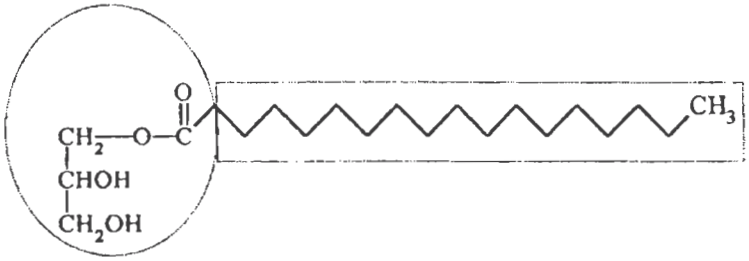



Figure 11 Structural formula of glyceryl monooleate

Glyceryl monooleate when placed in water reorganizes into lipid bilayers forming a reversed micellar phase ($L_2$) and three types of liquid crystalline phases (lamellar, reversed hexagonal and cubic phase) depend upon the temperature and water content. The lamellar phase ($L_\alpha$) has a long-range order in one dimension. Its structure consists of a linear arrangement of alternating lipid bilayers and water channels. The reversed hexagonal phase ($H_{II}$) consists of infinite water rods arranged in a two-dimensional lattice and separated by lipid bilayers. The cubic phase (C) is usually observed between the lamellar and the reversed hexagonal phases as the water content is increased. The cubic phase consists primarily of two phases with similar structures, the cubic phase of the type G, also known as gyroid and the cubic phase D or diamond, although the structural similarity of these phases is such that in practice the two are usually considered to be equivalent. As seen from phase diagram of glyceryl monooleate-water system (Figure 12), with increased hydrocarbon chain disorder, obtained either by heating or increasing the water content, there is a transition from the $L_\alpha$ phase to the cubic phase and finally into the $H_{II}$ phase (Shah, Sadhale and Chilukuri, 2001).

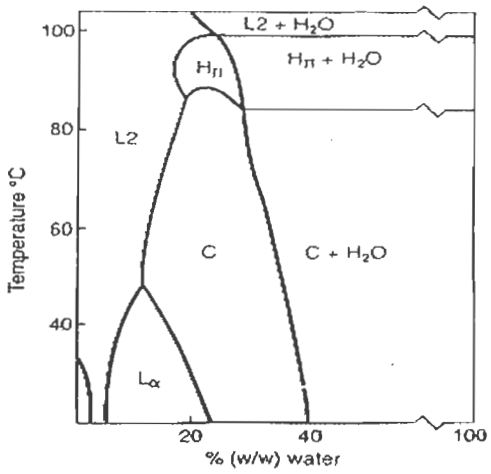



Figure 12 Binary phase diagram of glyceryl monooleate-water system

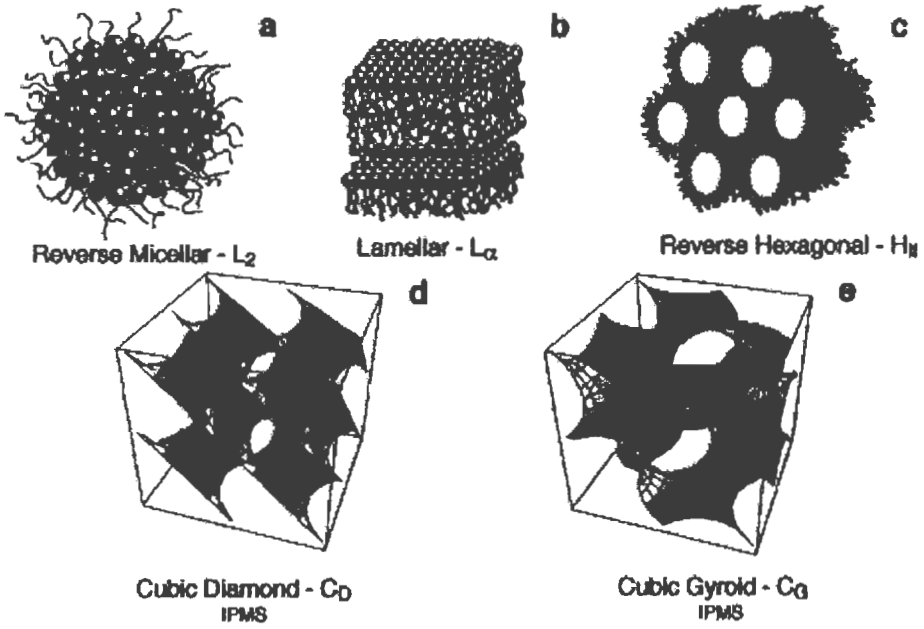



Figure 13 Schematic illustrations of a reversed micellar phase and liquid crystalline phases of glyceryl monooleate

The liquid crystalline phases formed by glyceryl monooleate in excess water have the potential of acting as an *in situ* forming biodegradable drug delivery system. The biodegradability arises from the fact that glyceryl monooleate is subject to lipolysis due to different kinds of esterase activity in different tissues. Moreover, glyceryl monooleate is a nontoxic and biocompatible material classified as GRAS (generally recognized as safe) and it is included in the FDA Inactive Ingredients Guide and in nonparenteral medicines licensed in the United Kingdom (Ganem-Quintanar, Quintanar-Guerrero and Buri, 2000).

The glyceryl monooleate-water system have been described its use as a sustained release carrier for both conventional and peptide or protein drugs (Leslie et al., 1996; Chang and Bodmeier, 1997, 1998). For oral delivery, the model drugs were dispersed in monoglycerides and filled into capsules and then transformed *in situ* into the cubic phase upon contact with gastrointestinal body fluids (Wyatt and Dorschel, 1992; Sallam et al., 2002). For buccal delivery, Lee and Kellaway (2000) suggested that the cubic and lamellar liquid crystalline phases can be considered as promising drug carriers for the buccal delivery of peptide drugs as well as acting as permeation enhancers. For vaginal delivery, glyceryl monooleate-water system was studied for vaginal delivery of antimuscarinic drugs to treat urinary incontinence. Geraghty et al. (1996) found that the release of the drugs *in vitro* was sustained over a period of 18 hr and followed square root of time kinetics, indicating that the rate of release was diffusion controlled. For parenteral delivery, the high viscosity and stiffness of the cubic phase gel limits its potential use as the delivery system by itself. However, the ability of the less viscous lamellar phase to form cubic phase gel upon absorbing more water has resulted in novel drug delivery. The studies have shown that cubic phase can be used intramuscularly and subcutaneously as a delivery system for peptide drugs such as somatostatin, desmopressin and insulin (Sadhale and Shah, 1999; Shah et al., 2001). Another interesting application of an *in situ* forming was for periodontal delivery of antibiotics for the treatment of infections. Norling et al. (1992) suggested that addition of triglyceride (sesame oil) into glyceryl monooleate could be lowering the melting point which was improved the flow properties of glyceryl monooleate and could be administered with a syringe into a periodontal pocket. Moreover, the liquid crystal structure of the gel would be the reversed hexagonal phase instead of the cubic phase. The *in vitro* release data showed that the reversed hexagonal form gave slower released of metronidazole benzoate when compared with the cubic form. Since the diffusion pathway is more obstructed in the reversed hexagonal form than in the cubic one, which has connected

water channels. The closed water channels of the reversed hexagonal phase slow down the diffusion of dissolved drug through the matrix. Komwatchara (1996) developed the mixtures of glyceryl monooleate and various vegetable oils containing the *Andrographis paniculata* extract for treatment of adult periodontitis. Upon contact with the gingival crevicular fluid, the reversed hexagonal matrix were formed and showed desirable release of the extract. The results suggested that soybean oil was the best formulation since it showed the most drug release and antimicrobial activity. In addition, soybean oil was not expensive, easily available and stable. In another study, viscous solutions prepared with either poloxamer or glyceryl monooleate were delivered by a syringe and needle into a periodontal pocket. Both formulations undergo a transformation to gel upon administration resulting in local drug delivery. While poloxamer undergoes thermoreversible gelling, glyceryl monooleate formed the viscous cubic phase *in situ* upon absorption of water. The results indicated that glyceryl monooleate showed slower release of the drug when compared with poloxamer (Esposito et al., 1996). The successful results of the above studies demonstrate an interesting application of liquid crystalline phases for periodontal drug delivery.

**IV Characterization of Films and Liquid Crystalline Phases**

The evaluation tests designed to study the characteristics and final properties of film bases are carried out by many utilizing laboratory techniques including thermal analysis, mechanical measurements, microscopic examination, and diffusion experiments (Peh and Wong, 1999; Mashru et al., 2005). The most commonly used methods are thermal analysis and mechanical property evaluations. Other test procedures are considered on the basis of needful information in that investigation.

**1. Thermal analysis**

Measurements of thermal analysis are conducted for the purpose of evaluating the physical and chemical changes that may take place in a heated sample. This requires that the operator interpret the observed events in a thermogram in terms of plausible reaction processes. The reactions normally monitored can be endothermic (melting, boiling, sublimation, vaporization, desolvation, solid-solid phase transitions, chemical degradation, etc.) or exothermic (crystallization, oxidative decomposition, etc.) in nature.

Thermal methods can be used to indicate the existence of possible drug-excipient interactions in a formulation and evaluate compound purity, polymorphism, solvation, degradation (Brittain, 1995).

Thermal properties of the films were examined by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and thermomechanical analysis (TMA). Differential scanning calorimetry is an extremely useful technique for measuring glass transition temperature (Tg), that means the temperature at which a glassy polymer becomes rubbery on heating and a rubbery polymer reverts to a glassy one on cooling. Whereas thermomechanical analysis measures deformation of a substance under a non-oscillatory load and can also conveniently measure transition from a glassy to a rubbery polymer.

Polymers in the rubbery state are very viscous liquids, with relatively high freedom of rotation around the carbon-carbon bonds in the backbone within the constraint of the tetrahedral bond angle. The temperature is high enough so that most bonds can overcome the potential energy barrier against rotation. Rotational freedom results in very flexible chains, segmental or micro-Brownian motion, and changing chain conformations as discussed for polymer solutions. Segmental mobility is considerably smaller in rubbery, liquid bulk polymers than in their solutions because of the much higher viscosity of the former (Martin, Swarbrick, and Cammarata, 1983).

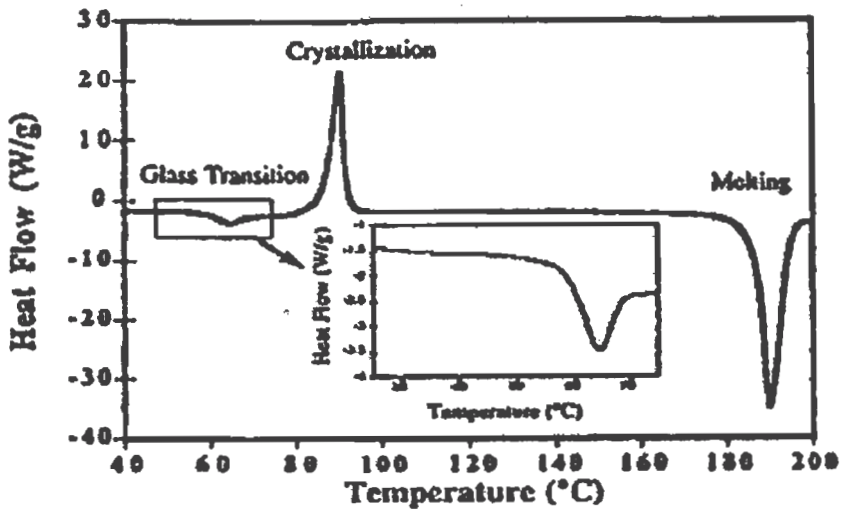



Figure 14 Typical DSC thermogram

**2. Mechanical properties of film** (Aulton and Abdul-Razzak, 1981; Martin, Swarbrick, and Cammarata, 1983)

The mechanical properties, tensile strength, percentage of elongation at break and Young's modulus of various films can be evaluated by using a tensile-strength tester. The tensile testing process is to apply increasing tensile load at s constant rate to a film strip which know dimension perpendicular to the cross-section of the film strip until the failure takes place. The load at film failure will be measured in term of force per unit cross-section area of the film.

Polymers are divided into five categories according to a qualitative description of their mechanical behavior and corresponding stress-strain characteristics as showed in the Table 5.

Table 5 Qualitative description of polymer and its stress-strain characteristics

| Polymer description | Characteristics of stress-strain curve | | | |
|---|---|---|---|---|
| | Young's modulus | Yield stress | Tensile strength | Elongation to break |
| Soft, weak | Low | Low | Low | Low to moderate |
| Soft, tough | Low | Low | Moderate | Very high (20-1000%) |
| Hard, brittle | High | None (break around yield point) | Moderate to high | Very low (<2%) |
| Hard, strong | High | High | High | Moderate (~5%) |
| Hard, tough | High | High | High | High (cold drawing or "necking") |

**3. Polarizing Microscopy**

As a result of their molecular ordering, anisotropic liquid crystalline phases, such as the hexagonal, the lamellar, and the reversed hexagonal phases, are optically birefringent (Figure 17). This property can be used for studying such phases with polarizing microscopy. The lamellar phase usually yields mosaic patterns under the polarizing microscope, whereas the hexagonal phases normally show nongeometric textures. The occurrence of crystals may also be identified by this method. On the other hand, isotropic phases (e.g., micellar and reversed micellar solutions and cubic phases) are nonbirefringent and generate a dark background when investigated under the polarizing microscope (Malmsten, 2002).

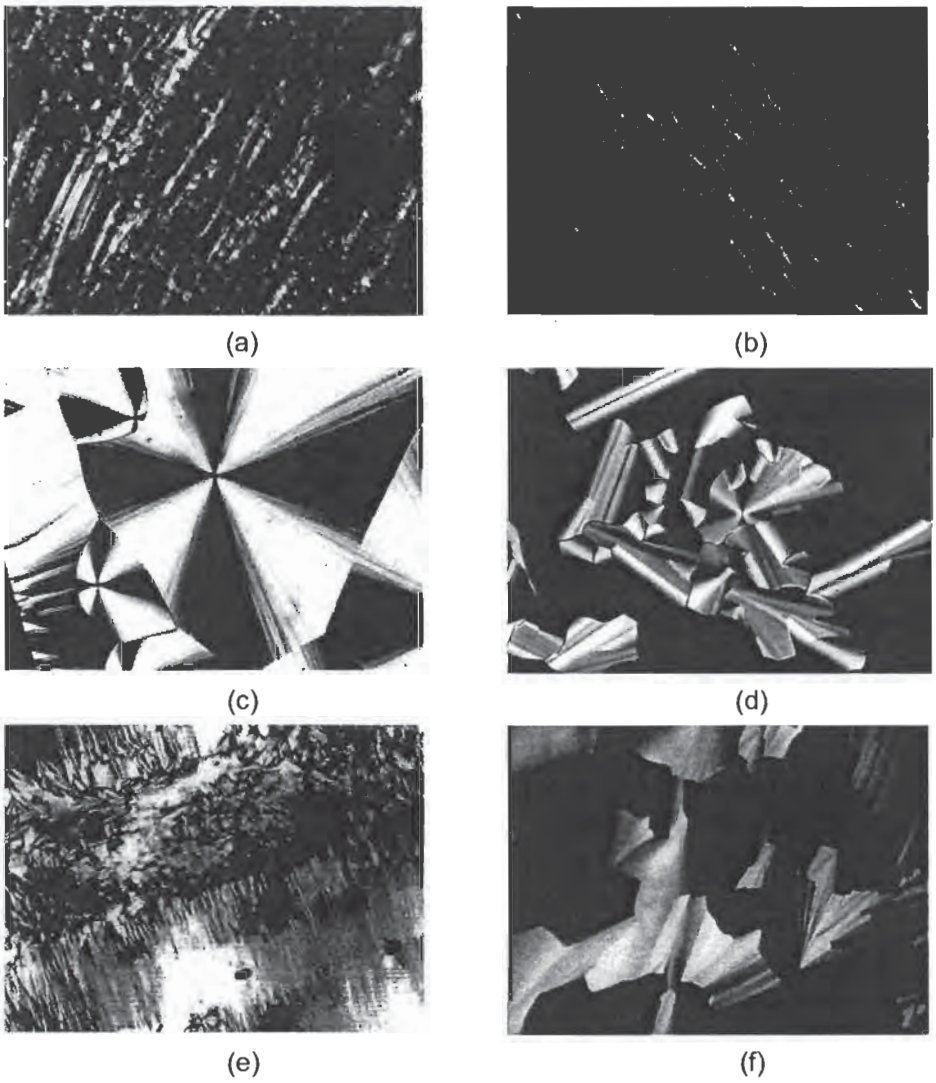



Figure 15 Polarizing microscopic textures of anisotropic liquid crystalline phases; (a) and (b) lamellar phases; (c) and (d) hexagonal phases; and (e) and (f) reversed hexagonal phase (Geraghty et al., 1996, Schwarzwälder and Meier, 1997; Makai et al., 2003; Goldmann et al., 2004; Barauskas et al., 2005)

# CHAPTER II

# MATERIALS AND METHODS

## PART I FAST DISSOLVING ORAL STRIPS

### MATERIALS

1. Plant material: *Garcinia mangostana* Linn. (purchased in Bangkok, Thailand in July, 2004).
2. Standard mangostin
3. Acesulfame potassium (Rama production, Thailand, lot no. 0311040).
4. Cetylpyridinium chloride (BDH chemicals, England, lot no. 6152940).
5. Chlorhexidine diacetate (Imperial chemical industries, Great Britain, lot no. 43132314).
6. Clotrimazole (S. Tong Chemicals, Thailand, lot no. 20010605).
7. Disodium hydrogen orthophosphate (Merck, Germany, lot no. F974786 538).
8. Ethanol absolute, AR grade (Merck, Germany, lot no. K32751783 349).
9. Ethyl acetate, AR grade (Labscan Asia, Thailand, lot no. 04 07 0073).
10. Eucalyptus oil (Srichand United Dispensary, Thailand, lot no. 030115).
11. Glycerin (Srichand United Dispensary, Thailand, lot no. 8-03).
12. Hydroxypropyl cellulose (LV) (Pharmaserv, Thailand, lot no. 8019201).
13. Hydroxypropyl methylcellulose (3 cps.) (Shin-etsu, Japan, lot no. 112530)
14. Hydroxypropyl methylcellulose (5 cps.) (Colorcon, USA., lot no. RJ08012405).
15. Commercial product strips A (containing essential oils).
16. Commercial mouth wash solution B (containing essential oils).
17. Commercial mouth wash solution C (containing 0.12% w/v of chlorhexidine gluconate).
18. Menthol (Srichand United Dispensary, Thailand, lot no. 036597).
19. Methanol, HPLC grade (Labscan Asia, Thailand, lot no. 05 03 0064).
20. Mueller Hinton broth (Difco, USA, lot no.4104193).
21. Mueller Hinton agar (Merck, Germany, lot no. VL798237).
22. Polyethylene glycol 400 (Srichand United Dispensary, Thailand, lot no. 419070103).
23. Potassium dihydrogen phosphate (Merck, Germany, lot no. A315973 127).
24. Propylene glycol (Srichand United Dispensary, Thailand, lot no. 1752124024).
25. Sodium chloride (Merck, Germany, lot no. K28555404 049).
26. Sodium lauryl sulfate (S-Tong Chemical, Thailand, lot no. 10420).
27. *Staphylococcus aureus* ATCC 25923 (Department of Microbiology, Faculty of Pharmaceutical science, Chulalongkorn University, Thailand).
28. *Streptococcus mutans* ATCC $KPSK_2$ (Department of Microbiology, Faculty of Dentistry, Chulalongkorn University, Thailand).

**INSTRUMENTS**

1. Analytical balance (Model AG285, Mettler Toledo, Switzerland).
2. Autoclave (Model HA-3D, Hirayama, Japan).
3. Botanical grinder (Retsch GmbH SK1, Germany).
4. Centrifuged (model4206, Milano, Italy).
5. Differential scanning calorimeter (DSC822$^{e}$, Mettler Toledo, Switzerland).
6. Disposable syringe filter nylon 13 mm, 0.45 μm (Chrom Tech, USA, lot no. 0811).
7. Fluorescence analysis cabinet (Model CM-10, Spectroline, USA).
8. High performance liquid chromatography (HPLC) (Perkin Elmer, Germany) equipped with
   - Automatic sample injector (SIL-10A, Shimadzu, Japan).
   - Communications bus module (CBM-10A, Shimadzu, Japan).
   - Liquid chromatography pump (LC-10AD, Shimadzu, Japan).
   - UV-VIS Detector (SPD-10A, Shimadzu, Japan).
   - Column (BDS Hypersil C18, 5 μm, 250 x 4.6 mm, Thermo Electron Corporation England, lot no. 6596).
   - Precolumn (μBondapack C18, 10 μm, 125A$^{o}$, Water Corporation, Ireland, lot no. W2336B1).
9. Hot air oven (Model B40, Memmert, Germany).
10. Incubator (Model B60, Memmert, Germany).
11. Laminar sir flow (Model BV-126, ISSCO, USA).
12. Magnetic stirrer (Stuart, England).
13. Micrometer (Starrett, USA.).
14. Microscope (Nikon eclipse E200, Japan).
15. Modified dissolution tester.
16. pH meter (Orion model 420A, Orion Research Inc., USA).
17. Rotary evaporator (Rotavapor RE-120, Buchi, Switzerland).
18. Stability cabinet (Eurotherm Axyos, Germany).
19. Stopwatch (Heuer, Switzerland).
20. Tensile testing machine (Model H5KS 1509, Tinius Olsen, England).
21. TLC alumina sheet silica gel 60F 254 20 x 20 cm (Merck, Germany, lot no. OB290814).
22. Tube rotary machine (model EW PC 9022T/R/P, Thailand).
23. UV-Visible spectrophotometer (UV-1601, Shimadzu, Japan).
24. Vernier caliper (Macoh, China).
25. Vortex mixer (Vortex Genies-2, Scientific Industries, Inc., USA).

**PART II MONOGLYCERIDE-BASED DRUG DELIVERY SYSTEMS (LIQUID CRYSTALLINE)**

**MATERIALS**

1. The fruits of *Garcinia mangostana* (purchased in Bangkok, Thailand in July, 2003)
2. Clotrimazole (S. Tong Chemicals Co., Ltd., Thailand, lot no. 20010605)
3. Disodium hydrogen orthophosphate (Merck, Germany, lot no. F974786 538)
4. Ethanol absolute, AR grade (Merck, Germany, lot no. K32751783 349 )
5. Ethyl acetate, AR grade (Labscan Asia, Thailand, lot no. 04070073)
6. Glyceryl monooleate (Donated from Hong Huat Co., Ltd., Thailand, lot no. 601)
7. Hexane, AR grade (Labscan Asia, Thailand, lot no. 03030145)
8. Lutrol F127 (BASF, Germany, lot no. WPHY615B)
9. Methanol, HPLC grade (Labscan Asia, Thailand, lot no. 04070120)
10. Mueller Hinton agar (Oxoid, England, CM0337)
11. n-Octanol (Asia Pacific Specialty Chemicals Ltd., Australia, lot no. H1F223)
12. Olive oil (Bertolli, Italy, lot no. L014BS)
13. Polyamide membrane filter 47 mm, 0.45 µm (Satorius AG, Germany, lot no. 0503 25006 0340093)
14. Potassium dihydrogen orthophosphate (Merck, Germany, lot no. A262673 045)
15. Sesame oil (Thai China Flavours & Fragrances Industry Co., Ltd., Thailand, lot no. 4607113/0807)
16. Sodium chloride, AR grade (Merck, Germany, lot no. K28555404 049)
17. Soybean oil (Thai Vegetable Oil Public Co., Ltd., Thailand, lot no. 310546)
18. *Streptococcus mutans* $KPSK_2$ (Department of Microbiology, Faculty of Dentistry, Chulalongkorn University, Thailand)
19. Syringe filter cellulose acetate 13 mm, 0.45 µm (Chrom Tech Inc., USA, lot no. 100301)
20. TLC Alumina sheet silica gel 60F 254 20 × 20 cm (Merck, Germany, lot no. OB290814)
21. Whatman filter paper No.1, 150 mm (Whatman International Ltd., England, lot no. A815891)

**INSTRUMENTS**

1. Analytical balance (Model AG285, Mettler Toledo, Switzerland)
2. Botanical grinder (Retsch GmbH SK1, Germany)
3. Cone and plate viscometer (Brookfield viscometer, Scientific Industries, Inc., USA)
4. $CO_2$ water jacketed incubator (Forma Scientific, Inc., USA)
5. Differential scanning calorimeter (DSC822$^e$, Mettler Toledo, Switzerland)
6. Digital camera (Coolpix 5400, Nikon, Japan)
7. High performance liquid chromatography
   - Auto Injector (SIL-10A, Shimadzu, Japan)
   - Communications bus module (CBM-10A, Shimadzu, Japan)
   - Liquid chromatograph pump (LC-10AD, Shimadzu, Japan)
   - UV-VIS detector (SPD-10A, Shimadzu, Japan)

- Column (BDS Hypersil C18, 5 µm, 250 × 4.6 mm, Thermo Electron Corporation, England, lot no.6596)
- Precolumn (µBondapack C18, 10 µm, 125A°, Water Corporation, Ireland, lot no. W2336B1)

8. Hot air oven (Memmert, Germany)
9. Laminar air flow (BH 2000 series, Clyde-Apac, Australia)
10. Microscope (Eclipse E200, Nikon, Japan)
11. Modified Franz diffusion cells (Crown Glass Company, USA)
12. pH Meter (Model 420A, Orion, USA)
13. Refrigerated incubator (FOC 225I, VELP Scientifica, Italy)
14. Rotary evaporator (Rotavapor RE-120, Buchi, Switzerland)
15. Ultrasonicator (Crest Ultrasonics, Malaysia)
16. UV-visible spectrophotometer (UV-1601, Shimadzu, Japan)
17. Vacuum pump (CB169 Vacuum system, Buchi, Switzerland)
18. Vortex mixer (Vortex Genies-2, Scientific Industries, Inc., USA)

## METHODS

## PART I: Extraction, Identification, Quanlitative and Quantitative Analysis And Antibacterial activity of *Garcinia mangostana* fruit hulls

### A Extraction of Active Constituents from *Garcinia mangostana* fruit hulls

The fruit hulls of *Garcinia mangostana* were cut into small pieces about 1 x 1 inch, and then dried at the temperature 45±0.5 °C in a hot air oven for 48 hours. The dried powder fruit hulls (4345.3 g.) were extracted by maceration method with ethyl acetate at room temperature overnight. The ethyl acetate extract was filtered and evaporated using rotary evaporator at 30°C. Then the crude extract was allowed to crystallize at room temperature. The yield was kept in a desiccator and used for further studies (modified from Hiranras, 2001).

### B Identification and Determination of Active Constituents from *Garcinia mangostana*

#### 1. Differential scanning calorimetric (DSC) method

The DSC thermogram was determined by using differential scanning calorimeter (DSC822$^e$, Mettler Toledo, Switzerland) to detect melting point. An accurately weighed amount 3 mg of the crude extract was placed in an aluminum pan. Then the aluminum pan was sealed with the lid under the pressure of the plunger by using crucible-sealing press. After sealing, the aluminum pan was placed on a DSC sensor in the furnace. The scan rate was performed at 10 °C/min over the temperature range of –50 to 220 °C under the nitrogen atmosphere. The experiment was performed in triplicate.

#### 2. Thin layer chromatographic (TLC) method

TLC is the most versatile and flexible chromatographic method. It is rapid and gives highest sample throughput because many samples and standards can be applied to a single plate and separated at the same time.

The extract was dissolved with ethyl acetate and spotted on TLC alumina sheet, compared with standard mangostin. After that the alumina sheet was placed in a closed chamber saturated with vapor of ethyl acetate: hexane (3:1) as mobile phase. When the mobile phase had moved to an appropriate distance, the alumina sheet was removed and dried. The alumina sheet was detected under UV light at the wavelength of 254 nm (Hiranras, 2001). The basic parameter used to describe the migration is the Rf value, where

$$Rf = \frac{\text{distance moved by solute}}{\text{distance moved by mobile phase front}}$$

**3. High performance liquid chromatographic (HPLC) method**

The determination of active constituents from *Garcinia mangostana* was performed by HPLC method because of specificity and high sensitivity.

**3.1 HPLC condition**

From the preliminary study of mangostin assayed with UV spectrophotometer, the scanning spectra of mangostin in 87% v/v methanol in water was obtained. From the spectra, the maximum absorbance was found at the wavelength of 243 nm (Figure 16). Therefore, the detection of mangostin was performed at this wavelength (Hiranras, 2001).

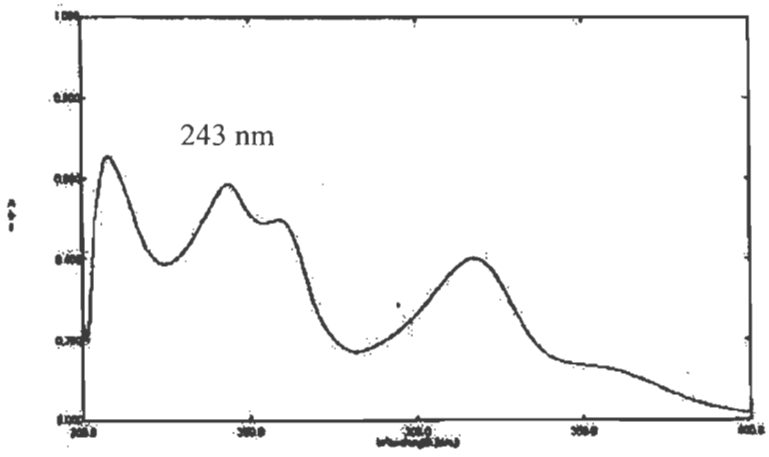



Figure 16 Absorption spectra of mangostin in 87% v/v methanol in water.

The HPLC conditions for the analysis of mangostin were as follows (modified from Hiranras, 2001):

| | | |
|---|---|---|
| Column | : | BDS Hypersil® C18, 5 μm, 250 x 4.6 mm |
| Precolumn | : | μBondapack C18, μ10 m, 125A° |
| Mobile phase | : | methanol: water (87:13) |
| Injection volume | : | 20 μl |

| | | |
|---|---|---|
| Flow rate | : | 1 ml/min |
| Detector | : | UV detector at 243 nm |
| Temperature | : | ambient |
| Run time | : | 13 min |
| Internal standard | : | clotrimazole |

The mobile phase was freshly prepared by using methanol, HPLC grade and ultrapure water with ratio of 87:13 v/v. The solution was thoroughly mixed, filtered through 0.45 μm membrane filter and then degassed by sonication for 30 min prior to use.

**3.2 Standard solutions for HPLC**

**3.2.1 Preparation of internal standard solutions**

In the preliminary of HPLC study, clotrimazole solution (400 μg/ml) was used as an internal standard due to its appropriate retention time and optimal resolution from mangostin peak.

A stock solution of clotrimazole was prepared by accurately weighed 200 mg of clotrimazole into a 50 ml volumetric flask, diluted and adjusted to volume with mobile phase. The final concentration of clotrimazole stock solution was 4,000 μg/ml.

**3.2.2 Preparation of standard solutions**

An accurate weight 20 mg amount of mangostin was placed into a 10 ml volumetric flask and diluted to volume with mobile phase. This stock solution had a final concentration of 2,000 μg/ml. Then 5.0 ml of this solution was transferred into a 100 ml volumetric flask and was diluted with mobile phase to give a solution of 100 μg/ml. The solution of 0.1, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 ml and 1.0 ml of internal standard stock solution were added into 10 ml volumetric flask. The dilution to volume with mobile phase gave 1, 5, 10, 15, 20, 30, and 40 μg/ml of mangostin, respectively and 400 μg/ml of clotrimazole. Three sets of standard solution were prepared for each HPLC run. As a result, the standard curve of mangostin between concentration and peak area ratio was plotted.

**3.2.3 Preparation of sample solutions**

The sample stock solution was prepared by accurately weighing 10 mg of *Garcinia mangostana* extract into a 50 ml volumetric flask. Mobile phase was added to dissolve and the solution was adjusted to final volume. This stock solution had a final concentration of 200 μg/ml. Then 1.0 ml of stock solution and 1.0 ml of clotrimazole stock solution were transferred into a 10 ml volumetric flask. The solutions were adjusted to volume with mobile phase to give the concentrations of *Garcinia mangostana* extract and clotrimazole of 20 μg/ml and 400 μg/ml, respectively.

**3.3 Validation of the HPLC method**

The analytical parameters used in the assay validation for the HPLC method were specificity, linearity, precision and accuracy.

**3.3.1 Specificity**

Under the chromatographic conditions used, the peak of mangostin must be completely separated from and not be interfered by the peaks of other components in the sample.

**3.3.2 Linearity**

Three sets of seven standard solutions were prepared and analyzed. Linear regression analysis of the peak area ratios versus their concentrations was performed. The linearity was determined from the coefficient of determination ($R^2$).

**3.3.3 Accuracy**

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined from the percentage of recovery. Five sets of three concentrations at 8, 25 and 35 μg/ml were prepared and analyzed. The percentage of recovery of each concentration was calculated from the ratio of inversely estimated concentration to known concentration multiplied by 100.

**3.3.4 Precision**

**a) Within run precision**

The within run precision was determined by analyzing five sets of three concentrations at 8, 25 and 35 μg/ml in the same day. Peak area ratios of mangostin to clotrimazole were calculated and the percent coefficient of variation (%CV) of each concentration was determined.

**b) Between run precision**

The between run precision was determined by analyzing three concentrations at 8, 25 and 35 μg/ml on five different days. The coefficient of variation (%CV) of mangostin of each concentration was determined.

**Acceptance criteria:**

For accuracy, the percentage of recovery should be within 98-102 % of each nominal concentration, whereas the percent coefficient of variation for both within run precision and between run precision should be less than 2%.

**4. UV Spectrophometric methods**

**4.1 Preparation of calibration curve UV spectrophotometry**

A stock solution of mangostin was prepared by accurately weighed 12 mg of mangostin into a 10 ml volumetric flask, then dissolved and adjusted to volume with absolute ethanol. Then pipette 1.0 ml of this standard stock solution into 100 ml volumetric flask. This standard solution had a final concentration of 12 μg/ml. After pipette 2, 3, 4, 5, 6, 7, and 8 ml of this solution into 10 ml volumetric flask, diluted and adjusted to volume with the absolute ethanol. The final concentrations of mangostin were 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, and 9.6 μg/ml, respectively. Then the standard solutions were analyzed using the UV

spectrophotometer at 243 nm. The standard curve of mangostin between concentration and absorbance was plotted.

**4.2 Validation of UV spectrophotometric method**

The analytical parameters used for the assay validation were specificity, linearity, accuracy and precision.

**4.2.1 Specificity**

Under the conditions used, the absorbance of mangostin must not be interfered by the absorbance of other components in the sample.

**4.2.2 Linearity**

Three sets of six standard solutions of mangostin ranging from 2.4 to 9.6 μg/ml were prepared and analyzed. Linear regression analysis of the absorbances versus their concentrations was performed. The linearity was determined from the coefficient of variation ($R^2$).

**4.2.3 Accuracy**

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined from the percentage of recovery. Five sets of three concentrations at 3.0, 5.4 and 7.8 μg/ml were prepared and analyzed. The percentage of recovery of each concentration was calculated from the ratio of inversely estimated concentration to known concentration multiplied by 100.

**4.2.4 Precision**

**a) Within run precision**

The within run precision was determined by analyzing five sets of three concentrations at 3.0, 5.4 and 7.8 μg/ml in the same day. The percent coefficient of variation (%CV) of mangostin of each concentration was determined.

**b) Between run precision**

The between run precision was determined by analyzing three concentrations at 3.0, 5.4 and 7.8 μg/ml on five different days. The percent coefficient of variation (%CV) of mangostin of each concentration was determined.

**Acceptance criteria:**

For accuracy, the percentage of recovery should be within 98-102 % of each nominal concentration, whereas the percent coefficient of variation for both within run precision and between run precision should be less than 2%.

**C Determination of Solubility of *Garcinia mangostana* Extract**

**Solubility study of the extract in ethanol: water system**

Excess amount of extract (mangostin) was put into each test tube containing 5 ml of hydroalcoholic mixture 40, 50, 60, 70 and 80 % v/v of ethanol. These suspensions were equilibrated by constant rotating in a tube rotary machine at ambient temperature for 24, 36,

and 48 hours. Then suspensions were centrifuged and the supernatants were filtered through 0.45 μm nylon membrane, appropriately diluted and analyzed by UV spectrophotometry. All solubility determinations were carried out in triplicate.

**D Determination of Antimicrobial Activities of Extract from *Garcinia mangostana***

The antimicrobial activity of extract was determined by the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) tests against the standard strain of *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$.

**1. Determination of minimal inhibitory concentration (MIC)**

The minimal inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial agent that inhibits growth of the microorganism under test and is generally recorded in mg/L or μg/ml (Denyer, Hodges, and Gorman, 2004).

The principle of the broth dilution method is the inhibition of growth of the bacteria by an antimicrobial agent incorporated in the broth medium.

The extract was tested for its activities against *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$ using broth dilution method (Lorian, 1991; Mahon and Manuselis, 2000; Hiranras, 2001).

**1.1 Preparation of double strength Mueller Hinton broth medium**

Double strength Mueller Hinton broth medium was prepared by suspending 21 g of the broth powder to 500 ml of distilled water and warmed to dissolve the medium completely. After that the sterilization was performed by autoclaving at $121 \pm 0.5$ $^{\circ}C$ for 15 min.

**1.2 Preparation of *Garcinia mangostana* extract stock solution**

The working stock solution of extract was prepared by accurately weighed 76.8, 32.0, and 25.6 mg, respectively, of extract into 10 ml volumetric flask diluted to volume with 95% v/v ethanol. The 1.0 ml solution was pipetted into 10 ml volumetric flask and diluted to volume with 95 % ethanol so that the final concentration of this solution was 768, 320, and 256 μg/ml, respectively.

**1.3 Preparation of inoculated suspension of bacteria**

The inoculum was prepared by picking bacteria colonies on the surface of subculture bacterial agar slant using sterile loop into test tube with sterile normal saline solution. The inoculum was standardized by adjusting turbidity of inoculated suspension with sterile normal saline solution until the turbidity equivalent to McFarland standard No. 0.5, since the concentration of standardized inoculated suspension was $1 \times 10^8$ cells/ml. Then the suspension was diluted with sterile normal saline solution to the final concentration of $1 \times 10^6$ cells/ml before use.

**1.4 Method for the MIC test**

The experiment was performed triplicately (using serial two fold-dilution method) by three series of 15 sterile test tubes. Every tube except the first were filled with 1.0 ml of double strength Mueller Hinton broth. Then 1.0 ml of extract solution was pipetted into the first and second tubes, mixed by vortex mixer, and 1.0 ml from the second tube was transferred to the third tube, repeated the procedure to tube no.13. The 1.0 ml in tube no. 13 was discarded, then 1.0 ml of inoculated suspension ($1 \times 10^6$ cells/ml) was pipetted into tube no. 1-14 and 1.0 ml of sterile normal saline solution was pipetted into tube no 15. Therefore tube no. 14 is a growth control tube (broth with inoculum) and tube no. 15 is an uninoculated control tube (broth with sterile normal saline solution).

All tubes were incubated for a period of time for 24 hr at 37 $^{\circ}$C for *Staphylococcus aureus* ATCC 25923 and 48 hr, 37 $^{\circ}$C in $CO_2$ for *Streptococcus mutans* ATCC $KPSK_2$. The MIC was determined visually as the lowest concentration that inhibited bacterial growth, as demonstrated by the absence of turbidity.

**2. Determination of minimal bactericidal concentration (MBC)**

The minimal bactericidal concentration (MBC) is the lowest concentration of antimicrobial agent that results in $\geq$ 99.9% killing of the bacteria under test (Denyer, Hodges, and Gorman, 2004).

**2.1 Preparation of Mueller Hinton agar medium**

The MBC of *Garcinia mangostana* extract was determined by using Mueller Hinton Agar. Mueller Hinton Agar medium was prepared by adding 34 g of the agar powder to 1,000 ml of distilled water and boiled to dissolve the medium completely. After that the sterilization was performed by autoclaving at 121$\pm$0.5 $^{\circ}$C for 15 min. Each agar plate was prepared by pipetting 25 ml of the medium to Petri dish with diameter of 10 cm and allowed to solidify at room temperature. The depth of agar was approximately 3 mm.

**2.2 Method for the MBC test**

The MBC was determined by dipping a sterile loop into each tube of no growth broth dilution test from 1.4, then the sterile loop was streaked on the surface of Mueller Hinton agar plate and incubated at the same condition as the MIC test. After incubation, the MBC was determined visually as the lowest concentration that the absence of colony of bacteria occurred on surface of agar.

The MIC and MBC studies for *Staphylococcus aureus* ATCC 25923 were performed in triplicate with another series of 128 µg/ml and 160 µg/ml of extract solution, so that the series were 128 µg/ml to 0.5 µg/ml and 160 µg/ml to 0.625 µg/ml, respectively.

The MIC and MBC studies for *Streptococcus mutans* ATCC $KPSK_2$ were performed in triplicate with another series of 64 µg/ml and 80 µg/ml of extract solution, so that the series were 64 µg/ml to 0.5 µg/ml and 80 µg/ml to 0.625 µg/ml, respectively.

**3. The effect of solvent on antimicrobial activity**

Because mangostin is a poor water solubility substance, so 95% ethanol was selected as solvent to solubilize the extract. Ethanol is a solvent that has inhibitory and bactericidal effect for many species of bacteria. Therefore, the effect of this solvent for *Garcinia mangostana* extract (95% ethanol) on the inhibition of 2 types of test bacteria were examined using a serial two fold-dilution method.

**3.1 Method for the MIC test**

The experiment was performed duplicately (using serial two fold-dilution method) by two series of 15 sterile test tubes. Every tube except the first was filled with 1.0 ml of double strength Mueller Hinton broth. Then 1.0 ml of 95% ethanol was pipetted into the first and second tubes, mixed by vortex mixer, and 1.0 ml from the second tube was transferred to the third tube, repeated the procedure to tube no.13. The 1.0 ml in tube no. 13 was discarded, then 1.0 ml of inoculated suspension ($1 \times 10^6$ cells/ml) was pipetted into tube no. 1-14 and 1.0 ml of sterile normal saline solution was pipetted into tube no 15. Therefore tube no. 14 is a positive control tube (broth with inoculum) and tube no. 15 is a negative control tube (broth with sterile normal saline solution).

All tubes were incubated in the same manner as in 1.4. After incubation, the MIC of 95% ethanol was determined visually as the lowest concentration that inhibited bacterial growth, as demonstrated by the absence of turbidity.

**3.2 Method for the MBC test**

The MBC was determined by dipping a sterile loop into each tube of no growth broth dilution test, then the sterile loop was streaked on the surface of Mueller Hinton agar plate and incubated at the same condition as the MIC test. After incubation, the MBC was determined visually as the lowest concentration that the absence of colony of bacteria occurred.

## PART II FAST DISSOLVING ORAL STRIPS CONTAINING *Garcinia mangostana* EXTRACT

### E. Preparation of Orally Fast Dissolving Films

**1. Preparation of cellulose derivatives film bases**

From preliminary studies, some polymers, which could be prepared with appropriate properties including good appearance, integrity, and easy to remove from a glass mold without defect or breakage were explored. These polymers included HPMC 3 and 5 cps, and HPC. The formulas are presented in Table 9. The procedures for preparing were as follows:

1) The polymer was dispersed in hydroalcoholic mixture at 60% v/v of ethanol, sealed the top of beaker with paraffin film to prevent evaporation of solvent. The dispersion was left at room temperature for 12 hours in order to remove entrapped air bubbles and allowed the polymer to completely hydrate and swell.

2) The additives, which are acesulfame potassium, menthol, and eucalyptus oil were added and mixed consequently into the polymer dispersion. The mixture was stirred gently to prevent air entrapment and left until air bubbles were removed.

3) The film was prepared by pouring the mixture into a hollow space of an aluminum box placed on a dry and clean surface of flat glass plate with area 20 x 20 $cm^2$. The aluminum box was adjusted at height of 0.5 mm. Then it was dragged towards the right side of the flat glass plate. The film was allowed to completely dry on a leveled flat surface in a hot air oven at 45 $^{\circ}C$ for 2 hours (Figure 19).

4) The film was carefully removed from the glass and cut to 2 x 3 $cm^2$. The final weight and the thickness of the film were measured with an analytical balance (Mettler Toledo, Switzerland) and a micrometer (Starrett, USA), respectively.

In this experiment, the films were prepared in various formulations to study the effect of plasticizer and effect of polymer mixture on mechanical properties. The formulations are depicted in Tables 6 and 7

All formulations were consisted of 0.3% w/w of acesulfame potassium as sweetener, 1.5% w/w of menthol and 0.6% w/w of eucalyptus oil as flavoring agents.

Table 6 Composition of cellulose derivatives film bases (HPMC 3 cps) with plasticizers.

| Polyethylene glycol 400 | Propylene glycol | glycerin |
|---|---|---|
| - | - | - |
| + | - | - |
| + | + | - |
| + | + | + |
| - | + | - |
| - | - | + |
| - | + | + |
| + | - | + |

+ quantity of plasticizer 5% w/w of polymer

- quantity of plasticizer 0% w/w of polymer

Table 7 Composition of cellulose derivatives film bases.

| composition | Concentration in solution (%w/w) | Formulation codes |
|---|---|---|
| HPMC (3 cps) | 12 | E3 |
| HPMC (5 cps) | 12 | E5 |
| HPC (LV) | 12 | HPC |
| HPMC 3 cps : HPC LV (2:1) | 8 : 4 | E3HPC (2:1) |
| HPMC 3 cps : HPC LV (3:1) | 9 : 3 | E3HPC (3:1) |
| HPMC 3 cps : HPC LV (4:1) | 9.6 : 2.4 | E3HPC (4:1) |
| HPMC 3 cps : HPC LV (5:1) | 10 : 2 | E3HPC (5:1) |
| HPMC 3 cps :HPMC 5 cps (1:1) | 6 : 6 | E3E5 (1:1) |
| HPMC 3 cps :HPMC 5 cps (2:1) | 8 : 4 | E3E5 (2:1) |
| HPMC 3 cps :HPMC 5 cps (3:1) | 9 : 3 | E3E5 (3:1) |
| HPMC 3 cps :HPMC 5 cps (5:1) | 10 : 2 | E3E5 (4:1) |
| HPMC 5 cps: HPC LV (1:1) | 6 : 6 | E5HPC (1:1) |
| HPMC 5 cps: HPC LV (2:1) | 8 : 4 | E5HPC (2:1) |
| HPMC 5 cps: HPC LV (3:1) | 9 : 3 | E5HPC (3:1) |
| HPMC 5 cps: HPC LV (5:1) | 10 : 2 | E5HPC (5:1) |

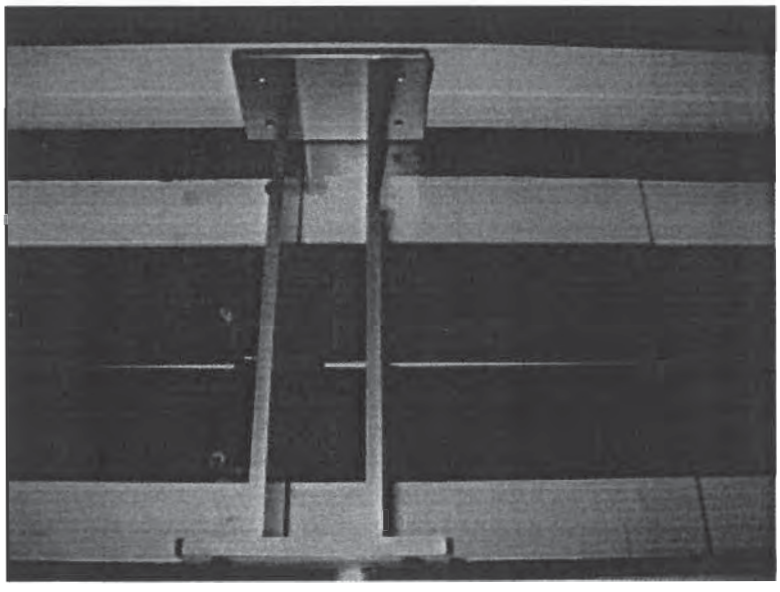


Figure 17 Film casting apparatus (assembled in the laboratory).

**Table 8 Formulations of orally fast dissolving films (% w/w)**

| Rx | Formulas | HPMC 3 cps | HPMC 5 cps | HPC LV | PEG 400 | PG | Glycerin | Ace- K | Menthol | Eucalyptus oil | 60% ethanol |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | PG | 12 | - | - | - | 0.6 | - | 0.3 | 1.5 | 0.6 | 85.0 |
| 2 | PEG | 12 | - | - | 0.6 | - | - | 0.3 | 1.5 | 0.6 | 85.0 |
| 3 | GLY | 12 | - | - | - | - | 0.6 | 0.3 | 1.5 | 0.6 | 85.0 |
| 4 | PG/GLY | 12 | - | - | - | 0.6 | 0.6 | 0.3 | 1.5 | 0.6 | 84.4 |
| 5 | PEG/GLY | 12 | - | - | 0.6 | - | 0.6 | 0.3 | 1.5 | 0.6 | 84.4 |
| 6 | PEG/PG | 12 | - | - | 0.6 | 0.6 | - | 0.3 | 1.5 | 0.6 | 84.4 |
| 7 | PEG/PG/GLY | 12 | - | - | 0.6 | 0.6 | 0.6 | 0.3 | 1.5 | 0.6 | 83.8 |
| 8 | E3 | 12 | - | - | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 9 | E5 | - | 12 | - | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 10 | HPC | - | - | 12 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 11 | E3HPC (2:1) | 8 | - | 4 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 12 | E3HPC (3:1) | 9 | - | 3 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 13 | E3HPC (4:1) | 9.6 | - | 2.4 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 14 | E3HPC (5:1) | 10 | - | 2 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 15 | E3E5 (1:1) | 6 | 6 | - | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 16 | E3E5 (2:1) | 8 | 4 | - | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 17 | E3E5 (3:1) | 9 | 3 | - | - | - |  | 0.3 | 1.5 | 0.6 | 85.6 |
| 18 | E3E5 (5:1) | 10 | 2 | - | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 19 | E5HPC (1:1) | - | 6 | 6 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 20 | E5HPC (2:1) | - | 8 | 4 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 21 | E5HPC (3:1) | - | 9 | 3 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |
| 22 | E5HPC (5:1) | - | 10 | 2 | - | - | - | 0.3 | 1.5 | 0.6 | 85.6 |

**F *In vitro* Evaluation of Orally Fast Dissolving Films**

**1. Physical appearances**

Color, transparency, flexibility and integrity of the film bases were visually observed. Ease of detachment from glass in the preparation process was also investigated.

**2. Determination of film thickness**

The thickness of 2 x 3 $cm^2$ films was determined using a micrometer (Starrett, USA), which has a sensitivity of 0.001 mm. Thickness was measured at five different points, one point at film center and others around the central point. The results were recorded as the mean of five measurements.

**3. Determination of film weight**

The weight of film bases was investigated using an analytical balance (Mettler Toledo, Switzerland), that has a sensitivity of 0.00001 g. The measurement was performed in 5 samples and the results were showed in the mean of five measurements.

**4. Determination of surface and cross section morphology**

The surface and cross section morphology of polymer films was observed by using a scanning electron microscope (Joel, Japan). The films were mounted on a metal stub and coated with gold. The films were imaged with a 15 kV electron beam with an appropriate magnification.

**5. Determination of mechanical properties of film bases**

Mechanical properties of test films were measured by using a tensile testing machine (Tinius Olsen®, Model H5KS 1509) equipped with 10 N tension load cell. The mechanical properties studied included the tensile strength, percent elongation at break, work of failure, and Young's modulus. The procedure employed was based on the guideline of the American Society for Testing and Material (1995).

The film specimens were cut into small strips 2 x 30 mm by using a standard knife. The specimens were left to expose to room humidity for 1 hour before tested. The thickness of each strip was the average value of five separate measurements taken along the length of the sample by using micrometer. The test specimen was carefully clamped by an upper and lower flat-faced grip and was extended by the test machine with the condition as follows:

| | | |
|---|---|---|
| Rate of grip separation | = | 3 mm/min |
| Gauge length | = | 5 mm |
| Load range | = | 8 Newton |
| Temperature | = | $25 \pm 2\ ^{\circ}C$ |
| Relative humidity | = | $40 \pm 5$ % |

In this experiment, at least five specimens were examined for one film formulation. After the specimen was ruptured the breaking force and the change in length at the moment of rupture were analyzed by the software-controlled program, QMat 4.10 S-Series-

5K). The acceptable data were only those obtained from the strip that ruptured at the bilateral section.

The tensile stress was plotted against the percent strain to give stress-strain curve, and ultimate tensile strength as well as elongation at break was reported. The mean and standard deviation of the values were obtained from five determinations. The ultimate tensile strength and percent elongation at break were calculated from the following formulas.

$$\text{Tensile strength (MPa)} = \frac{\text{maximum load}}{\text{original minimum cross-sectional area of the specimen}}$$

$$\text{Percent elongation} = \frac{\text{extension at the moment of rupture} \times 100}{\text{initial gauge length of the test specimen}}$$

$$\text{Work of failure (mJ)} = \text{area of a curve plotting between force and extension}$$

$$\text{Young's modulus(MPa)} = \frac{\text{tensile stress}}{\text{elastic strain in tension}}$$

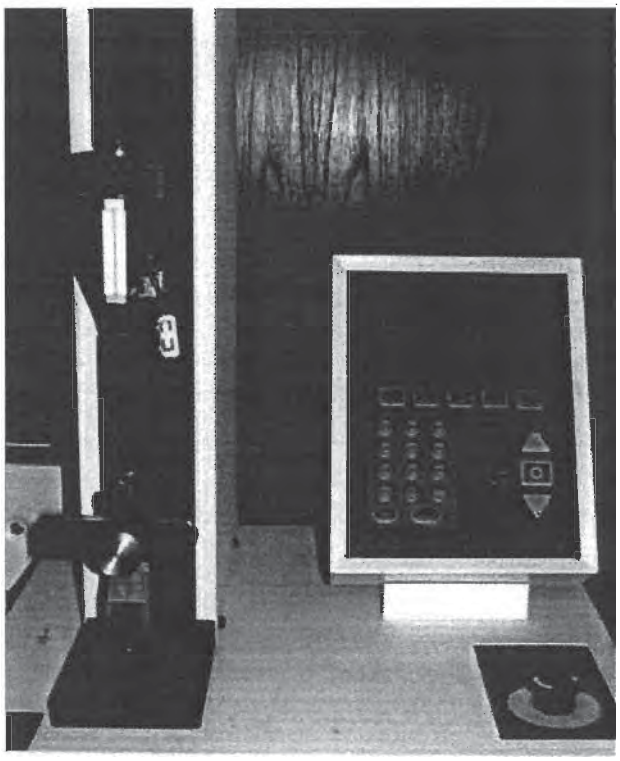


Figure 18 Tensile testing apparatus (H5KS 1509, Tinius® Olsen, England)

**6. *In vitro* determination of dissolution time of film bases**

The dissolution time of film bases was determined using a modified version of the speed of dissolution of the film in water described by Xu et al. (2002). The apparatus consisted of glass beaker containing isotonic phosphate buffer saline pH 6.2 and stirred with a magnetic bar at 60 $\pm$ 5 cycles per minute, a heating unit equipped with a thermostat controlling the temperature at 37 $\pm$ 1 $^{\circ}$C. A fast dissolving film base was secured on the surface of fluid and recorded the dissolution time when the film disappeared or completely disintegrated using stopwatch (Heuer, Switzerland), which has a sensitivity of 0.2 second. The apparatus employed in this study is displayed in Figure 21-23.

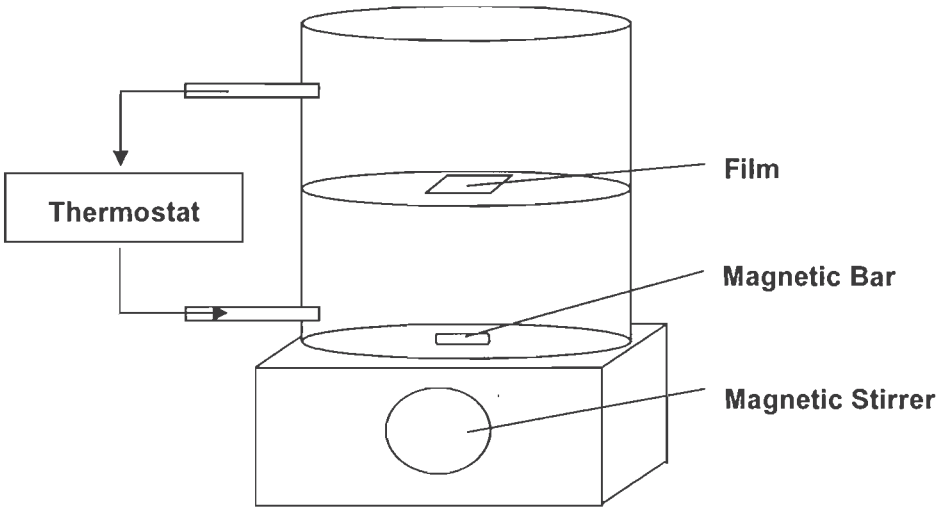



Figure 19 Schematic illustration of dissolution time testing apparatus (assembled in the laboratory).

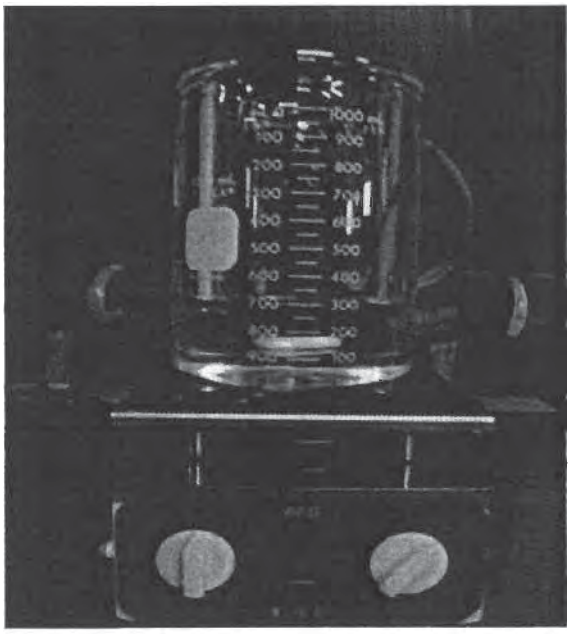


Figure 20 Dissolution time testing apparatus (assembled in the laboratory).

Figure 21 Dissolution time testing apparatus; top view (assembled in the laboratory)

## G Formulation of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract

From the evaluation of film bases (physical, mechanical, and dissolution time), it was found that all formulations of the film consisting of HPMC 3 cps and HPC LV as polymer mixture had appropriate properties both mechanical properties and fast dissolution time and thus were selected to use in the further formulation.

According to the microbiological study of the *Garcinia mangostana* extract, the optimal concentration in the film against *Staphylococcus aureus* ATCC 25923 was obtained from the dose of about 100 times of MIC in a specimen of 2 x 3 $cm^2$ size. The formulations of orally fast dissolving film containing *Garcinia mangostana* extract are shown in Table 10.

Table 9 The formulations of orally fast dissolving films containing *Garcinia mangostana* extract consisting of HPMC 3 cps and HPC LV as polymers. All substances are shown in % w/w.

| Substances (%w/w) | Formulation code | | | |
|---|---|---|---|---|
| | E3HPC 2:1 | E3HPC 3:1 | E3HPC 4:1 | E3HPC 5:1 |
| HPMC 3 cps | 8 | 9 | 9.6 | 10 |
| HPC LV | 4 | 3 | 2.4 | 2 |
| *Garcinia mangostana* extract | 0.216 | 0.216 | 0.216 | 0.216 |
| Acesulfame K | 0.3 | 0.3 | 0.3 | 0.3 |
| Menthol | 1.5 | 1.5 | 1.5 | 1.5 |
| Eucalyptus oil | 0.6 | 0.6 | 0.6 | 0.6 |
| 60% v/v ethanol | 85.4 | 85.4 | 85.4 | 85.4 |

**H Characterization of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract**

**1. Determination of general characteristics of films**

Physical appearances, thickness, weight, surface morphology, mechanical properties and dissolution time of the films containing *Garcinia mangostana* extract were investigated as the same apparatus and procedures of film bases.

**2. Determination of differential scanning calorimetric thermograms**

Differentials scanning calorimetric thermograms were recorded with a differential scanning calorimeter (DSC822$^e$, Mettler Toledo, Switzerland), which is instrument for thermal analysis. A highly sensitive ceramic sensor in DSC instrument is used to measure the difference between the heat flows to the sample and reference crucibles. The standard type pan for DSC measurement is aluminum crucible standard 40 µl. Indium (melting point 156.6 $^{\circ}$C) was used to calibrate the instrument. An accurately weighed amount of 3-5 mg of the sample was placed into an aluminum crucible. Then the aluminum crucible was sealed with the lid under the pressure of the plunger by using crucible sealing press. After sealing, the aluminum crucible was placed on a DSC sensor in the furnace. Samples were heated ranging from –50 to 220 $^{\circ}$C at a heating rate of 10 $^{\circ}$C/min. All tests were performed under a nitrogen atmosphere.

Figure 22 Differential scanning calorimeter (DSC822$^e$, Mettler Toledo, Switzerland).

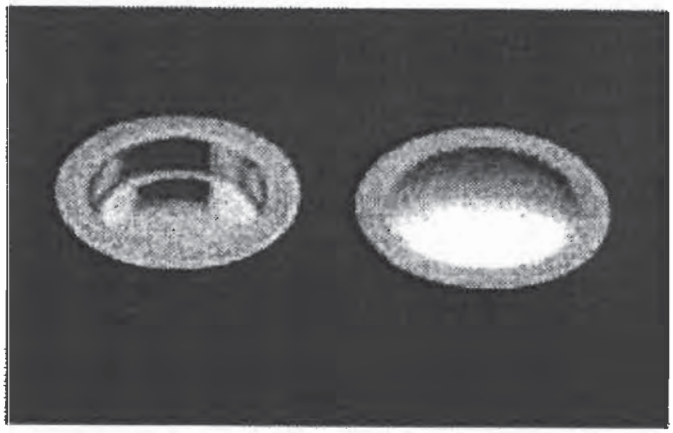


Figure 23 Aluminum crucible standard 40 µl (a) and crucible sealing press (b).

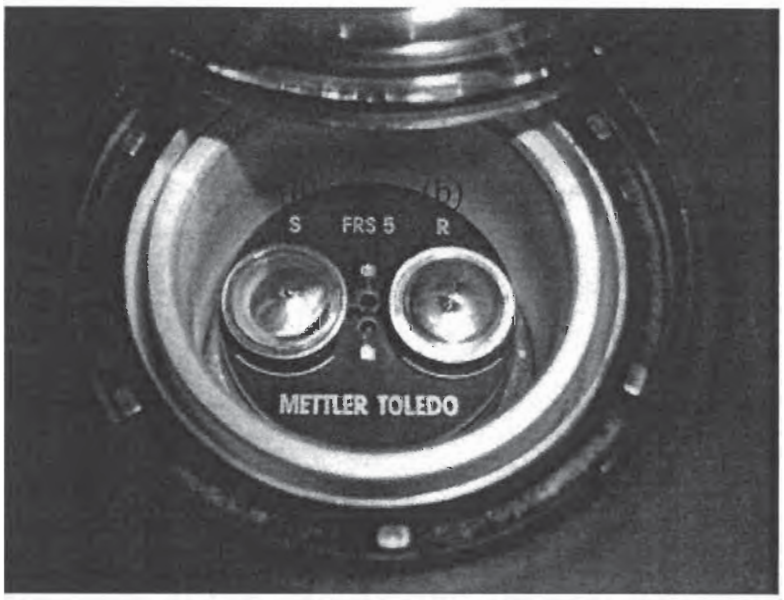



Figure 24 DSC sensor (a) sample pan and (b) reference pan

**3. *In vitro* dissolution study of *Garcinia mangostana* extract orally fast dissolving films**

**3.1 Select medium for dissolution study**

Selection of an appropriate dissolution medium is important to maintain sink condition during the dissolution study. Sodium lauryl sulfate was used to enhance drug solubility in dissolution medium. The solubility study of extract was performed by adding excess amount of the extract into each screw-capped tube containing 5 ml of isotonic phosphate buffer pH 6.2 and sodium lauryl sulfate ranging from 0 to 1.0 % w/v. The tubes were continuously rotated using rotary shaker at 37±0.5 °C for 72 hr to achieve equilibrium. Then the suspensions were centrifuged and the supernatants were withdrawn and filtered through 0.45 µm nylon membrane syringe filters. The filtrates were appropriately diluted and analyzed by HPLC. All solubility determinations were carried out in triplicate.

**3.2 *In vitro* dissolution study**

Dissolution study of orally fast dissolving films containing *Garcinia mangostana* extract was determined using a modified dissolution testing apparatus. The apparatus consisted of a glass beaker containing 20 ml of 1.0% sodium lauryl sulfate in isotonic phosphate buffer pH 6.2 as the dissolution medium and stirred with a magnetic bar at 100 ± 5 cycles per minute, a heating unit equipped with a thermostat controlling the temperature at 37 ± 1 °C. A film product (2 x 3 cm) was secured by placing in a basket before being immersed into the glass beaker and a 3 ml of the dissolution medium was sampled at 1, 3, 5, 7, 10, 15, 30, 45 and 60 minutes. Then the sample was cooled down to ambient temperature, 2.0 ml of sample was pipetted into test tube containing 0.4 ml of clotrimazole stock solution and 1.6 ml of methanol. The solution was filtered through 0.45 µm nylon membrane and analyzed for mangostin content using HPLC as the same previously described conditions. The apparatus employed in this study is displayed in Figure 27.

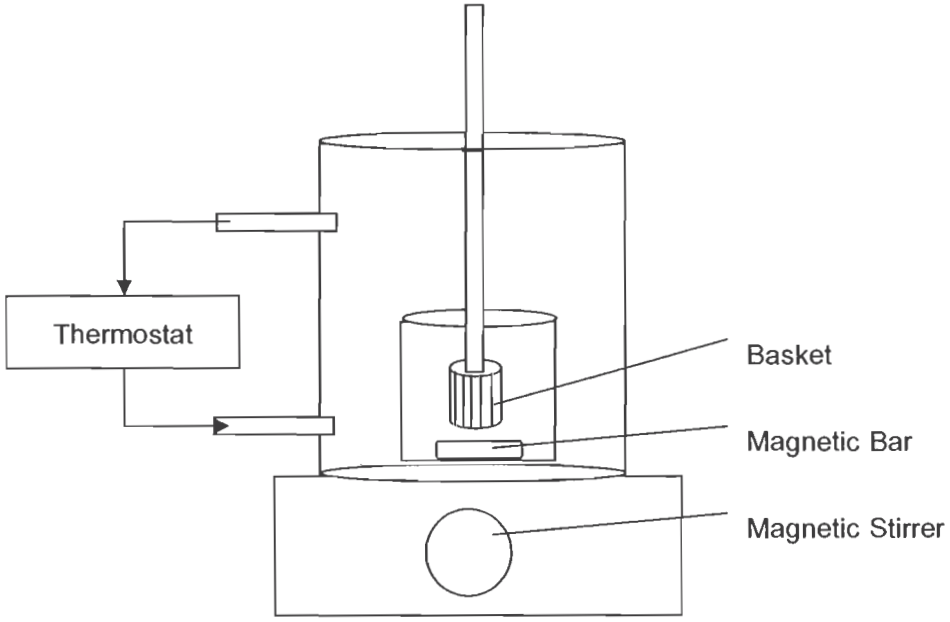



Figure 25 Modified dissolution testing apparatus (assembled in the laboratory)

**4. Determination of content uniformity**

The content uniformity of *Garcinia mangostana* extract in the orally fast dissolving films was quantitatively determined by mean of peak area ratio by HPLC method. The film was cut into ten small pieces (2 x 3 $cm^2$) with an accurate weight. Each piece was determined for the amount of mangostin. They are individually tested for their content uniformity. Each piece was analyzed by dissolving in 10 ml volumetric flask containing mobile phase and shaken at room temperature until the film was completely dissolved. Then 1.0 ml of stock internal standard was added into the flask, finally adjusted volume to 10 ml. The solution was filtered through 0.45 µm nylon membrane and analyzed using the HPLC method described previously. The percentage of active drug within the test films was calculated from the calibration curve.

**I Stability Study of Mangostin in Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract**

The film preparations (2 x 3 $cm^2$) were stored in glass vials, which were tightly sealed with rubber closure and aluminum caps at 40±2 °C and the humidity at 75±5 %RH for three months (Carstensen, 1990). The amounts remaining of mangostin were determined in triplicate at initial, 1, 2, and 3 month periods. The analysis of mangostin in film samples followed the previously described HPLC method. Triplicate samples of each test preparation were randomly drawn and analyzed compared with purified extract solution.

**J *In Vitro* Antimicrobial Activity of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract**

All formulations of orally fast dissolving films containing *Garcinia mangostana* extract were evaluated for their *in vitro* antimicrobial effect against *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$ that are commonly microorganisms found in oral cavity.

**1. Antimicrobial activity of films**

Antimicrobial activity of films was determined by agar diffusion using Mueller Hinton agars (Difco, USA). The method was previously standardized by adjusting the microbial incubation at 0.5 Mcfarland, 1 x $10^8$ cells/ml.

After adjusting the turbidity of the inoculum suspension, a sterile swab was dipped into the suspension and pressed the swab on the inside wall of tube to remove excess inoculum from the swab. The inoculum was swabbed over the entire agar surface in three different directions to ensure an even distribution of the inoculum. Five-mm diameter disks were cut from different films and placed on Mueller Hinton agars using sterile forceps. The agar plates were cooled down at 25 °C for 3 hours to allow diffusion of the extract and then incubated at 37 °C, 24 hours for *Staphylococcus aureus* ATCC 25923 and 48 hr, 37 °C in $CO_2$ for *Streptococcus mutans* ATCC $KPSK_2$. Each formulation was performed in triplicate. After incubation, the diameters of the zones produced by antimicrobial inhibition of bacterial growth were measured using Vernier caliper (Macoh, China). Data are expressed as growth inhibitory zone diameter in millimeter (Lorian, 1991; Mahon and Manuselis, 2000; Sebti, Broughton, and Coma, 2003).

The negative control groups of this experiment were polymer films without any additives and films base formulations without extract. Commercial product strips A, and the prepared film of cetylpyridinium chloride and chlorhexidine diacetate at the same concentration of extract were the positive control.

**2. Antimicrobial activity of solution of films**

The films were cut into 2 x 3 cm and dissolved with 2 ml of isotonic phosphate buffer pH 6.2 as simulated saliva. The films solutions were dropped into the sterile stainless steel cups that placed on Mueller Hinton agars swapped with inoculum (1 x $10^8$ cells/ml).

The plates were cooled down at 25 °C for 3 hours to allow diffusion of the extract and then incubated at 37 °C, 24 hr for *Staphylococcus aureus* ATCC 25923 and 48 hr, 37 °C in $CO_2$ for *Streptococcus mutans* ATCC $KPSK_2$. Each formulation was performed in triplicate. After incubation, the inhibition zone diameters were determined by Vernier caliper in millimeter.

The antimicrobial activity of orally fast dissolving films containing extract were compared with positive control as commercial product strips A, commercial mouth wash product B, containing essential oils, and commercial mouth wash product C, contained 0.12% w/v of chlorhexidine gluconate, prepared film with cetylpyridinium chloride and film with chlorhexidine diacetate at the same concentration with extract. The negative control was isotonic phosphate buffer pH 6.2, polymer film without any additives, and film base without *Garcinia mangostana* extract.

**K Statistical Analysis**

The data of dissolution time, tensile strength, percent elongation, work of failure, percentage of drug dissolution and amount of mangostin remaining after the stability study were analyzed by statistically using one-way analysis of variance (ANOVA). When a significant difference ($p<0.05$) was indicated, the data were subjected to multiple comparison by Duncan test to compare the difference. The statistical package for the social sciences (SPSS) program version 11.0 was used in this study.

## PART III MONOGLYCERIDE-BASED DRUG DELIVERY SYSTEMS (LIQUID CRYSTALLINE) CONTAINING *Garcinia mangostana* EXTRACT

**L . Preparation of Monoglyceride-Based Drug Delivery System**

**1. Monoglyceride-Based Drug Delivery System**

Monoglyceride-based drug delivery system is based on the ability of mixture of monoglyceride and triglyceride to form liquid crystals when in contact with water. Monoglyceride-based drug delivery systems were developed by using glyceryl monooleate, triglycerides and water. Triglycerides used in this study were sesame oil, soybean oil and olive oil.

Monoglyceride-based drug delivery systems were prepared on a weight by weight (w/w) basis by weighing the three components in screw-capped tubes and vigorously mixed using vortex mixer for 5 min. The samples were heated at 45±0.5 ºC and occasionally mixed for 30 min. The samples were stored at room temperature for 1 week before further studies.

**2. Physical Characterization**

**2.1 Polarized Light Microscopy**

A drop of samples was placed on a glass slide and examined for the liquid crystalline phases under the polarized light microscope (Eclipse E200, Nikon, Japan). Anisotropic liquid crystalline phases, such as hexagonal, lamella and reversed hexagonal

phases, are optically birefringent. This property can be used for studying such phases with polarizing microscopy. On the other hand, isotropic phases (e.g. micellar and reversed micellar solutions and cubic phases) are nonbirefringent and generate a dark background when investigated under the polarizing microscope.

**2.2 Physical Stability**

The samples that could form reversed hexagonal phases were evaluated for their physical stability by heating-cooling cycle. The samples were stored in a hot air oven at 45±0.5 ºC for 48 hr, and then placed in a refrigerator at 4±0.5 ºC for 48 hr. The heating-cooling cycle was repeated for 6 cycles.

**M. Formulation of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

The most stable samples from the study in topic C 2.2, with the equilibrium water content, were selected. Since a mixture of glyceryl monooleate and oil with drug comes into contact with water, hydration progresses and the formulation turns into a liquid crystalline state with high viscosity. The equilibrium water content means the maximum water uptake without causing phase separation. Monoglyceride-based drug delivery systems were prepared by weighing the three components and 2% *Garcinia mangostana* extract in screw-capped tubes and then followed method as described in C1.

**N. Characterization of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

**1. Determination of Physicochemical Properties**

The physicochemical properties of formulations before and after incorporating the *Garcinia mangostana* extract were determined as follows:

**1.1 Physical Appearances**

The physical appearances of formulations such as color, clarity and phase separation were observed.

**1.2 pH Measurement**

The pH of formulations was measured by using pH meter (Model 420A, Orion, USA). The measurements were done in triplicate.

**1.3 Viscosity Measurement**

The viscosity of formulations was determined by using cone and plate viscometer (Brookfield viscometer, Scientific Industries, USA.). The determination of the viscosity was performed by applying about 0.5 ml of sample to the lower plate of the viscometer. The cone CP-40 was used. The measurements were performed in triplicate.

**1.4 Polarized Light Microscopy**

A drop of samples was placed on a glass slide and examined for the liquid crystalline phases under polarized light microscope. The polarized light photomicrographs

were recorded by digital camera (Coolpix 5400, Nikon, Japan) to compare the liquid crystalline phases before and after adding the extract.

**2. Determination of Injectability through the Syringe**

The injectability through the syringe was performed to ensure that the formulations could be administered by syringe into a periodontal pocket. Since the high-viscous formulations had stiffness and could not be administered by syringe, the low-viscous formulations were used in this study. When they are in contact with the fluid in the oral cavity, the formulation turns into a liquid crystalline state and changes to a semi-solid at the injection sites.

The water-free formulations with low viscosity were evaluated for injectability by administering through a syringe with 23-gauge tip needle. The viscosity of formulations was also determined by using cone and plate viscometer in triplicate.

**3. *In vitro* Liquid Crystalline Phase Formation Study**

This study was performed to ensure that the formulations could form liquid crystalline phases with high viscosity upon dilution with water. The study was modified from Scherlund et al. (2001). The test was conducted within a screw-capped tube at the temperature 37±0.5 ºC using water bath. The water-free formulations 0.5 ml was added into 2 ml of water then the liquid crystal formation was detected by polarized light microscopy.

**4. *In vitro* Release Study**

**4.1 Solubility Study of the Receiver Fluid**

Selection of an appropriate receiver fluid is important to maintain sink condition during the release study. Ethanol was used to enhance drug solubility in receiver fluid. Excess amount of the extract was put into each screw-capped tube containing 5 ml of pH 7.4 phosphate buffer and ethanol in pH 7.4 phosphate buffer ranging from 0 to 35%. The tubes were continuously rotated at 37±0.5 ºC for 48 hr to achieve equilibrium. Then the suspensions were centrifuged and the supernatants were withdrawn and filtered through 0.45 µm cellulose acetate syringe filters. The filtrates were appropriately diluted and analyzed by UV spectrophotometer (UV-1601, Shimadzu, Japan). All solubility determinations were carried out in triplicate.

**4.2 *In vitro* Release Study**

The *in vitro* release study was performed using modified Franz diffusion cell which consists of a donor compartment and a receiver compartment (Figure 26). The cellophane membrane was placed between two compartments of modified Franz diffusion cell. The membrane was soaked in the medium before use. The receiving compartment contained 14 ml of 35% v/v ethanol in pH 7.4 phosphate buffer which was maintained at 37±0.5 ºC by a circulating water jacket. The receptor fluid and membrane were equilibrated to the desire temperature for 1 hr before the release study. After equilibration, 0.1 g of the sample was carefully placed into the donor compartment using a syringe and then covered

with paraffin film to prevent evaporation. The receptor fluid was continuously mixed by magnetic stirring bar at 600 rpm throughout the time of release study. Any air bubbles formed under the preparation had necessarily been removed before the experiment was started. Four specimens of each formulation were examined.

Samples of 5 ml were taken from the receiver medium at certain time intervals (1, 3, 6, 9, 12, 24, 36 and 48 hr) via the sampling port of diffusion cell. The receiver medium was removed by using a syringe and the receiver compartment was replaced with the same amount of medium to keep the constant volume during the experiment. All receiver solutions taken were analyzed concentrations of mangostin in the medium by using UV spectrophotometer at 243 nm.

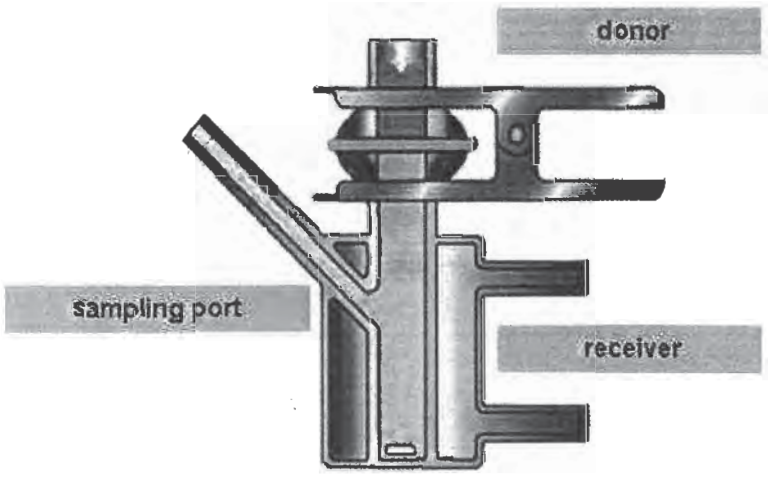



Figure 26 A schematic illustration of the two compartment of Franz diffusion cell

The amount of drug release was calculated by multiplying the drug concentration with the receiver volume. The percentage of drug release was calculated by the following equation:

$$\% \text{ drug release} = (A_t / A_0) \times 100$$

where $A_t$ is the cumulative released amount of drug at a particular time; $A_0$ is the initial amount of drug.

**4.3 Assay of *In vitro* Release**

The determination of amount of mangostin release was performed by UV spectrophotometry due to rapidity and convenience.

**5. Differential Scanning Calorimetric (DSC) Method**

The DSC thermogram of samples was determined by using differential scanning calorimeter (DSC822$^e$, Mettler Toledo, Switzerland). A highly sensitive ceramic sensor in DSC instrument is used to measure the difference between the heat flows to the sample and reference crucibles. The standard type of pan for DSC measurement is aluminum

crucible standard 40 µl. An accurately weighed amount of 3-5 mg of the samples was placed in an aluminum crucible. Then the aluminum crucible was sealed with the lid under the pressure of the plunger by using crucible sealing press. After sealing, the aluminum crucible was placed on a DSC sensor in the furnace. Samples were heated from -20 to 250 °C at a heating rate of 10 °C/min. All tests were performed under a nitrogen atmosphere.

**6. Determination of Antimicrobial Activity of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

*Streptococcus mutans* $KPSK_2$ is a representative of bacteria in the dental plaque. The effectiveness of formulations against *Streptococcus mutans* $KPSK_2$ was evaluated using agar diffusion method. The efficacy was determined by the diameter of inhibition zone.

The principle of agar diffusion method is dependent upon the inhibition of the growth of bacteria on the surface of an inoculated agar plate, by the antimicrobial agent that diffuses into the surrounding medium.

Mueller Hinton Agar medium was prepared by adding 19 g of the agar to 500 ml of distilled water and boiled to dissolve the medium completely. After that the sterilization was performed by autoclaving at 121±0.5 °C for 15 min. Each agar plate was prepared by pouring 30 ml of the medium to Petri dish with a diameter of 10 cm and allowed to solidify at room temperature. The depth of agar was approximately 4 mm. Then the agar plates were perforated by using a 5 mm diameter cylindrical tube to make a hole for filling the samples.

The inoculum was prepared by picking the isolated 48 hr colonies of *Streptococcus mutans* $KPSK_2$ into broth and letting the inoculum grow for 12 hr. Then 5 ml of the inoculum was transferred to new broth (100 ml) and grown for 4 hr to reach mid-exponential growth phase. The inoculum was compared for the turbidity equivalent to the McFarland No.0.5 standard ($1.5\times10^8$ CFU/ml) to provide an appropriate growth of the microorganism for testing the antimicrobial activity. The method for determining the turbidity of McFarland No.0.5 standard was done by using spectrophotometer. The cuvette of inoculum was placed in the spectrophotometer and read the absorbance at 600 nm, the absorbance of the inoculum should be approximately 0.015. If the suspension was too dense, the inoculum was diluted with additional sterile broth. The standardized inoculum suspensions should be used within 15 min of preparation.

After adjusting the turbidity of the inoculum suspension, a sterile swab was dipped into the suspension and rotated the swab several times with a firm pressure on the inside wall of the flask above the fluid level to remove excess inoculum from the swab. The inoculum was swabbed over the entire agar surface in three different directions to ensure an even distribution of the inoculum. The plate was allowed to dry at room temperature for 5-10 min. The formulations were placed into the holes of agar about 4-5 holes/plate. Each formulation was performed in triplicate. Then the plates were incubated at 37±0.5 °C for 24

hr in $CO_2$ water jacketed incubator (Forma Scientific, Inc., USA). After incubation, the diameters of the zones produced by antimicrobial inhibition of bacterial growth were measured using Vernier caliper (Komsri, 1997; Mahon and Manuselis, 2000).

**7. Determination of Partition Coefficient of *Garcinia mangostana* Extract**

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water

$$P_{ow} = \frac{C_{n\text{-octanol}}}{C_{water}}$$

The partition coefficient therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (log P).

The most common method for determining partition coefficient is the shake flask method. In this technique, the candidate drug is shaken between n-octanol and water layers, from which an aliquot is taken and analyzed.

Before the partition coefficient was determined, the two solvents were mutually saturated by shaking at the room temperature. Two stock bottles were shaken for 24 hr on a mechanical shaker, one containing n-octanol and a sufficient quantity of water, and the other containing water and sufficient quantity of n-octanol. Then the two bottles were let to allow the phase separation.

The test solution was prepared in n-octanol with a concentration of 5 mg/ml of mangostin. Five millilitres of the two solvents prepared as described above were filled into screw-capped tubes. The tubes were continuously rotated at room temperature for 48 hr to achieve equilibrium. Then the tubes were let to allow the phase separation. The partition coefficient was determined by analyzing the concentrations of mangostin in both phases by using UV spectrophotometer at 243 nm. All partition coefficient determinations were carried out in triplicate (OECD 107, 1995).

**O. Stability Study of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

The stability study was performed by heating-cooling cycle. The samples were placed in a closed container and stored in a hot air oven at 45±0.5 ºC for 48 hr, and then placed in a refrigerator at 4±0.5 ºC for 48 hr. The heating-cooling cycle was repeated for 6 cycles. The physicochemical properties were determined in the same method as described in E1 and the determination of amount of mangostin was performed by HPLC method. The analysis of samples followed the method described in B3. The amount of mangostin was determined in triplicate at the initial and after the stability study.

**P. Statistical Analysis**

The data of viscosity, percentage of drug release, coefficient of determination of the release kinetic, the Higuchi release rate constant and amount of mangostin remaining after the stability study were analyzed by one-way analysis of variance (ANOVA). When a significant difference ($P<0.05$) was indicated, the data were subjected to multiple comparison by Fisher's least significant difference (LSD) test to compare the difference. The statistical package for the social sciences (SPSS) program version 12.0 was used in this study.

# CHAPTER III

# RESULTS AND DISCUSSION

## PART I: Extraction, Identification, Quanlitative and Quantitative Analysis And Antibacterial activity of *Garcinia mangostana* fruit hulls

### A. Extraction of Active Constituents from *Garcinia mangostana*

The crude extract of *Garcinia mangostana* was obtained by maceration of four kilograms of the dried fruit hull powder with ethyl acetate for extraction of non polar substances. The ethyl acetate extract was brownish viscous liquid, which was concentrated by using a rotary evaporator. Then the extract was crystallized into yellow crystal and ground into fine powder for further studies (Figure 28). The photomicrograph of yellow bright needle shaped crystalline of mangostin obtained is shown in Figure 27. The weight of final yield was 324.5 g, which was calculated as 7.47% yield.

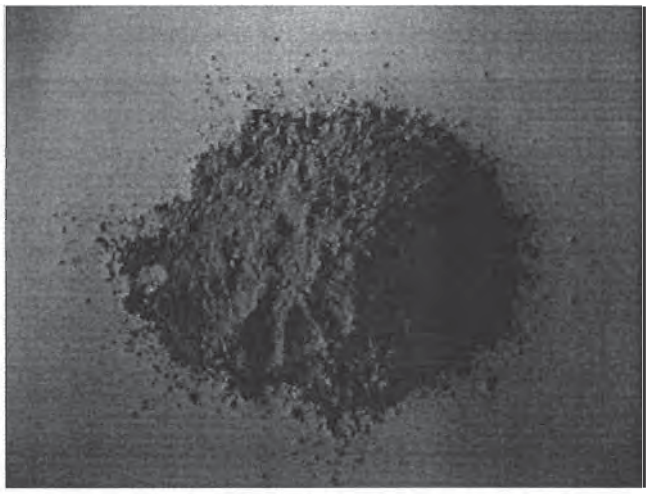


Figure 27 Photograph of fine powder of *Garcinia mangostana* extract

### B. Identification and Determination of Active Constituents from *Garcinia mangostana*

#### 1. Differential scanning calorimetric (DSC) method

The information from differential scanning calorimetric (DSC) method can be used for compound identification or in an estimation of purity. The melting point of a substance is defined as the temperature at which the solid phase exists in equilibrium with its liquid phase (Brittain, 1995).

The accurately weighed amount of the extract was placed into an aluminum pan and the run was performed at the heating rate of 10 $^{\circ}$C/min in the temperature ranging from –50 to 220 $^{\circ}$C under nitrogen atmosphere. The experiment was performed in triplicate. The DSC thermogram of the extract showed an endothermic melting peak with a broad range of temperature at 165.04-166.80 $^{\circ}$C (Figure 28), while the melting point of mangostin which was reported as 181.6-182.6 $^{\circ}$C (Gopalakrishnan, Banumathi, and Suresh, 1997; Budavari,

2001). This result indicated that the extract might contain a mixture of other constituents such as 8-desoxygartanin (mp 155-156 °C), BR-xanthone (mp 180-182 °C), gartanin (mp 164-166 °C), or β-mangostin (mp 178-179 °C).

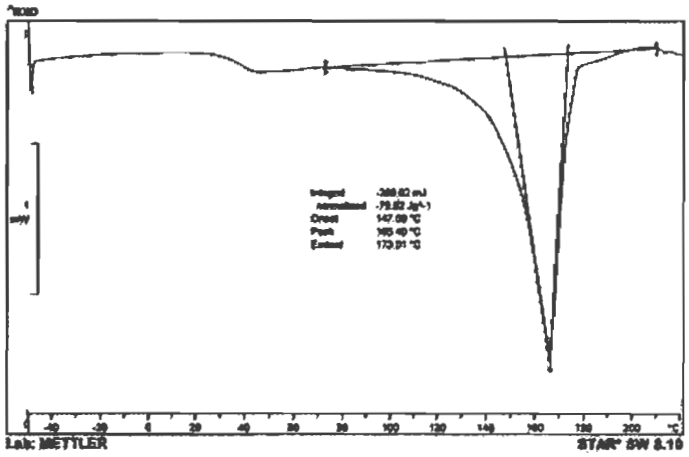


Figure 28 DSC thermogram of *Garcinia mangostana* extract (sample weight 3.27 mg)

**2. Thin layer chromatographic (TLC) method**

The isolated compound from dried fruit hull extract was identified by TLC. The chromatogram of the compound was compared to the standard mangostin as shown in Figure 29. In this chromatogram, the Rf value of the extract was equal to the standard mangostin at the value of 0.60. Based on this data, major composition of this extract was mangostin.

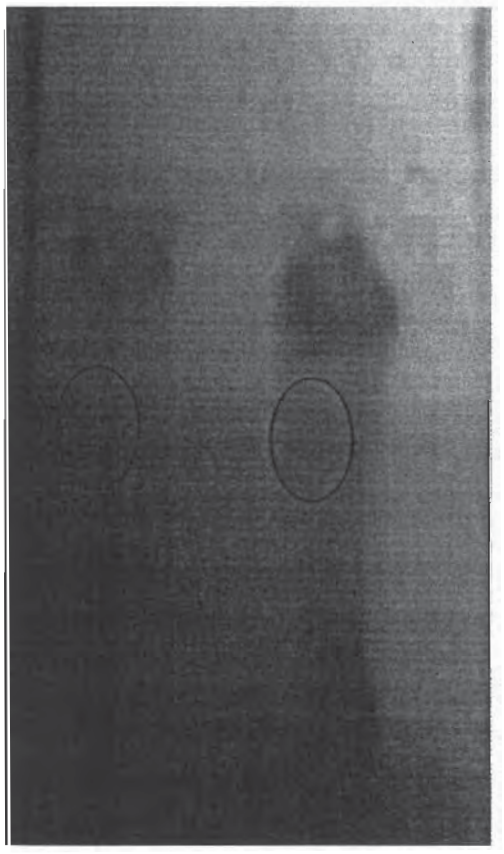


Figure 29 TLC chromatogram of (a) standard mangostin and (b) *Garcinia mangostana* extract.

**3. High performance liquid chromatographic (HPLC) method**

The developed HPLC system was applied to analyze the extract from dried fruit hulls of *Garcinia mangostana*. It was found that the extract had similar chromatogram to the standard mangostin. However, in the same concentration the extract gave the peak area ratio of 55.86 $\pm$ 0.22 % of the standard mangostin. This result is consistent with the information from DSC that the extract might contain a mixture of other compounds.

**3.1 Validation of HPLC method**

The validation of analytical method is the process by which it is established that the performance characteristics of the method meet the requirements for the intended analytical applications. The performance characteristics are expressed in term of analytical parameters. For HPLC assay validation, these include specificity, linearity, accuracy and precision.

**3.1.1 Specificity**

The specificity of an analytical method is its ability to measure the analyte accurately and with specificity in the presence of other components in the sample.

The internal standard technique was performed by determining the peak area ratio of mangostin to clotrimazole (internal standard) to give the complete separation, appropriate resolution and sharp peaks of all components. The methanol-water mixture of 87% by volume was used as the mobile phase. The typical chromatograms of blank solution, internal standard solution, mangostin standard solution and *Garcinia mangostana* extract solution are shown in Figure 30-35.

The retention times of internal standard solution, mangostin standard solution and *Garcinia mangostana* extract solution were around 5.073, 8.844, 8.872 min, respectively. In addition, there was no interference from other components in the chromatogram.

**3.1.2 Linearity**

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical ralationship from test results obtained by the analysis of samples with varying concentrations of analyte. The calibration plot of mangostin concentrations versus the peak area ratios of mangostin and its internal standard, clotrimazole illustrated the linear correlation in the concentration range studied of 1-40 µg/ml (Figure 37). The coefficient of determination ($R^2$) of this line was 0.9999. These results indicated that HPLC method was acceptable for quantitative analysis of mangostin in the range studied.

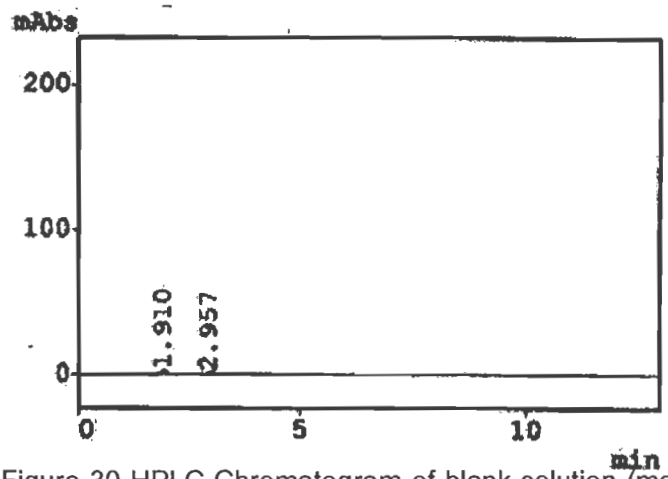



Figure 30 HPLC Chromatogram of blank solution (mobile phase).

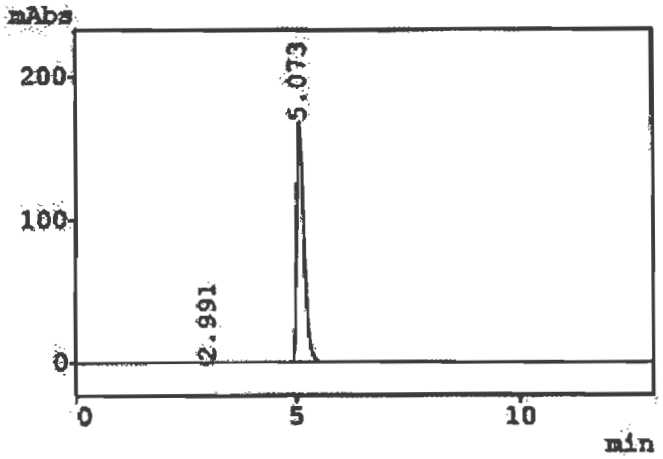



Figure 31 HPLC chromatogram of internal standard solution (clotrimazole).

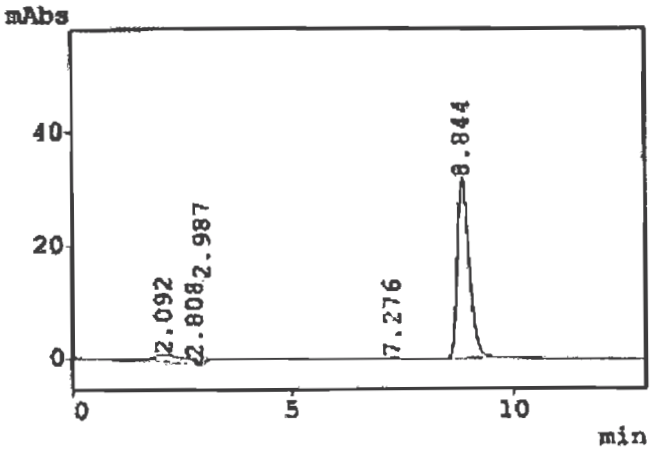



Figure 32 HPLC chromatogram of mangostin standard solution.

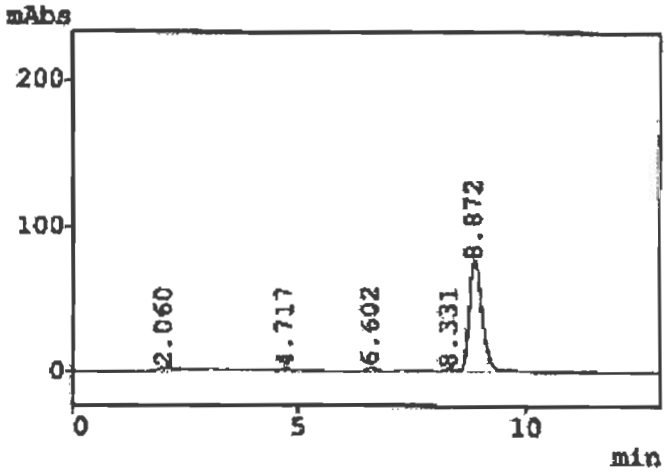



Figure 33 HPLC chromatogram of *Garcinia mangostana* extract solution.

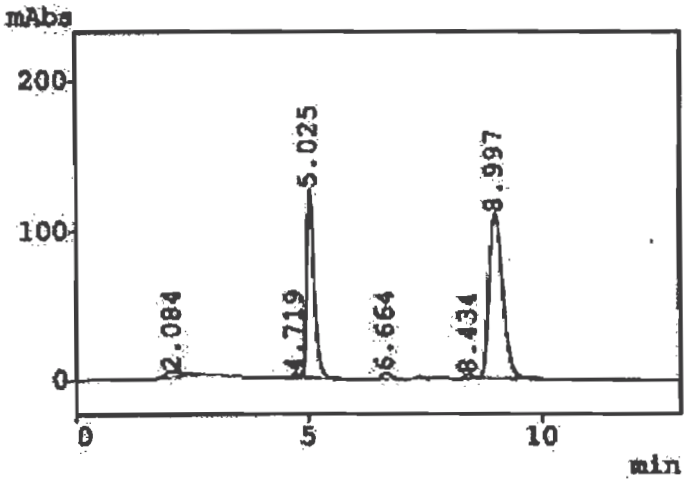



Figure 34 HPLC chromatogram of mixture of the extract and internal standard.

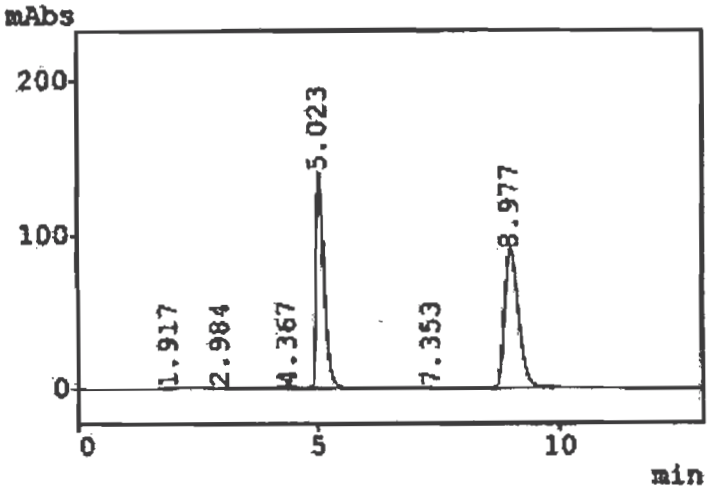



Figure 35 HPLC chromatogram of mixture of mangostin and internal standard.

### 3.1.3 Accuracy

The determination of accuracy was performed by analyzing five sets of three concentration (8.0, 25.0, 35.0 μg/ml). The inversely estimated concentration and percentages of analytical recovery of each drug concentration are shown in Table 11 and Table 12, respectively. All percentages of analytical recovery were in the range of 98.82 –

100.42 %, which indicated that this method could be used for analysis of mangostin in all concentrations studied with high accuracy.

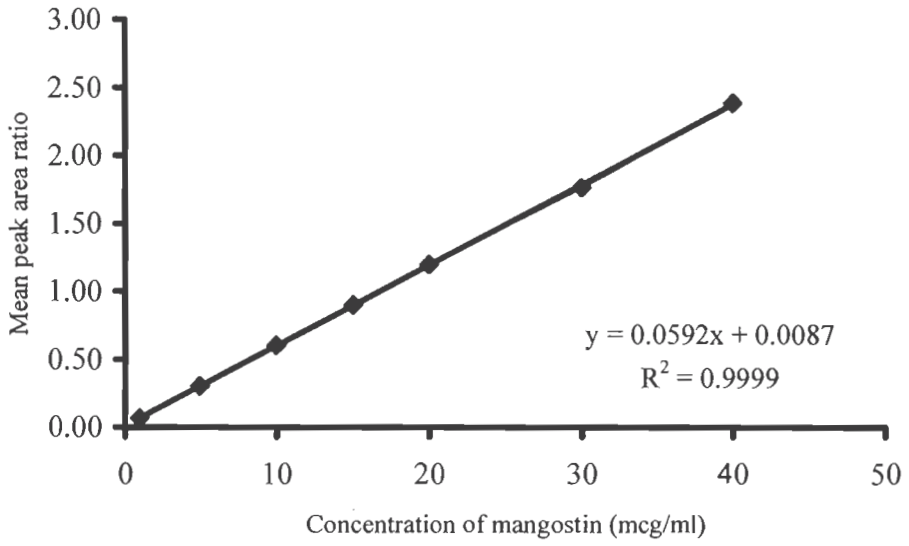



Figure 36 Calibration curve of mangostin by HPLC method

Table 10 The inversely estimated concentrations of mangostin by HPLC method

| Concentration (μg/ml) | Inversely estimated concentration (μg/ml) | | | | | Mean ± SD |
|---|---|---|---|---|---|---|
| | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | |
| 8 | 7.9687 | 8.0675 | 7.9516 | 8.1124 | 8.0675 | 8.0335±0.07 |
| 25 | 24.7167 | 24.7683 | 24.8054 | 24.5531 | 24.6809 | 24.7049±0.10 |
| 35 | 34.6363 | 34.6929 | 34.8002 | 34.5115 | 34.6929 | 34.6668±0.11 |

Table 11 The percentage of analytical recovery of mangostin by HPLC method

| Concentration (μg/ml) | % Analytical recovery | | | | | Mean ± SD |
|---|---|---|---|---|---|---|
| | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | |
| 8 | 99.61 | 100.84 | 99.39 | 101.40 | 100.84 | 100.42±0.87 |
| 25 | 98.87 | 99.07 | 99.22 | 98.21 | 98.72 | 98.82±0.39 |
| 35 | 98.96 | 99.12 | 99.43 | 98.60 | 99.12 | 99.05±0.30 |

### 3.1.4 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Table 12 and Table 13 illustrate the data of within run precision and between run precision, respectively. All coefficient of variation values were small, 0.32-0.85% and 0.66-1.43%, respectively. The coefficient of variation of an analytical method should generally be less than 2%. Therefore, the HPLC method was precise for quantitative analysis of mangostin in the range studied.

Table 12 Data of within run precision by HPLC method

| Concentration | Peak area ratio | | | | | Mean | SD | %CV |
|---|---|---|---|---|---|---|---|---|
| (μg/ml) | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | | | |
| 8 | 0.4804 | 0.4863 | 0.4794 | 0.4890 | 0.4863 | 0.4843 | 0.00 | 0.85 |
| 25 | 1.4719 | 1.4750 | 1.4772 | 1.4622 | 1.4698 | 1.4712 | 0.01 | 0.39 |
| 35 | 2.0592 | 2.0625 | 2.0689 | 2.0518 | 2.0625 | 2.0610 | 0.01 | 0.30 |

Table 13 Data of between run precision by HPLC method

| Concentration | Peak area ratio | | | | | Mean | SD | |
|---|---|---|---|---|---|---|---|---|
| (μg/ml) | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | | %CV | |
| 8 | 0.4876 | 0.4783 | 0.4696 | 0.4796 | 0.4734 | 0.4777 | 0.01 | 1.43 |
| 25 | 1.4648 | 1.4785 | 1.4832 | 1.4688 | 1.4595 | 1.4710 | 0.01 | 0.66 |
| 35 | 2.0946 | 2.0523 | 2.0548 | 2.0685 | 2.0536 | 2.0648 | 0.02 | 0.87 |

In conclusion, the analysis of mangostin by HPLC method developed in this study showed good specificity, linearity, accuracy and precision. Thus this method was used for the determination of the content of mangostin in this study.

**4. UV Spectrophotometric Method**

**1. Validation of UV spectrophotometric method**

The validation of analytical method is the process for evaluation that the method is suitable and reliable for the intended analytical applications. The analytical parameters used for the UV spectrophotometric assay validation were specificity, linearity, accuracy and precision.

**1.1 Specificity**

The UV validation absorption spectrum of mangostin is shown the maximum absorbance at the wavelength of 243 nm. Therefore, the detection of mangostin was performed at this wavelength (Hiranras, 2001).

**1.2 Linearity**

The calibration curve of mangostin in absolute ethanol was shown in Figure 40. Linear regression analysis of the absorbances versus the corresponding concentrations was performed and the coefficient of determination ($R^2$) was calculated as 0.9999. The calibration data were found to be linear with excellent coefficient of determination. These results indicated that UV spectrophotometric method was acceptable for quantitative analysis of mangostin in the range studied.

**1.3 Accuracy**

Mangostin solutions were prepared at the concentration of 3.0, 5.4, 7.8 µg/ml in five sets. Each individual sample was analyzed by UV spectrophotometer. The inversely estimated concentrations and percentages of analytical recovery of each drug concentration are shown in Table 15 and 16, respectively. All percentages of analytical recovery were in the range of 98.54-99.45%, which indicated the high accuracy of this method. Thus, it could be used for analysis of mangostin in all concentrations studied.

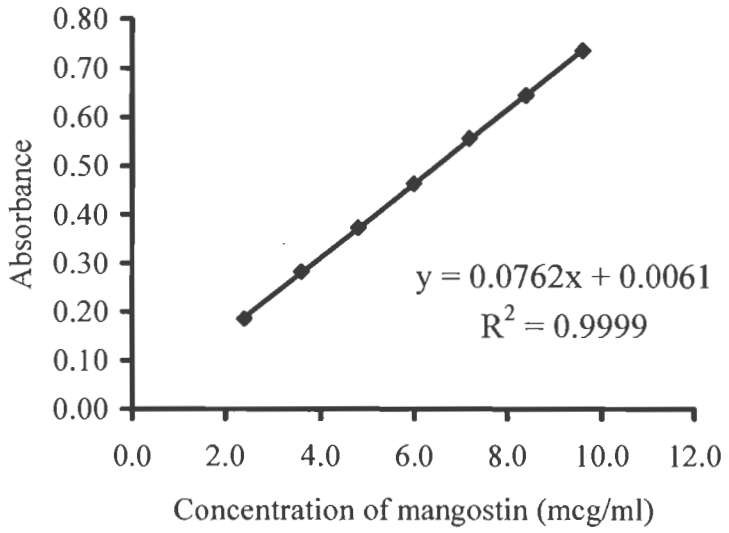



Figure 37 Calibration curve of mangostin by UV spectrophotometric method.

Table 14 The inversely estimated concentrations of mangostin by UV spectrophotometric method

| Concentration (µg/ml) | Inversely estimated concentration (µg/ml) | | | | | Mean ± SD |
|---|---|---|---|---|---|---|
| | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | |
| 3.0 | 2.9922 | 2.9778 | 2.9490 | 2.9974 | 2.9895 | 2.9812 ± 0.02 |
| 5.4 | 5.4092 | 5.3922 | 5.3490 | 5.4026 | 5.2980 | 5.3702 ± 0.05 |
| 7.8 | 7.6693 | 9.6915 | 7.6967 | 7.6641 | 7.7085 | 7.6860 ± 0.02 |

Table 15 The percentage of analytical recovery of mangostin by UV spectrophotometric method

| Concentration (µg/ml) | % analytical recovery | | | | | Mean ± SD |
|---|---|---|---|---|---|---|
| | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | |
| 3.0 | 99.74 | 99.26 | 98.30 | 99.91 | 99.65 | 99.37 ± 0.65 |
| 5.4 | 100.17 | 99.85 | 99.06 | 100.05 | 98.11 | 99.45 ± 0.86 |
| 7.8 | 98.32 | 98.61 | 98.68 | 98.26 | 98.83 | 98.54 ± 0.24 |

**1.4 Precision**

The precision of mangostin analyzed by UV spectrophotometric method were determined both within run precision and between run precision as illustrated in Tables 16-17. All coefficients of variation values were very low, as 0.24-0.87% and 0.62-1.13%, respectively. The coefficient of variation of an analytical method should generally be less than 2%. Therefore, the UV spectrophotometric method was precise for quantitative analysis of mangostin in the range studied.

Table 16 Data of within run precision by UV spectrophotometric method

| Concentration | Absorbance | | | | | Mean | | SD |
|---|---|---|---|---|---|---|---|---|
| (µg/ml) | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | %CV | | |
| 3 | 0.2294 | 0.2283 | 0.2261 | 0.2298 | 0.2292 | 0.2286 | 0.001 | 0.65 |
| 5.4 | 0.4143 | 0.4130 | 0.4097 | 0.4138 | 0.4058 | 0.4109 | 0.004 | 0.87 |
| 7.8 | 0.5872 | 0.5889 | 0.5893 | 0.5868 | 0.5902 | 0.5885 | 0.001 | 0.24 |

Table 17 Data of between run precision by UV spectrophotometric method

| Concentration | Absorbance | | | | | Mean | | SD |
|---|---|---|---|---|---|---|---|---|
| (µg/ml) | Set 1 | Set 2 | Set 3 | Set 4 | Set 5 | %CV | | |
| 3 | 0.2255 | 0.2248 | 0.2296 | 0.2231 | 0.2278 | 0.2262 | 0.003 | 1.13 |
| 5.4 | 0.413 | 0.4155 | 0.4201 | 0.4119 | 0.4162 | 0.4153 | 0.003 | 0.77 |
| 7.8 | 0.5877 | 0.5921 | 0.5861 | 0.5823 | 0.5848 | 0.5866 | 0.004 | 0.62 |

In conclusion, the analysis of mangostin in by UV spectrophotometric method developed in this study showed good specificity, linearity, accuracy and precision. Thus this method was used for the determination of the content of mangostin in the solubility study.

**C. Determination of Solubility of *Garcinia mangostana* Extract**

The solubility of *Garcinia mangostana* extract in hydroalcoholic mixtures at various concentrations of ethanol at ambient temperature are shown in Table 19. The solubility values expressed were the mean ± S.D. of three determinations. The sharp increase in the

solubility of extract was observed as increasing ethanol content as cosolvent to the medium (Figure 39).

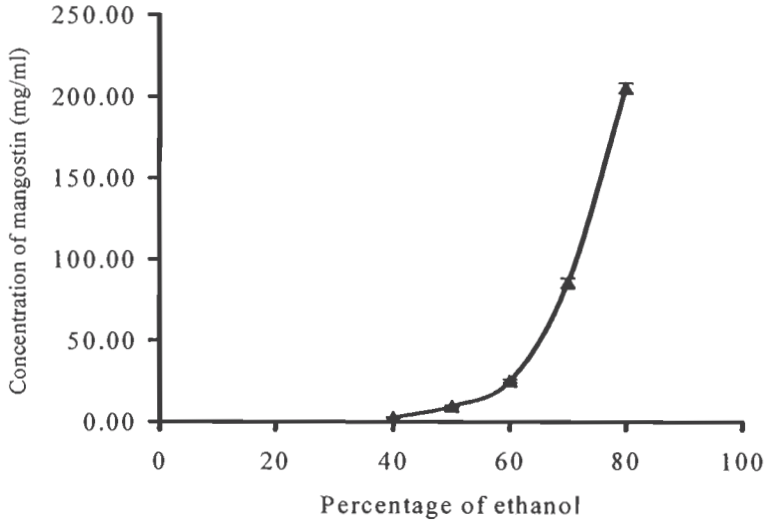



Figure 38 Solubility of mangostin of *Garcinia mangostana* extract in various concentrations of ethanol

Table 18 The solubility of *Garcinia mangostana* extract

| Solvent | Solubility (mg/ml) ± SD |
|---|---|
| 40% ethanol in water | 2.5399 ± 0.33 |
| 50% ethanol in water | 9.5396 ± 0.65 |
| 60% ethanol in water | 25.0200 ± 0.98 |
| 70% ethanol in water | 85.5552 ± 2.84 |
| 80% ethanol in water | 205.2323 ± 3.05 |

Figure 39 Solubility of mangostin of *Garcinia mangostana* extract in various concentrations of ethanol

**D. Determination of Antimicrobial Activities of *Garcinia mangostana* Extract**

**1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

The experiment was performed with three series of extract. After incubation the MIC was determined visually as the lowest concentration that inhibited bacterial growth, which was demonstrated by the absence of turbidity. The MIC of the extract 3 μg/ml for *Staphylococcus aureus* ATCC 25923 and 1.5 μg/ml for *Streptococcus mutans* ATCC $KPSK_2$ (Table 20).

The MBC was determined the lowest concentration of an antimicrobial agent that killed the test bacteria. The MBC of the extract was 4 μg/ml for *Staphylococcus aureus* ATCC 25923 and 3 μg/ml for *Streptococcus mutans* ATCC $KPSK_2$ (Table 20).

From the study, it was interesting that the MIC and MBC values of the extract against *Streptococcus mutans* ATCC $KPSK_2$ were lower than that against *Staphylococcus aureus* ATCC 25923. This result implied that *Garcinia mangostana* extract had a higher activity against *Streptococcus mutans* ATCC $KPSK_2$ than against *Staphylococcus aureus* ATCC 25923. This finding is consistent to the previous study reported by Hiranras (2001).

**2. Determination of solvent effect on bacterial growth inhibition**

From the previous study, it was found that mangostin has poor solubility in water and high solubility in ethanol (Budavari, 2001; Hiranras, 2001). Thus, in this present work, ethanol was selected to solubilize the extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol against *Staphylococcus aureus* ATCC 25923 was 5.9375% v/v and 23.75% v/v, respectively. The minimum concentration of ethanol that showed the inhibitory effect against *Streptococcus mutans* ATCC $KPSK_2$ was found to be 11.875% v/v and showed bactericidal effect at 23.75% v/v. The MIC and MBC of ethanol against two types of tested bacteria was higher than of the extract. These results showed that the ethanol content of the extract solution in this experiment did not interfere the MIC and MBC of the extract.

Table 19 The MIC and MBC of extract and ethanol on various types of bacteria.

| Types of bacteria | MIC (μg/ml) | MBC (μg/ml) | MIC of ethanol (%v/v) | MBC of ethanol (%v/v) |
|---|---|---|---|---|
| *Staphylococcus aureus* ATCC 25923 | 3 | 4 | 5.9375 | 23.75 |
| *Streptococcus mutans* $KPSK_2$ | 1.5 | 3 | 11.875 | 23.75 |

## PART II FAST DISSOLVING ORAL STRIPS CONTAING *Garcinia mangostana* EXTRACT

### E. Formulation of Orally Fast Dissolving Films Base

From the preliminary studies, appropriated type and concentration of cellulose polymers were investigated to obtain fast dissolving films with satisfactory appearance, good integrity, and which were easily detached from the glassy plate after drying with flexibility and no breakage. Consequently, three types of cellulose were employed for the study including HPMC 3 cps, HPMC 5 cps, and HPC.

**1. *In vitro* evaluation of orally fast dissolving films**

### 1.1 Physical characteristics of orally fast dissolving film bases

In this experiment, the films were prepared by solvent casting method on glassy plates. The films were cut into 2 x 3 cm that appropriate for place on the tongue (Xu et al., 2002; Szeles et al., 2004). The physical characteristics of orally fast dissolving films were transparent, glossy, flexible, sticky and easy to peel off from the glass flat plate as shown in Table 21.

From the result, it was found that the film formula that consisted of 3 plasticizers, PEG 400, PG and glycerin at 15% total amount of plasticizers per total amount of polymers was too moist and soft that caused self-adhering characteristic. It might be that all plasticizers are polyol substances, thus they have moisture absorption characteristic. Additionally, the film formulations already incorporated volatile oil which may soften and moisten the film. This finding is consistent with a previous study that the pullulan film products containing sorbitol, glycerin or both were easily broken into pieces, or could be too moist and/or self-adhering but they could produce films that rapidly dissolved in the oral cavity (Mcgregor, Homan, and Gravina, 2004).

The characteristics of film consisting of only HPC as film former were translucent, too soft, highly sticky and difficult to detach from flat glass plate but they were flexible. While the HPMC film was transparent and easy to peel. Therefore, for improving ease of peeling of HPC film, the combination of film formers between HPC and HPMC could provide the films that were easier to detach than HPC film alone. These findings agree with the previous study by Taweekunthum (2001), that used the combined polymers between HPMC 15 cps and HPC HV (high viscosity).

Focus on HPMC films, this study found that the film prepared from HPMC 5 cps provided more transparent and colorless than one from HPMC 3 cps. It may be due to the yellow-white color of HPMC 3 cps powder. Additionally, HPMC 5 cps (E5) film was more flexible than HPMC 3 cps (E3) film.

### 1.2 Thickness of orally fast dissolving films

There are many studies that discuss the thickness of fast dissolving or breath freshening films. Many studies reported that the appropriate thickness of the fast dissolving film should be in range from 35 to 45 μm and not more than 70 μm (Marco and Fausto, 2004). While another patent claimed that the film preferably has a thickness about 40-60 μm (Szeles et al., 2004). Additionally, Xu et al. (2002) reported that the film has a thickness ranging from about 15 to about 80 μm, and preferably about 30 to 60 μm.

Table 20 Physical characteristics of fast dissolving film bases

| Formulas | Transparency | Glossiness | Flexibility | Stickiness | Ease of peeling |
|---|---|---|---|---|---|
| PEG/PG | ++ | ++ | +++ | ++ | +++ |
| PEG/GLY | ++ | ++ | ++ | ++ | +++ |
| PEG/PG/GLY | ++ | ++ | ++ | +++ | +++ |
| PEG | ++ | ++ | ++ | + | +++ |
| PG/GLY | ++ | ++ | ++ | ++ | +++ |
| GLY | ++ | ++ | ++ | + | +++ |
| PG | ++ | ++ | +++ | + | +++ |
| E3 | ++ | ++ | + | + | +++ |
| E5 | +++ | +++ | +++ | + | +++ |
| HPC | + | + | +++ | +++ | + |
| E3HPC (2:1) | ++ | ++ | +++ | + | +++ |
| E3HPC (3:1) | ++ | ++ | +++ | + | +++ |
| E3HPC (4:1) | ++ | ++ | +++ | + | +++ |
| E3HPC (5:1) | ++ | ++ | +++ | + | +++ |
| E3E5 (1:1) | ++ | +++ | +++ | + | +++ |
| E3E5 (2:1) | ++ | +++ | +++ | + | +++ |
| E3E5 (3:1) | ++ | +++ | +++ | + | +++ |
| E3E5 (5:1) | ++ | +++ | +++ | + | +++ |
| E5HPC (1:1) | ++ | +++ | +++ | ++ | ++ |
| E5HPC (2:1) | ++ | +++ | +++ | + | +++ |
| E5HPC (3:1) | ++ | +++ | +++ | + | +++ |
| E5HPC (5:1) | ++ | +++ | +++ | + | +++ |

The symbols of (+) and (-) mean the appearance and no appearance, respectively. The number of the symbols of (+) means a degree of the appearance of the specified property.

**1.3 Weight variation of orally fast dissolving films**

Many patents claimed that the weight of orally fast dissolving films should be in the range of 30 to 100 mg (Pearce, 2003) but another patent claimed that weight variation should be between 25 and 35 mg (Marco and Fausto, 2004). It shows in Table 23 shows the average weights of all test films which ranged from 20.18±0.50 to 31.60±1.62 mg.

Table 21 The average thickness of the prepared fast dissolving film bases (n=5, each sample was measured at 5 locations).

| Formulas | Average thickness ± SD. (μm) | | | | | Mean |
|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | ± SD |
| PEG/PG | 40.2±1.30 | 40.0±1.22 | 37.8±2.17 | 37.8±1.92 | 40.2±0.45 | 39.2±1.28 |
| PEG/GLY | 41.2±2.59 | 42.6±1.95 | 39.0±2.45 | 41.2±1.79 | 39.2±1.30 | 40.6±1.52 |
| PEG/PG/GLY | 44.8±1.30 | 50.4±2.61 | 48.8±1.30 | 49.0±1.58 | 46.0±2.55 | 47.8±2.32 |
| PG | 41.8±1.48 | 39.4±1.14 | 38.4±1.14 | 39.0±1.87 | 38.4±1.14 | 39.4±1.41 |
| PG/GLY | 39.2±1.64 | 38.2±1.30 | 35.4±0.55 | 37.4±2.51 | 37.8±0.84 | 37.6±1.40 |
| GLY | 35.6±0.55 | 36.0±0.71 | 36.2±0.84 | 34.6±0.55 | 38.0±1.00 | 36.1±1.24 |
| PEG | 34.0±0.00 | 33.6±0.55 | 32.4±2.19 | 33.2±1.10 | 35.8±2.84 | 33.8±1.26 |
| E3 | 38.6±1.82 | 40.6±2.19 | 39.6±1.95 | 42.8±1.30 | 42.2±1.48 | 40.8±1.75 |
| E5 | 38.0±0.71 | 37.8±0.84 | 38.0±1.00 | 40.0±1.41 | 40.4±1.34 | 38.8±1.25 |
| HPC | 39.6±2.88 | 38.2±1.92 | 39.6±2.07 | 39.6±1.67 | 40.2±1.30 | 39.4+0.74 |
| E3HPC (2:1) | 36.2±0.84 | 38.4±0.55 | 39.2±0.84 | 38.4±1.14 | 37.8±0.45 | 38.0±1.12 |
| E3HPC (3:1) | 35.2±0.45 | 35.4±0.55 | 39.8±1.30 | 38.0±1.00 | 38.4±0.55 | 37.4±2.00 |
| E3HPC (4:1) | 35.0±0.00 | 34.6±0.55 | 34.0±0.71 | 34.2±0.84 | 35.0±2.35 | 34.6±0.46 |
| E3HPC (5:1) | 37.8±1.48 | 35.8±0.84 | 35.4±1.14 | 34.8±0.84 | 36.4±0.55 | 36.0±1.14 |
| E3E5 (1:1) | 37.0±1.10 | 37.2±1.00 | 37.8±1.10 | 38.4±0.55 | 36.6±1.52 | 37.4±0.71 |
| E3E5 (2:1) | 34.0±0.71 | 36.4±0.55 | 35.0±0.71 | 38.0±1.58 | 34.8±1.48 | 35.6±1.58 |
| E3E5 (3:1) | 40.6±0.55 | 34.8±0.45 | 35.8±1.30 | 36.6±1.67 | 37.0±1.00 | 37.0±2.20 |
| E3E5 (5:1) | 38.0±0.00 | 37.6±1.14 | 38.6±1.52 | 36.2±2.17 | 35.4±1.14 | 37.2±1.32 |
| E5HPC (1:1) | 36.4±0.55 | 36.4±2.30 | 35.8±1.10 | 38.8±1.30 | 36.4±0.55 | 36.8±1.17 |
| E5HPC (2:1) | 36.6±1.14 | 36.0±1.22 | 35.8±1.30 | 41.0±0.00 | 36.8±0.45 | 37.2±2.14 |
| E5HPC (3:1) | 36.8±0.45 | 36.0±1.22 | 35.6±0.89 | 37.6±0.55 | 37.8±1.30 | 36.8±0.96 |
| E5HPC (5:1) | 32.2±0.45 | 33.8±0.45 | 33.0±1.22 | 33.2±0.84 | 32.6±0.55 | 33.0±0.61 |
| Commercial product strips A | 41.2±1.79 | 37.4±1.34 | 40.4±2.07 | 43.8±1.48 | 41.0±2.12 | 40.8±2.29 |

Table 22 Weight variation of orally fast dissolving films (n=5).

| Formulas | Weight (mg) ± SD | %CV |
|---|---|---|
| PEG/PG | 26.46 ± 0.11 | 4.20 |
| PEG/GLY | 25.90 ± 0.75 | 2.91 |
| PEG/PG/GLY | 31.60 ± 1.62 | 5.13 |
| PG | 24.34 ± 0.62 | 2.53 |
| PEG | 21.92 ± 0.97 | 4.42 |
| PG/GLY | 24.22 ± 1.66 | 6.85 |
| GLY | 24.08 ± 1.78 | 7.37 |
| E3 | 26.91 ± 1.21 | 4.48 |
| E5 | 23.53 ± 1.72 | 7.31 |
| HPC | 20.18 ± 0.50 | 2.46 |
| E3HPC (2:1) | 23.43 ± 0.29 | 1.24 |
| E3HPC (3:1) | 22.67 ± 1.46 | 6.43 |
| E3HPC (4:1) | 21.46 ± 1.12 | 5.23 |
| E3HPC (5:1) | 23.61 ± 1.04 | 4.43 |
| E3E5 (1:1) | 25.88 ± 0.83 | 3.19 |
| E3E5 (2:1) | 23.71 ± 1.22 | 5.15 |
| E3E5 (3:1) | 25.43 ± 1.96 | 7.70 |
| E3E5 (5:1) | 24.42 ± 1.80 | 7.35 |
| E5HPC (1:1) | 23.65 ± 0.86 | 3.63 |
| E5HPC (2:1) | 25.17 ± 1.54 | 6.11 |
| E5HPC (3:1) | 25.04 ± 0.55 | 2.20 |
| E5HPC (5:1) | 21.90 ± 0.25 | 1.13 |
| Commercial product strips A | 28.83 ± 0.77 | 2.67 |

### 1.4 Mechanical properties of orally fast dissolving films

Mechanical properties including percent strain at point of break, Young's modulus, ultimate tensile strength and work of failure are presented in Table 24 and Figures 40-47.

Focused on percent strain at point break, the results showed that the film from HPC had the high percent strain at break value. This represented to the elasticity characteristic but it was too soft when focused in tensile strength. This finding is consistent with the tensile property study that it had very low tensile strength and Young's modulus indicating it was soft, tough and weak (Taweekunthum, 2001).

The film consisted of HPMC 3 cps with 3 plasticizers including PEG 400, PG, and glycerin showed the lowest tensile strength and work of failure comparing with the HPMC 3 cps film without any plasticizer and with one or two types of plasticizers. This data

supported the physical characteristics of this film that these polyol type plasticizers made the film too soft and easily broke into pieces.

Focused on the films that were combination of HPMC 5 cps and HPC LV, it was found that the ultimate tensile strength and Young's modulus values increased as increasing the proportion of HPMC 5 cps in the films. Inversely, they showed decreasing of percentage of elongation significantly ($p<0.05$). Therefore, the combination of HPMC 5 cps and HPC LV as film formers provided the films that were more stronger and rigid than HPC alone.

While combining film with HPMC 3 cps and HPC LV exhibited increasing of tensile strength and Young's modulus, percent elongation at break and work of failure decreased. These results indicated that mixed polymers of HPMC 3 cps and HPC LV were more hard, rigid and brittle than HPC film. In addition, all ratios of these mixed polymers gave no remarkable difference in percent elongation at break of films ($p>0.05$).

The films that consisted of HPMC 3 cps and HPMC 5 cps showed sharply decreasing trend of tensile strength, percent elongation and work of failure when proportion of HPMC 3 cps increased. This finding revealed that the amount of HPMC 3 cps have a great effect on mechanical properties of films of HPMC 5 cps and 3 cps combination.

Table 23 Mechanical properties of orally fast dissolving films (n=5).

| Formulas | Tensile strength ±SD (MPa) | % Strain at point of break ± SD (%) | Young's modulus ± SD (MPa) | Work of failure ± SD.(mJ) |
|---|---|---|---|---|
| PEG/PG | 40.84 ± 1.24 | 16.57 ± 2.16 | 385.60 ± 15.20 | 1.65 ± 0.36 |
| PEG/GLY | 35.26 ± 1.50 | 11.91 ± 0.78 | 374.94 ± 9.90 | 0.86 ± 0.12 |
| PEG/PG/GLY | 24.70 ± 0.83 | 12.90 ± 1.00 | 323.14 ± 7.19 | 0.70 ± 0.12 |
| PG | 47.34 ± 1.59 | 19.76 ± 1.31 | 386.02 ± 10.58 | 2.38 ± 0.23 |
| PG/GLY | 34.46 ± 1.41 | 14.59 ± 1.21 | 344.96 ± 14.92 | 1.31 ± 0.23 |
| GLY | 52.24 ± 0.98 | 27.37 ± 0.44 | 412.12 ± 11.47 | 3.99 ± 0.23 |
| PEG | 46.53 ± 1.69 | 18.98 ± 1.41 | 376.74 ± 25.08 | 2.00 ± 0.37 |
| E3 | 43.59 ± 3.82 | 14.35 ± 0.51 | 431.50 ± 14.96 | 1.23 ± 0.14 |
| E5 | 72.38 ± 8.04 | 254.42 ± 4.76 | 442.14 ± 16.68 | 52.84 ± 7.86 |
| HPC | 10.43 ± 0.75 | 59.00 ± 1.96 | 179.56 ± 15.82 | 2.11 ± 0.14 |
| E3HPC (2:1) | 42.86 ± 1.61 | 14.90 ± 1.03 | 405.36 ± 10.26 | 1.21 ± 0.15 |
| E3HPC (3:1) | 45.17 ± 3.91 | 14.50 ± 0.98 | 397.40 ± 28.17 | 1.24 ± 0.27 |
| E3HPC (4:1) | 47.53 ± 0.77 | 15.04 ± 0.42 | 442.28 ± 9.08 | 1.23 ± 0.07 |
| E3HPC (5:1) | 47.13 ± 2.84 | 13.74 ± 1.23 | 443.80 ± 12.57 | 1.19 ± 0.18 |
| E3E5 (1:1) | 65.06 ± 4.03 | 220.84 ± 9.15 | 463.02 ± 17.20 | 46.13 ± 5.88 |
| E3E5 (2:1) | 59.40 ± 2.04 | 105.20 ± 7.06 | 440.22 ± 13.48 | 22.48 ± 7.42 |
| E3E5 (3:1) | 63.72 ± 3.16 | 53.32 ± 10.37 | 468.32 ± 24.83 | 9.64 ± 2.09 |
| E3E5 (5:1) | 54.80 ± 1.90 | 49.44 ± 4.16 | 433.06 ± 25.92 | 7.19 ± 0.99 |
| E5HPC (1:1) | 22.20 ± 0.36 | 18.02 ± 1.17 | 335.6 ± 22.30 | 1.03 ± 0.08 |
| E5HPC (2:1) | 32.75 ± 1.43 | 31.48 ± 1.45 | 379.9 ± 21.30 | 2.92 ± 0.25 |
| E5HPC (3:1) | 35.46 ± 0.57 | 27.12 ± 0.64 | 406.3 ± 13.49 | 2.57 ± 0.11 |
| E5HPC (5:1) | 49.10 ± 1.02 | 36.80 ± 1.67 | 452.12 ± 17.52 | 4.54 ± 0.25 |
| Commercial product strips A | 49.13 ± 1.42 | 16.74 ± 0.90 | 483.60 ± 10.07 | 1.97 ± 0.20 |

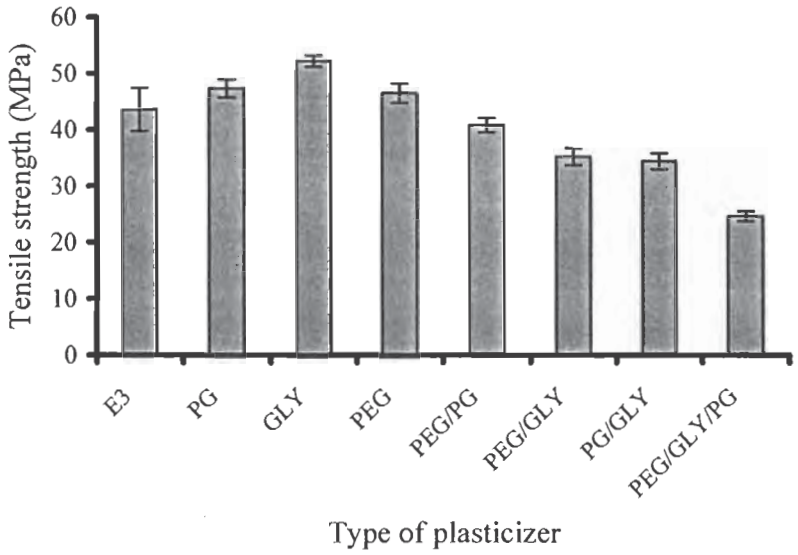



Figure 39 Tensile strength of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

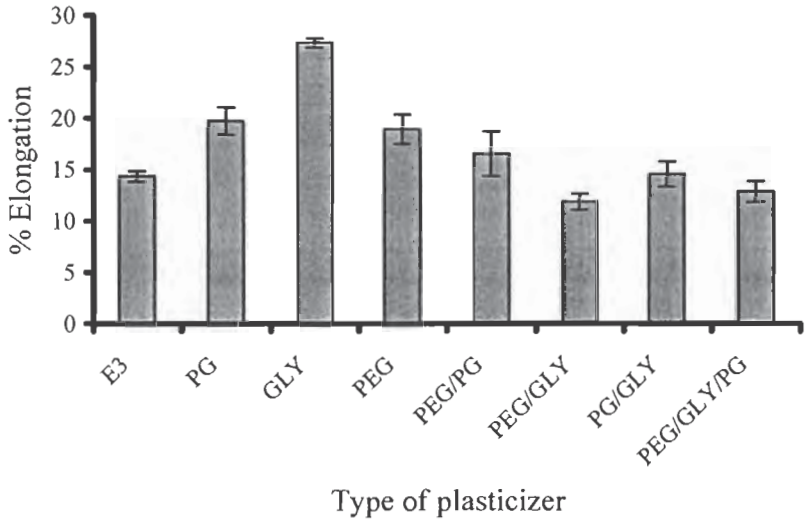



Figure 40 Percentage of elongation at break of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

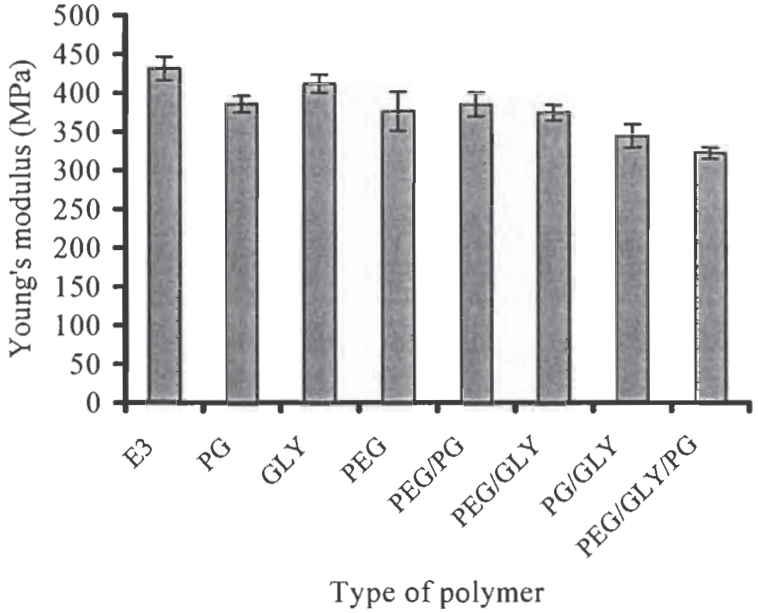



Figure 41 Young's modulus of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

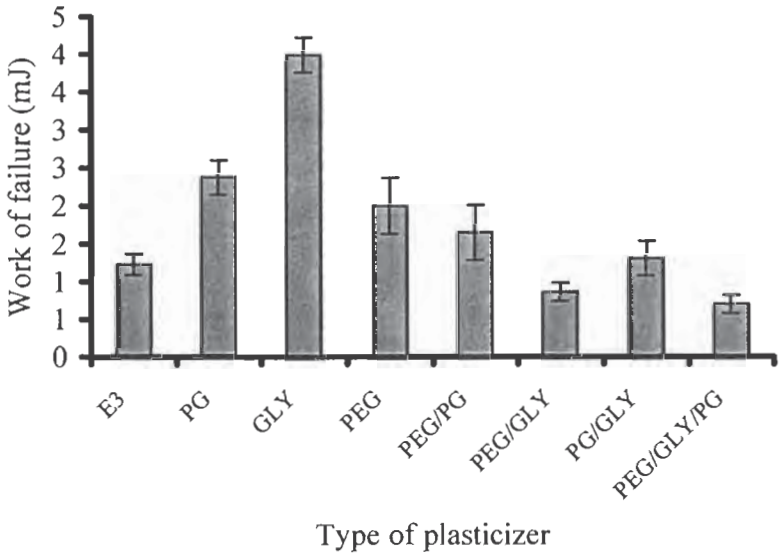



Figure 42 Work of failure of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

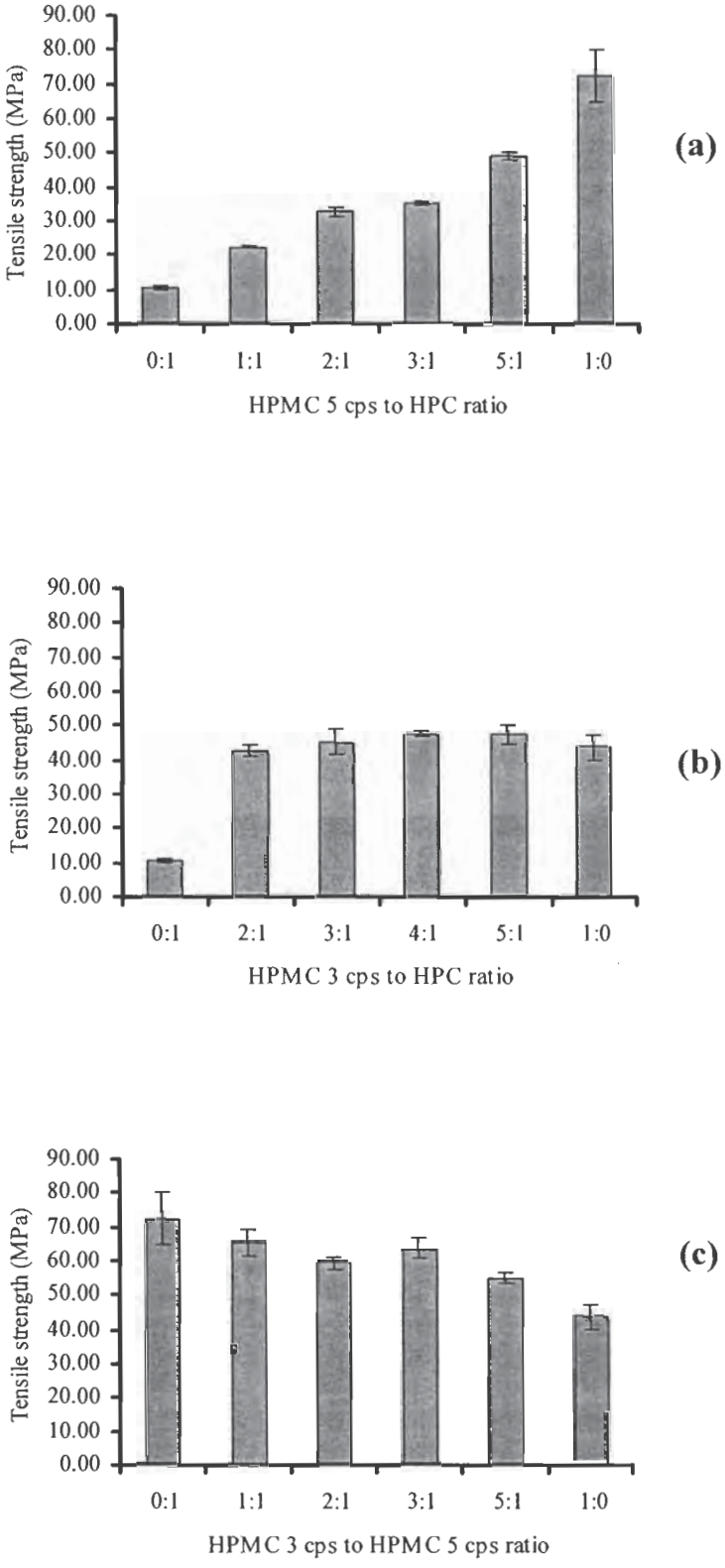



Figure 43 Tensile strength of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5

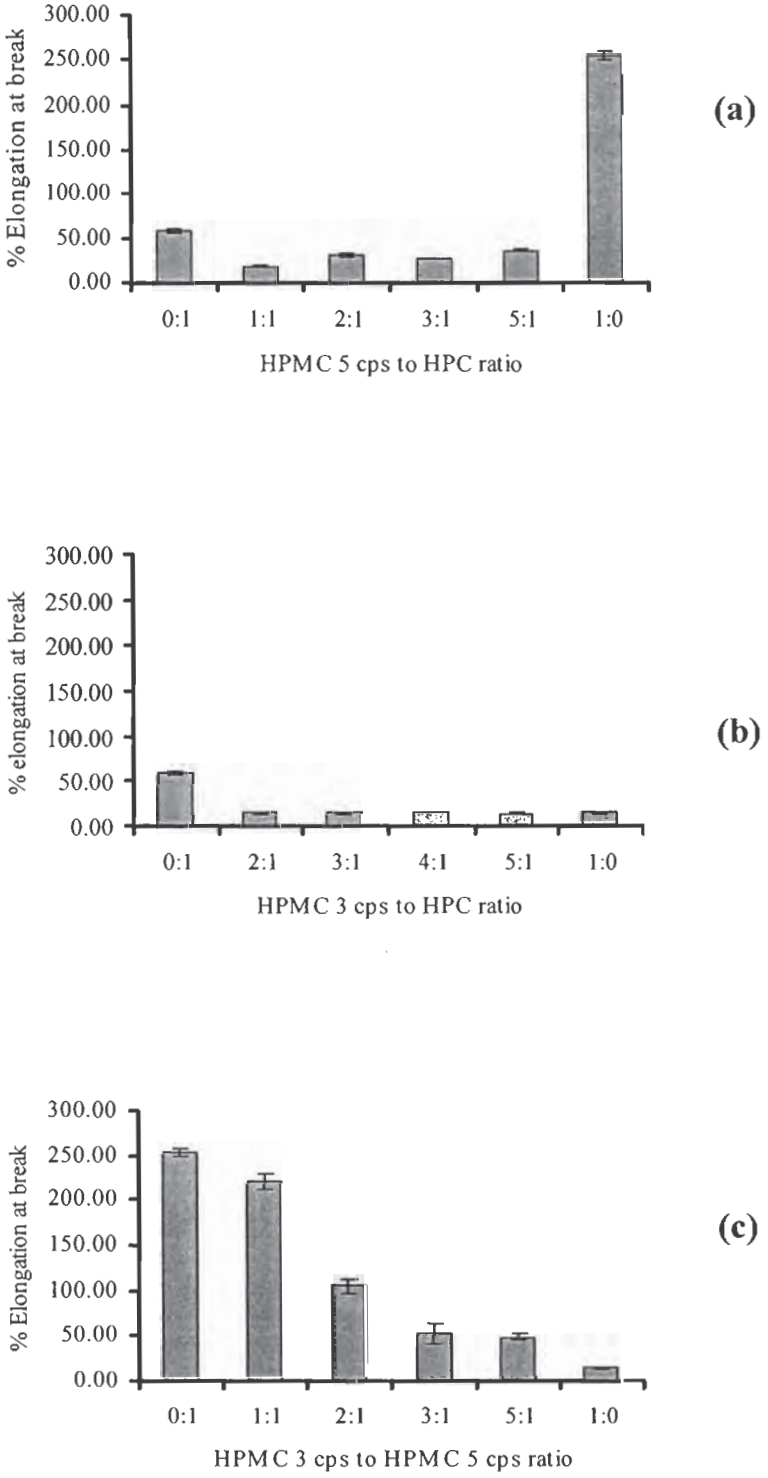
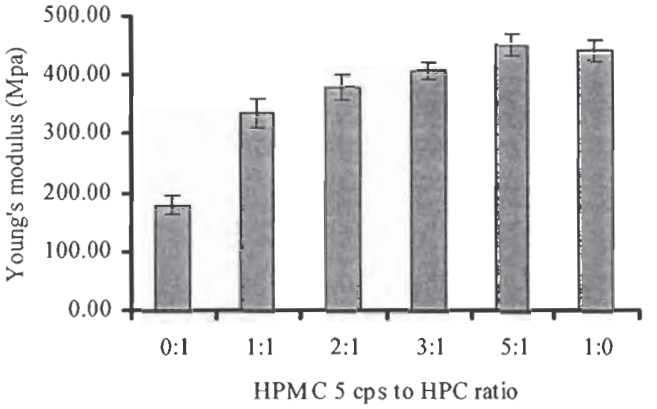
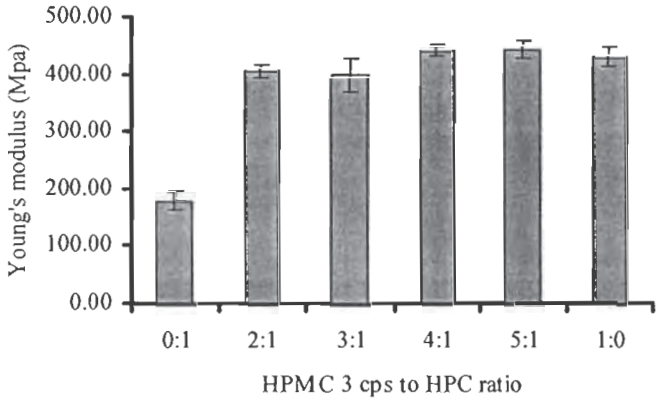


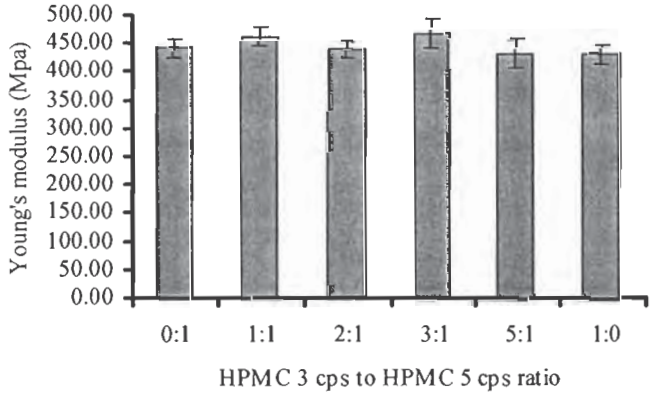

Figure 44 Percentage of elongation at break of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5




(a)




(b)




(c)

Figure 45 Young's modulus of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5

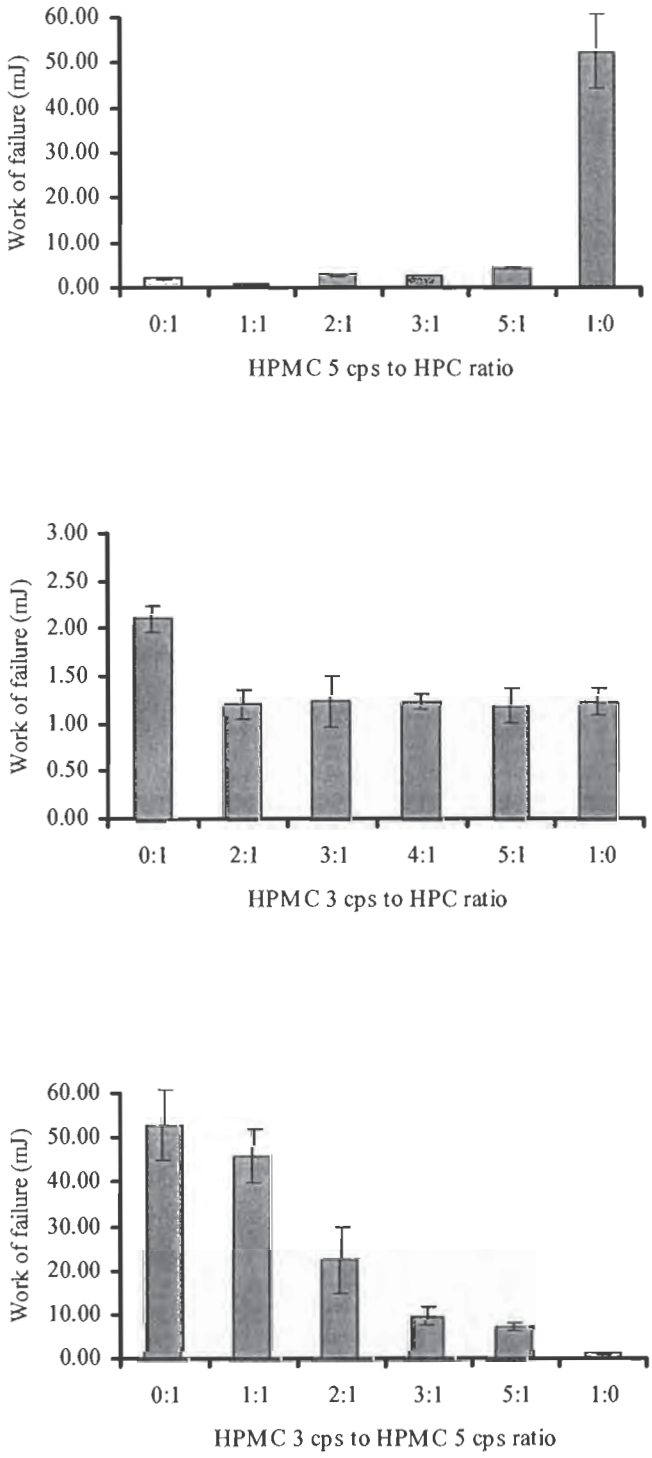



Figure 46 Work of failure of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5

### 1.5 Dissolution time of orally fast dissolving films

The dissolution time of the film is the most important properties for customer or patient's acceptance and it is a critical criteria for selecting film base to formulate the film containing *Garcinia mangostana* extract. Xu et al.(2002) defined that the fast dissolving strips were quickly dissolved in less than 30-40 seconds but in another patent claimed that the films should dissolved or disintegrated completely within 60 seconds in oral cavity (Szeles et al., 2004). In this experiment, *in vitro* speed of dissolution of films was determined by a modified method from Xu et al.(2002), explaining that the dissolution time was the time which the film disappeared or completely disintegrated. The dissolution time study was performed in 5 replications.

In this study, commercial product strips A, the commercial fast dissolving breath strip, was used as control for selecting the film base to formulate the fast dissolving film containing *Garcinia mangostana* extract. It was found that all formulations of E3HPC were not significantly different in dissolution time from commercial product strips A ($p>0.05$).

The film formula that gave the fastest dissolution was HPC and the film which gave the latest dissolution time was HPMC 5 cps and HPMC 3 cps dissolved faster than HPMC 5 cps about 6 times. This finding is similar to the result from the previous investigation by Xu et al.(2002) that films prepared using high viscosity grade of HPMC dissolved appreciably slower than a film prepared using lower viscosity grade.

## F. Formulation of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract

From the results of evaluation of film bases, the optimal formulas which appropriate properties such as good physical characteristic, suitable mechanical properties, and fast disintegrating time were selected to formulate orally fast dissolving films containing *Garcinia mangostana* extract. These formulas including E3HPC in proportion of 2:1, 3:1, 4:1, and 5:1 were selected. The concentration of extract employed in the formulations was about 0.15% w/w of dried film base. That is the film of 2 x 3 $cm^2$ contained an approximate weight of 181.61 µg of mangostin of extract. The physical properties of four formulations were depicted in Table 29. As a result, all formulations were clear and yellow color due to the color of extract. Other physical appearances of each formulation were similar to film bases.

Table 24 Dissolution time of fast dissolving films (n=5).

| Formulas | Dissolution time (sec) | | | | | Mean ± SD | % CV |
|---|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | | |
| PEG/PG | 191 | 186 | 191 | 167 | 173 | 181.6 ± 10.99 | 6.05 |
| PEG/GLY | 156 | 206 | 181 | 206 | 165 | 182.8 ± 22.99 | 12.58 |
| PEG/PG/GLY | 357 | 322 | 345 | 386 | 330 | 348.0 ± 25.17 | 7.23 |
| PG | 195 | 216 | 192 | 222 | 182 | 201.4 ± 16.91 | 8.39 |
| PG/GLY | 443 | 371 | 495 | 431 | 414 | 430.8 ± 45.08 | 10.46 |
| GLY | 280 | 266 | 330 | 320 | 328 | 304.8 ± 29.69 | 9.74 |
| PEG | 280 | 323 | 328 | 272 | 315 | 303.6 ± 25.77 | 8.49 |
| E3 | 205 | 182 | 224 | 182 | 211 | 200.8 ± 18.49 | 9.21 |
| E5 | 1278 | 1092 | 1228 | 1207 | 1270 | 1215.0 ± 74.76 | 6.15 |
| HPC | 73 | 80 | 64 | 53 | 55 | 65.0 ± 11.55 | 17.78 |
| E3HPC (2:1) | 133 | 118 | 129 | 123 | 104 | 121.4 ± 11.28 | 9.29 |
| E3HPC (3:1) | 82 | 100 | 99 | 130 | 126 | 107.4 ± 20.17 | 18.78 |
| E3HPC (4:1) | 83 | 90 | 123 | 88 | 128 | 102.4 ± 21.31 | 20.81 |
| E3HPC (5:1) | 125 | 131 | 141 | 139 | 130 | 133.2 ± 6.65 | 4.99 |
| E3E5 (1:1) | 218 | 232 | 205 | 352 | 245 | 250.4 ± 58.74 | 23.46 |
| E3E5 (2:1) | 143 | 200 | 179 | 191 | 158 | 174.2 ± 23.49 | 13.48 |
| E3E5 (3:1) | 161 | 188 | 202 | 204 | 219 | 194.8 ± 21.86 | 11.22 |
| E3E5 (5:1) | 197 | 285 | 179 | 195 | 190 | 209.2 ± 42.94 | 20.53 |
| E5HPC (1:1) | 168 | 204 | 213 | 149 | 141 | 175.6 ± 31.50 | 17.94 |
| E5HPC (2:1) | 267 | 193 | 197 | 211 | 201 | 213.8 ± 30.48 | 14.26 |
| E5HPC (3:1) | 200 | 249 | 320 | 200 | 316 | 257.0 ± 59.19 | 23.03 |
| E5HPC (5:1) | 235 | 269 | 265 | 160 | 305 | 246.8 ± 54.51 | 22.09 |
| Commercial product strips A | 110 | 109 | 132 | 158 | 96 | 121.0 ± 24.39 | 20.16 |

Table 25 Formulation of orally fast dissolving films containing *Garcinia mangostana* extract

| Composition | Formulation code (% w/w) | | | |
|---|---|---|---|---|
| | E3HPC 2:1 | E3HPC 3:1 | E3HPC 4:1 | E3HPC 5:1 |
| HPMC 3 cps | 8 | 9 | 9.6 | 10 |
| HPC LV | 4 | 3 | 2.4 | 2 |
| Acesulfame K | 0.3 | 0.3 | 0.3 | 0.3 |
| Menthol | 1.5 | 1.5 | 1.5 | 1.5 |
| Eucalyptus oil | 0.6 | 0.6 | 0.6 | 0.6 |
| *Garcinia mangostana* extract | 0.216 | 0.216 | 0.216 | 0.216 |
| 60% ethanol | 85.6 | 85.6 | 85.6 | 85.6 |

Table 26 Physical characteristics of orally fast dissolving films containing *Garcinia mangostana* extract

| Formulas | Transparency | Glossiness | Flexibility | stickiness | Ease of peeling |
|---|---|---|---|---|---|
| E3HPC (2:1) | ++ | ++ | +++ | + | +++ |
| E3HPC (3:1) | ++ | ++ | +++ | + | +++ |
| E3HPC (4:1) | ++ | ++ | +++ | + | +++ |
| E3HPC (5:1) | ++ | ++ | +++ | + | +++ |

The symbols of (+) and (-) show the appearance and no appearance, respectively. The number of the symbols of (+) means a degree of the appearance of the specified property.

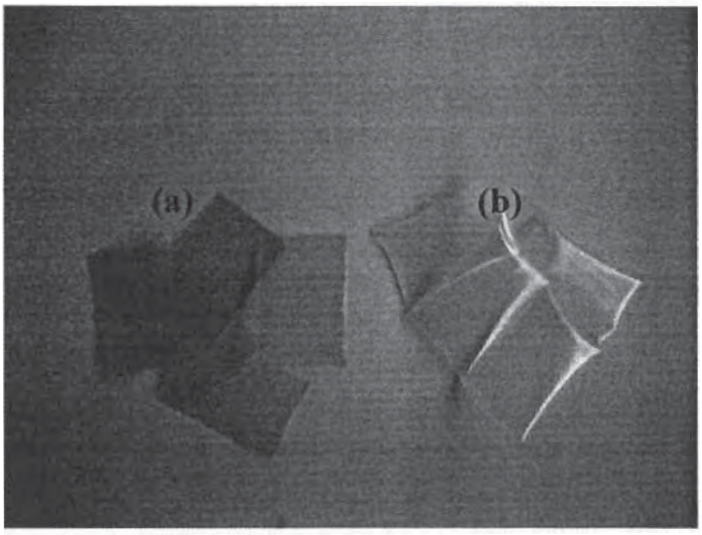



Figure 47 Orally fast dissolving films; (a) films containing *Garcinia mangostana* extract and (b) film bases

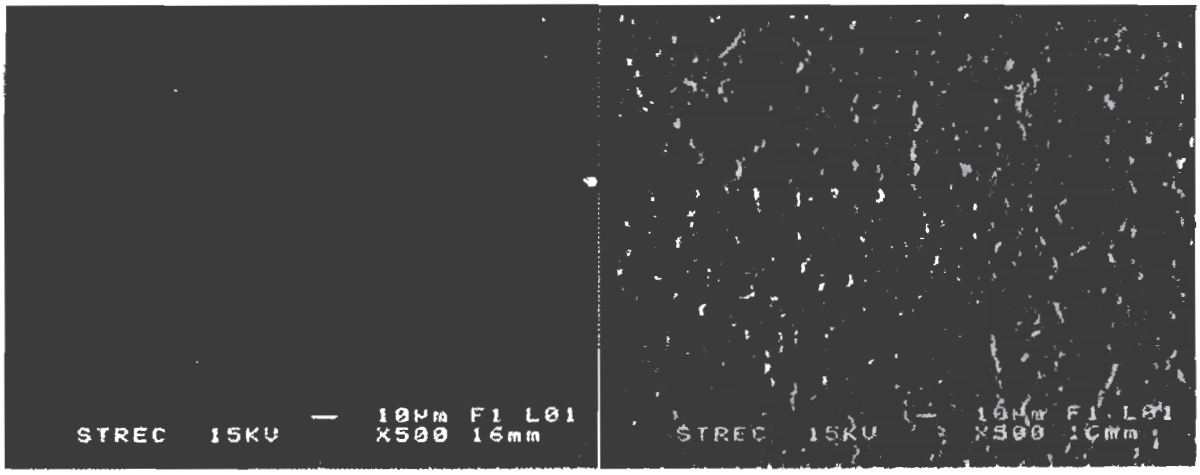
**G. Characterization of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract**

**1. Surface morphology**

The surface morphology of the four formulations containing *Garcinia mangostana* extract was observed by using scanning electron microscope (SEM). From the photomicrographs of cross-sectional area of E3HPC film bases, it was found that E3HPC (3:1) film exhibited dense, smooth and homogeneous texture.

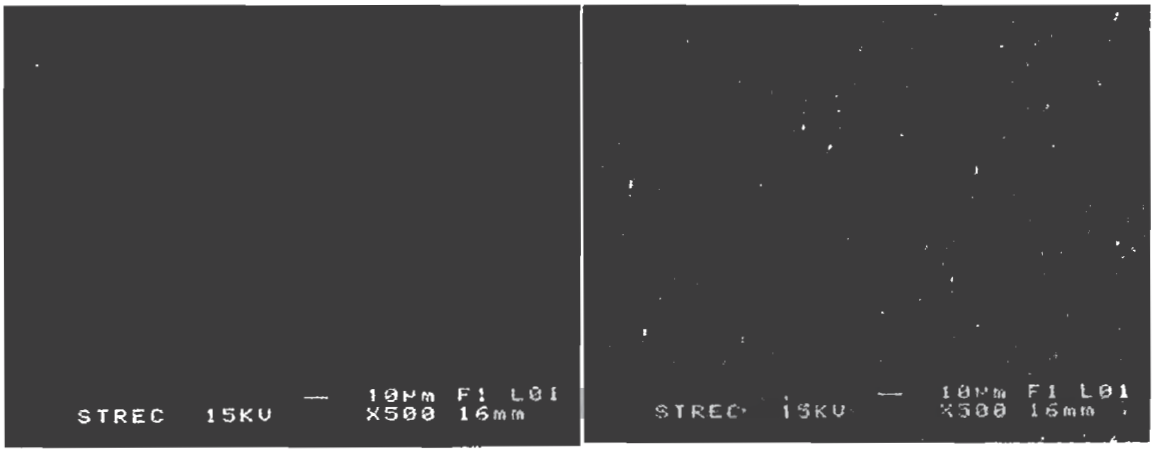
All formulations of the prepared films from combination of HPMC 3 cps and HPC LV showed rough surface with many pores. This result is consistent with the finding from Taweekunthum (2001) that observed surface topography of film consisted of HPMC 15 cps and HPC.

Comparison of fast dissolving film containing *Garcinia mangostana* extract with its film base in all formulations, it indicated that the incorporation of extract into films caused a slight increase of surface roughness and porosity (Figure 29-32).



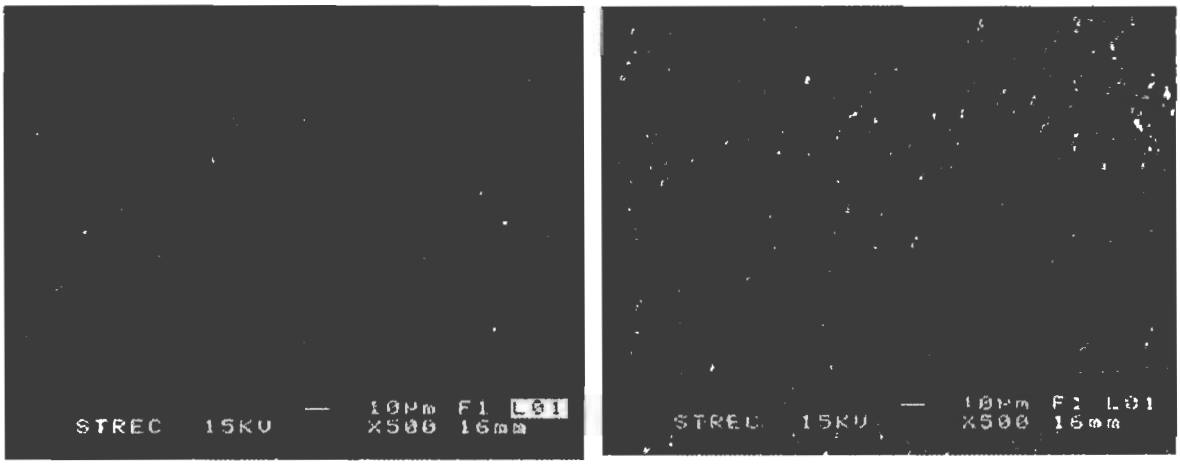

(a) (b)

Figure 48 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (2:1); (a) Film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)



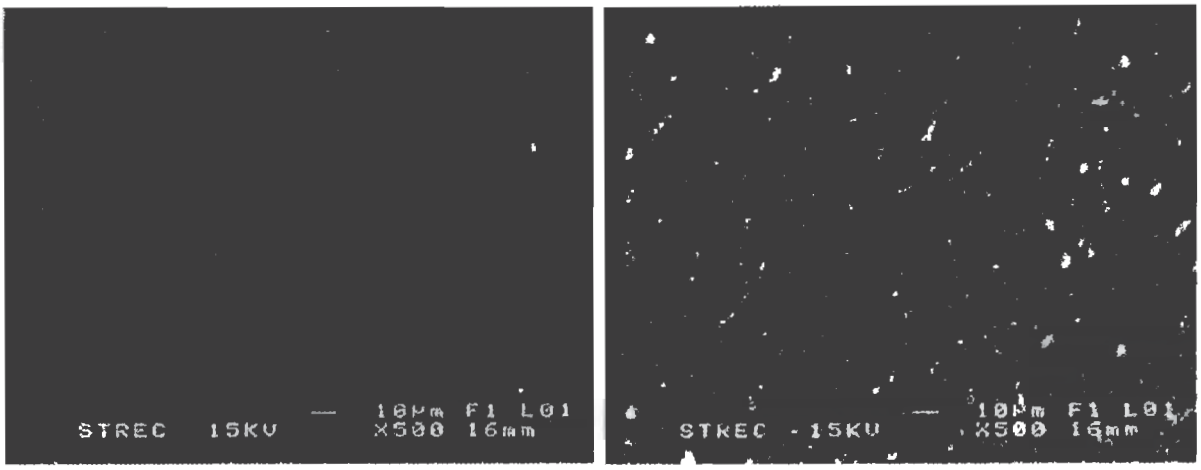

(a) (b)

Figure 49 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (3:1); (a) film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)




(a) (b)

Figure 50 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (4:1); (a) film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)




(a) (b)

Figure 51 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (5:1); (a) film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)

## 2. Physicochemical characterization

### 2.1 Differential thermal analysis

The DSC thermograms of the ingredients in formulation consisting of HPMC 3 cps, HPC LV, acesulfame potassium, menthol, and eucalyptus oil in temperature range of -50 to 220 °C, heating rate 10 °C/minute are shown in Figures 33-34. The physical mixtures of formulation without and with extract are shown in Figures 35 and 36. The DSC thermogram of *Garcinia mangostana* extract displayed its endothermic peak at 165.75 ± 0.93 °C (Figure 30). The DSC thermogram of menthol showed endothermic peak at 46.91 °C but no observation of melting point of acesulfame potassium due to its melting point is 250 °C (Rowe, Sheskey, and Weller, 2003). However, no peaks were found in the orally fast dissolving films with and without *Garcinia mangostana* extract (Figures 37-39). These results indicated that extract and other ingredients changed to either molecular dispersed or amorphous form.

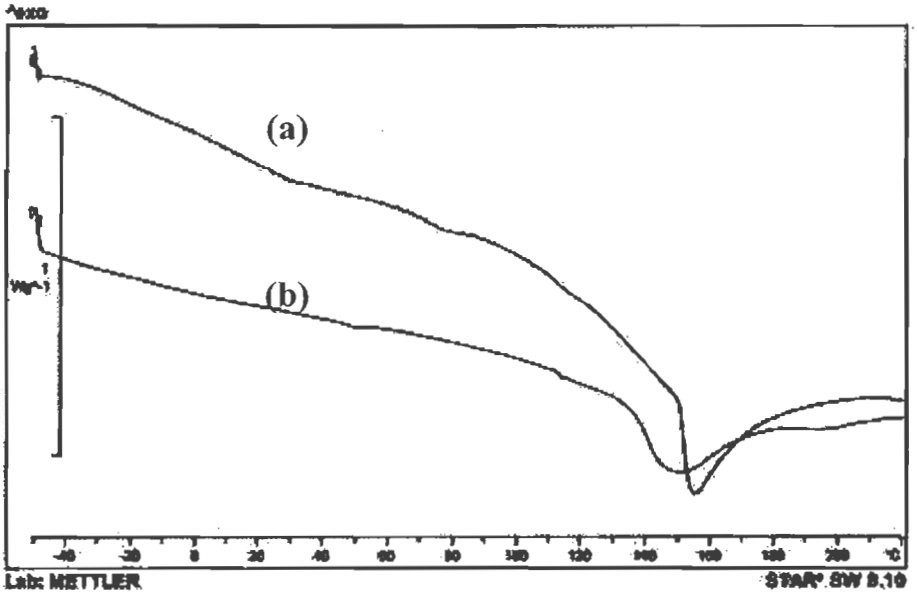



Figure 52 DSC thermograms of film former in powder form in the range of –50 to 220 °C; (a) HPMC 3 cps (sample weight 3.19 mg); (b) HPC LV (sample weight 3.15 mg)

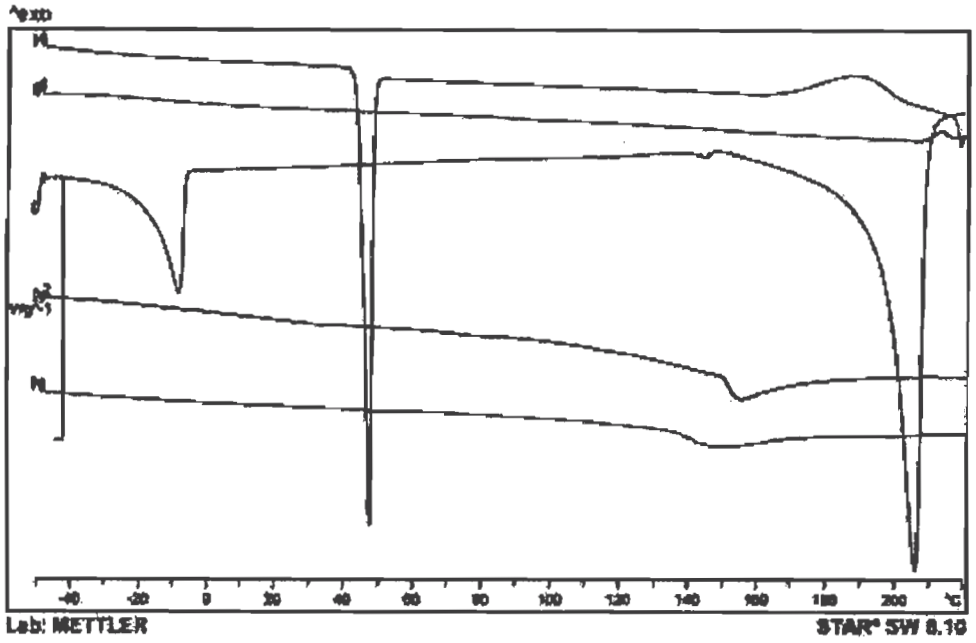



Figure 53 DSC thermograms of additive compounds in the range of –50 to 220 °C; (a) Menthol (sample weight 2.36 mg); (b) Acesulfame potassium (sample weight 3.32 mg) (c) Eucalyptus oil (sample weight 2.50 mg); (d) HPMC 3 cps (sample weight 3.19 mg); (e) HPC LV (sample weight 3.15 mg)

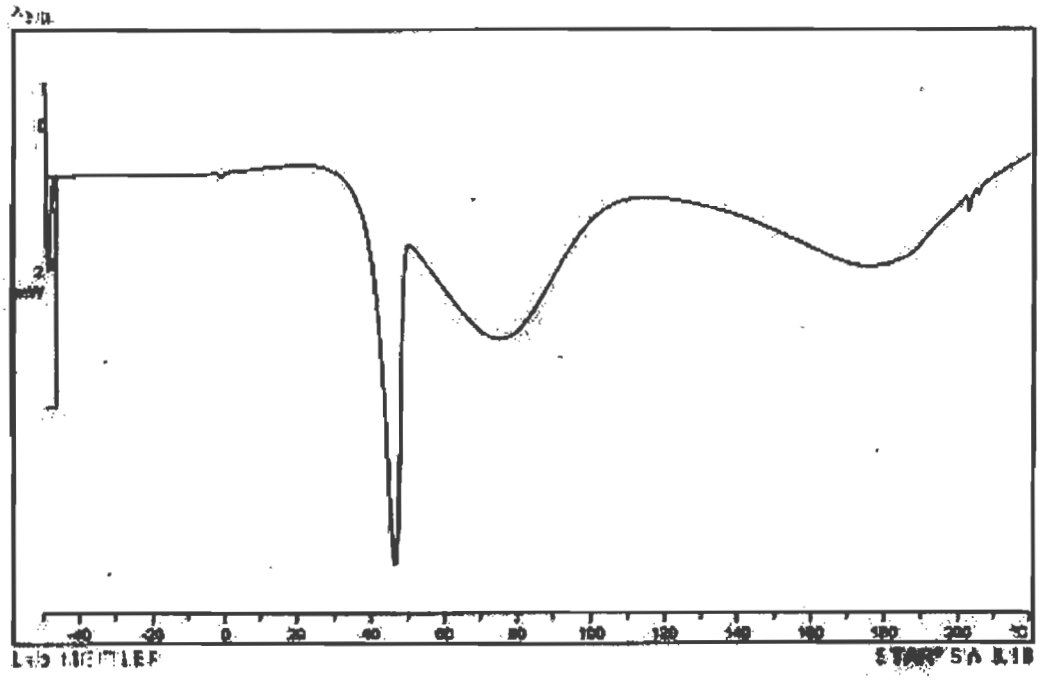


Figure 54 DSC thermograms of physical mixtures of substances in film base formula in the temperature range of –50 to 220 °C; consisted of HPMC 3 cps (sample weight 3.11 mg), HPC LV (sample weight 1.74), Acesulfame potassium (sample weight 0.64), Menthol (sample weight 1.06), and Eucalyptus oil (sample weight 0.32 mg)

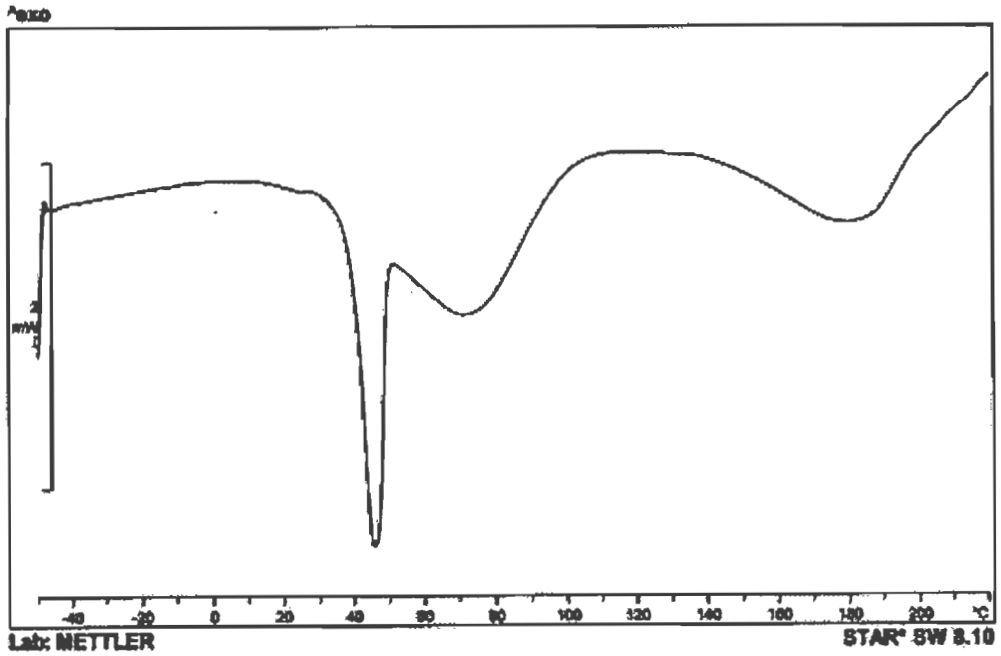



Figure 55 DSC thermograms of physical mixtures of substances in film containing extract formula in the temperature range of –50 to 220 °C; consisted of HPMC 3 cps (sample weight 2.73 mg), HPC LV (sample weight 1.16), Acesulfame potassium (sample weight 1.25), Menthol (sample weight 1.34), Eucalyptus oil (sample weight 0.44 mg), and *Garcinia mangostana* extract (sample weight 0.29 mg)

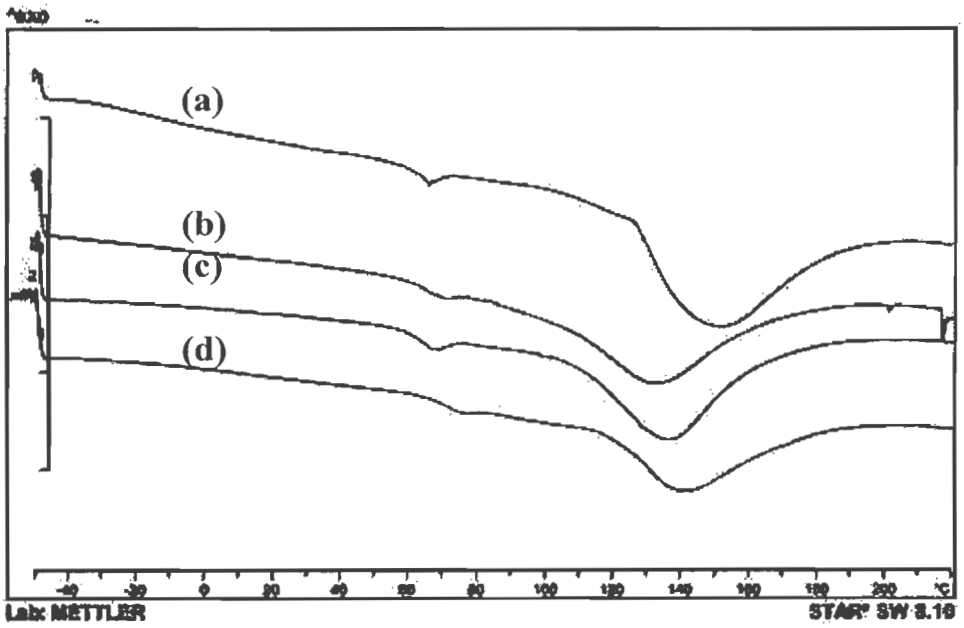



Figure 56 DSC thermograms of orally fast dissolving film bases in the temperature range of –50 to 220 °C; (a) E3HPC (2:1) (sample weight 5.07 mg); (b) E3HPC (3:1) (sample weight 4.70 mg); (c) E3HPC (4:1) (sample weight 4.96 mg); (d) E3HPC (5:1) (sample weight 4.93 mg)

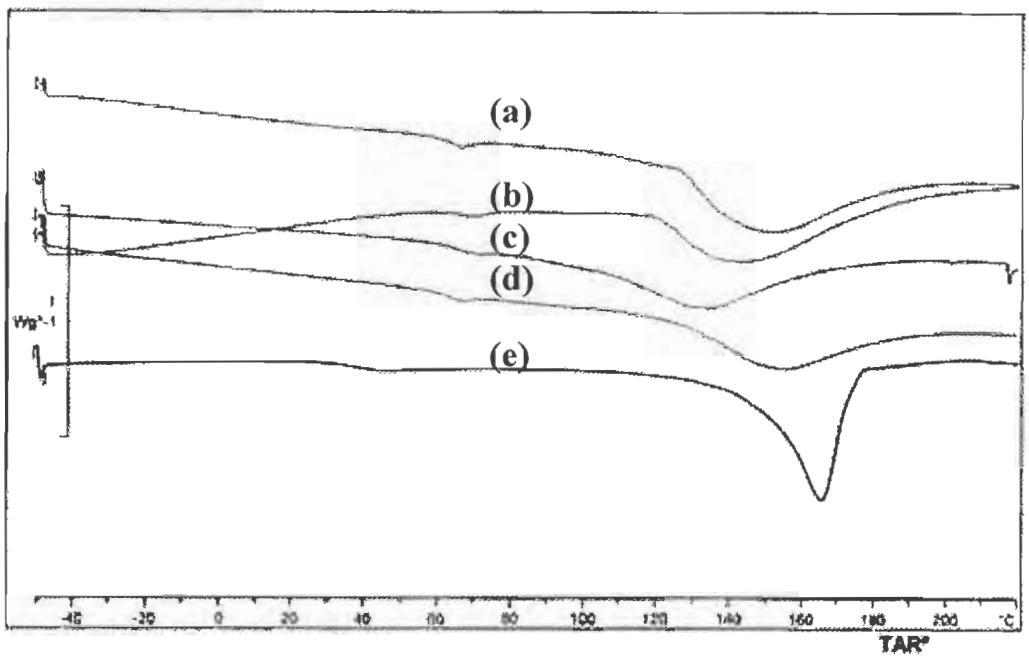



Figure 57 DSC thermograms of films in formulation of E3HPC (2:1) and (3:1) in the temperature range of –50 to 220 °C; (a) E3HPC (2:1) film base (sample weight 5.07 mg); (b) E3HPC (2:1) containing *Garcinia mangostana* extract (sample weight 4.60 mg); (c) E3HPC (3:1) film base (sample weight 4.70 mg); (d) E3HPC (3:1) containing *Garcinia mangostana* extract (sample weight 5.00 mg); (e) *Garcinia mangostana* extract (sample weight 3.27 mg)

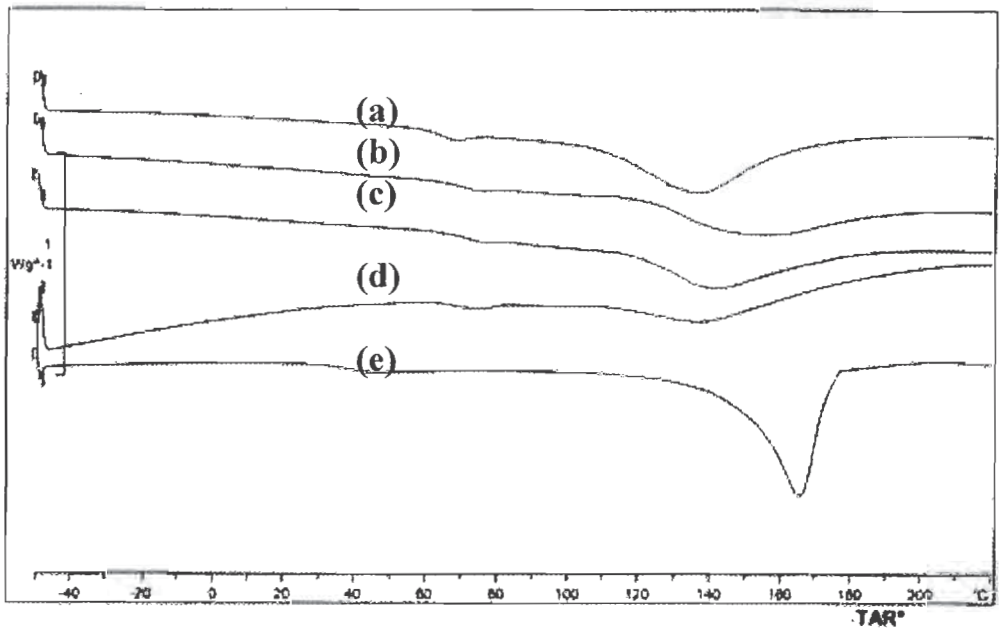



Figure 58 DSC thermograms of films in formulation of E3HPC (4:1) and (5:1) in the temperature range of –50 to 220 °C; (a) E3HPC (4:1) film base (sample weight 4.96 mg); (b) E3HPC (4:1) containing *Garcinia mangostana* extract (sample weight 5.32 mg); (c) E3HPC (5:1) film base (sample weight 4.93 mg); (d) E3HPC (5:1) containing *Garcinia mangostana* extract (sample weight 5.30 mg); (e) *Garcinia mangostana* extract (sample weight 3.27 mg)

**3. Weight variation of orally fast dissolving films containing *Garcinia mangostana* extract**

The results of weight variation are presented in Appendix C. It shows in Table 30 that the weight of all test films was within the limit as 21.25±0.52 to 24.39±0.56 mg.

Table 27 Weight variation of orally fast dissolving films containing *Garcinia mangostana* extract

| Formulas | Average weight ± SD (mg) | %CV |
|---|---|---|
| E3HPC (2:1) | 21.25 ± 0.52 | 2.43 |
| E3HPC (3:1) | 24.39 ± 0.56 | 2.31 |
| E3HPC (4:1) | 23.92 ± 0.82 | 3.43 |
| E3HPC (5:1) | 24.13 ± 0.65 | 2.71 |

**4. Thickness of orally fast dissolving films containing *Garcinia mangostana* extract**

The results of thickness of films are presented in Appendix C. It shows in Table 31 that the thickness of all test films was within the limit as 35.4±0.48 to 38.3±0.88 μm.

Table 28 The average thickness of the orally fast dissolving films containing *Garcinia mangostana* extract (n=5 each sample was measured at 5 locations).

| Formulas | Average thickness ± SD. (μm) | | | | | Mean |
|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | ± SD |
| E3HPC (2:1) | 35.2±1.10 | 35.6±1.52 | 36.2±1.30 | 35.0±0.71 | 35.2±0.45 | 35.4±0.48 |
| E3HPC (3:1) | 36.4±1.34 | 36.8±0.45 | 35.4±0.89 | 37.6±1.14 | 36.6±1.14 | 36.6±0.79 |
| E3HPC (4:1) | 36.8±1.10 | 39.0±1.00 | 38.6±0.89 | 38.8±1.10 | 38.2±1.10 | 38.3±0.88 |
| E3HPC (5:1) | 35.6±0.55 | 35.0±1.22 | 36.6±1.34 | 35.4±0.55 | 35.4±1.14 | 35.6±0.60 |

**5. Mechanical properties of orally fast dissolving films containing *Garcinia mangostana* extract**

Mechanical properties of films showed in values of tensile strength, percentage of elongation at break, Young's modulus and work of failure (Table 29). From the results, it was found that the ultimate tensile strength and Young's modulus values of fast dissolving films containing *Garcinia mangostana* extract were highly than of film bases in all

formulations. It indicated that the films containing *Garcinia mangostana* extract were rigid and harder than film bases. While percent elongation and work of failure values of film bases were more than of film containing extract. These results suggested that film bases were tougher than its film containing extract (Figure 61-64).

Table 29 Mechanical properties of orally fast dissolving films containing *Garcinia mangostana* extract (n=5).

| Formulas | Ultimate tensile strength ±SD (MPa) | % Strain at point of break ±SD (%) | Young's modulus ±SD (MPa) | Work of failure ±SD(mJ) |
|---|---|---|---|---|
| E3HPC (2:1) | 44.02 ± 1.01 | 13.36 ± 0.55 | 429.24 ± 13.58 | 1.04 ± 0.08 |
| E3HPC (3:1) | 49.10 ± 0.5 | 13.38 ± 0.34 | 465.72 ± 13.36 | 1.21 ± 0.08 |
| E3HPC (4:1) | 52.00 ± 2.24 | 13.62 ± 0.79 | 477.08 ± 14.02 | 1.22 ± 0.11 |
| E3HPC (5:1) | 47.44 ± 1.18 | 13.58 ± 0.79 | 443.72 ± 21.30 | 1.11 ± 0.09 |

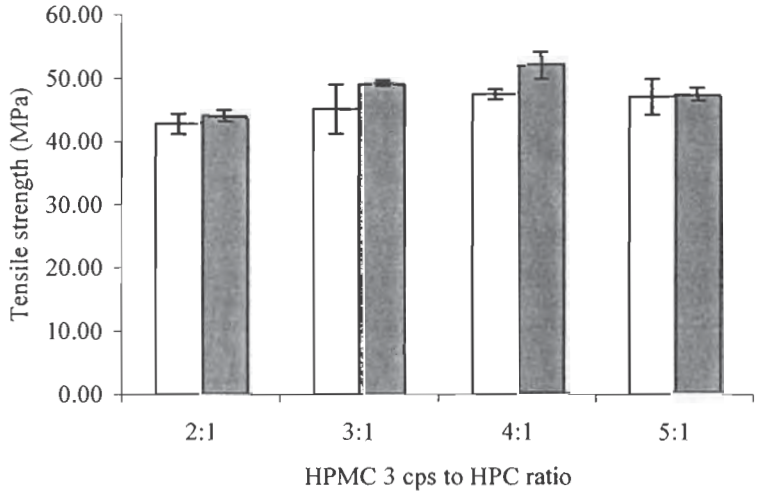



Figure 59 Tensile strength of orally fast dissolving films; ☐ film bases; ▩ film containing *Garcinia mangostana* extract

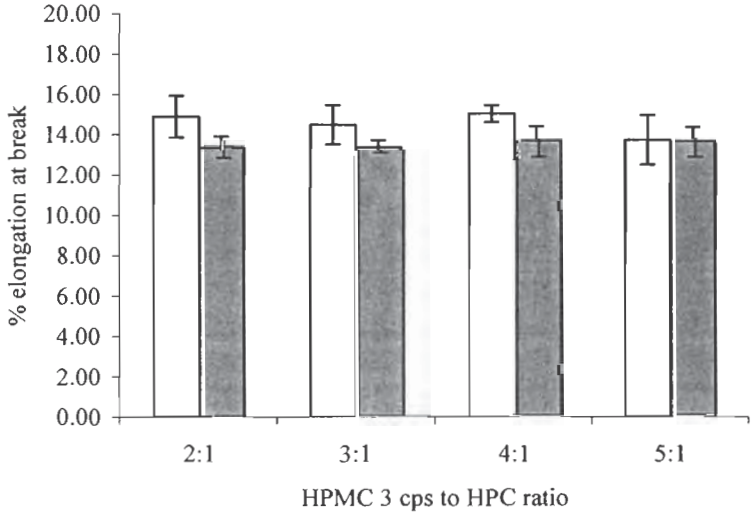



Figure 60 Percentage of Elongation at break of orally fast dissolving films; ☐ film bases; ▩ film containing *Garcinia mangostana* extract

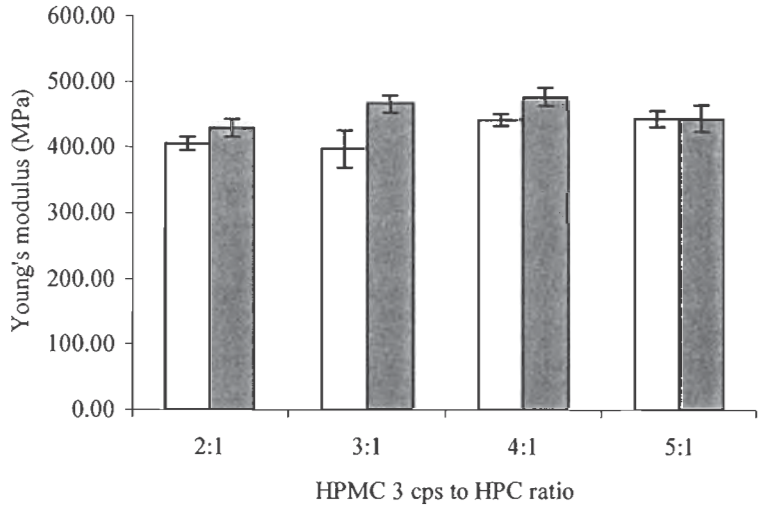



Figure 61 Young's modulus of orally fast dissolving films; □ film bases; ▩ film containing *Garcinia mangostana* extract

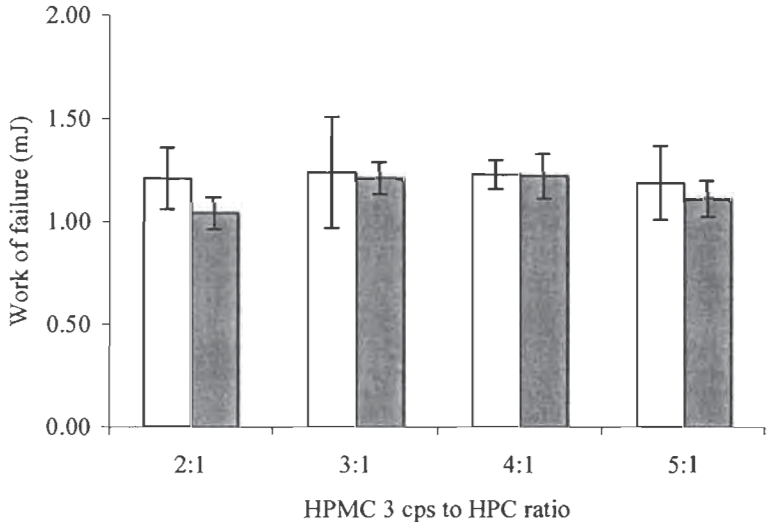



Figure 62 Work of failure of orally fast dissolving films; □ film bases; ▩ film containing *Garcinia mangostana* extract

**6. *In vitro* dissolution time of orally fast dissolving films containing *Garcinia mangostana* extract**

The method for observing the dissolution time of films in this study was modified from Xu et al.(2002). Unlike the film bases, when the film containing the extract floated on the surface of isotonic phosphate buffer pH 6.2 at temperature 37 $^{\circ}$C, it disintegrated into many small pieces and gave yellow and cloudy solution. This observation may be due to the extract has poor solubility in this buffer. Therefore, the dissolution time of the film containing extract in this study was the time that film completely disintegrated into small pieces. From the result of dissolution time of films, it was found that the films containing extract disintegrated slower than film bases in all formulations. It may be due to hydrophobic characteristic of the extract interfered hydrophilic group in polymers to bind with water molecule. Only E3HPC (2:1) films containing extract were not significantly different in dissolution time from commercial product strips A ($p$>0.05).

Table 30 Dissolution time of orally fast dissolving films containing *Garcinia mangostana* extract (n=5).

| Formulas | Dissolution time (sec) | | | | | Mean ± SD | % CV |
|---|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | | |
| E3HPC (2:1) | 102 | 128 | 140 | 132 | 114 | 123.2 ± 15.14 | 12.29 |
| E3HPC (3:1) | 460 | 432 | 485 | 490 | 507 | 474.8 ± 29.25 | 6.16 |
| E3HPC (4:1) | 367 | 435 | 384 | 402 | 430 | 403.6 + 29.19 | 7.23 |
| E3HPC (5:1) | 209 | 222 | 160 | 180 | 160 | 186.2 ± 28.34 | 15.22 |

**7. Content uniformity of orally fast dissolving films containing *Garcinia mangostana* extract**

The dosage-unit uniformity was determined by assay of 10 individual units (2 x 3 $cm^2$) using the HPLC method. The percentage content of mangostin was calculated based on mangostin content at 181.61 µg/strip. Table 31 informs that the drug content in the 10 individual units of prepared orally fast dissolving films lied within the range of 85.0-115.0% of labeled amount with the relative standard deviation (RSD) was less than 6.0%. Therefore, these fast dissolving films containing *Garcinia mangostana* extract met the requirement of content uniformity (USP 26/NF 21, 2003).

**8. *In vitro* dissolution study of orally fast dissolving films containing *Garcinia mangostana* extract**

**8.1 Selection of dissolution medium**

Table 31 The average of the percent content of mangostin in the prepared fast dissolving films (n=10).

| Formulas | %content ± SD | % Labeled amount ± SD | %RSD |
|---|---|---|---|
| E3HPC (2:1) | 170.68 ± 0.43 | 93.98 ± 2.37 | 2.52 |
| E3HPC (3:1) | 193.13 ± 0.75 | 106.34 ± 4.15 | 3.90 |
| E3HPC (4:1) | 179.50 ± 0.59 | 98.84 ± 3.26 | 3.30 |
| E3HPC (5:1) | 184.80 ± 0.58 | 101.75 ± 3.19 | 3.14 |

Due to the pH of saliva fluid was reported in the range from 5.8 to 7.4 (Rathbone, 1996), therefore, isotonic phosphate buffer pH 6.2 was used in this study. However, mangostin was poorly soluble in this medium, sodium lauryl sulfate was added into the medium to enhance drug solubility. In this study, sodium lauryl sulfate ranging form 0-1.0% was added into the dissolution medium and the solubility of mangostin was determined. The results showed that the solubility of mangostin was increased as a function of sodium lauryl sulfate concentration (Table 32). The solubility of mangostin in isotonic phosphate buffer pH 6.2 was 0.5606 μg/ml and in the present of 1.0% sodium lauryl sulfate, the solubility was increased to 952.314 μg/ml, which was the lowest concentration of sodium lauryl sulfate that was able to maintain the sink condition in the dissolution study.

Table 32 Solubility of mangostin in isotonic phosphate buffer pH 6.2 without and with various concentrations of sodium lauryl sulfate (SLS)

| Medium | Solubility (μg/ml) |
|---|---|
| isotonic phosphate buffer pH 6.2 | 0.5606 ± 0.095 |
| isotonic phosphate buffer pH 6.2 + 0.1% SLS | 73.339 ± 2.78 |
| isotonic phosphate buffer pH 6.2 + 0.2% SLS | 137.535 ± 3.64 |
| isotonic phosphate buffer pH 6.2 + 0.3% SLS | 145.247 ± 3.89 |
| isotonic phosphate buffer pH 6.2 + 0.4% SLS | 171.908 ± 9.52 |
| isotonic phosphate buffer pH 6.2 + 0.5% SLS | 241.123 ± 7.53 |
| isotonic phosphate buffer pH 6.2 + 0.75% SLS | 449.074 ± 3.11 |
| isotonic phosphate buffer pH 6.2 + 1.0% SLS | 952.314 ±15.57 |

**8.2 *In vitro* dissolution study**

*In vitro* drug release study are frequently used to gain information about the release profiles of active ingredients in the formulation development. In the present work, the release study of all formulations were carried out using a modified dissolution apparatus and isotonic phosphate buffer pH 6.2 with 1.0% sodium lauryl sulfate as a dissolution medium. The amounts of drug release were analyzed using HPLC. The validation results were shown in the previous part.

The dissolution profiles were plotted between the cumulative amounts of mangostin released from orally fast dissolving films in different ratios of HPMC 3 cps and HPC LV combination versus time as shown in Figures 44-47.

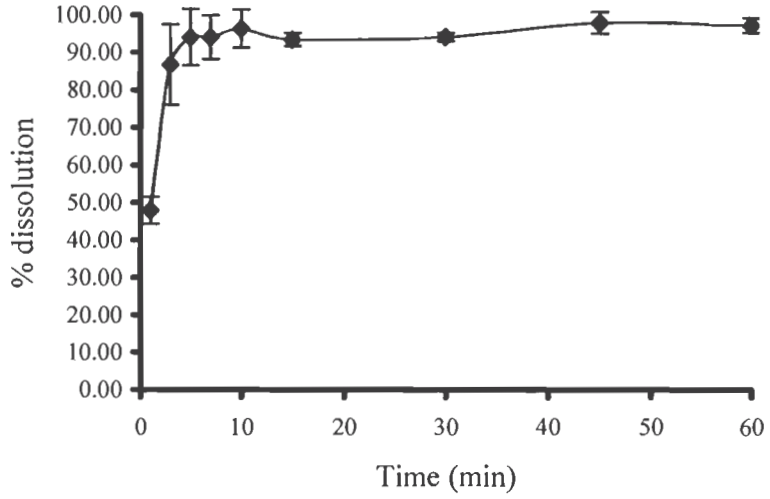



Figure 63 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (2:1)

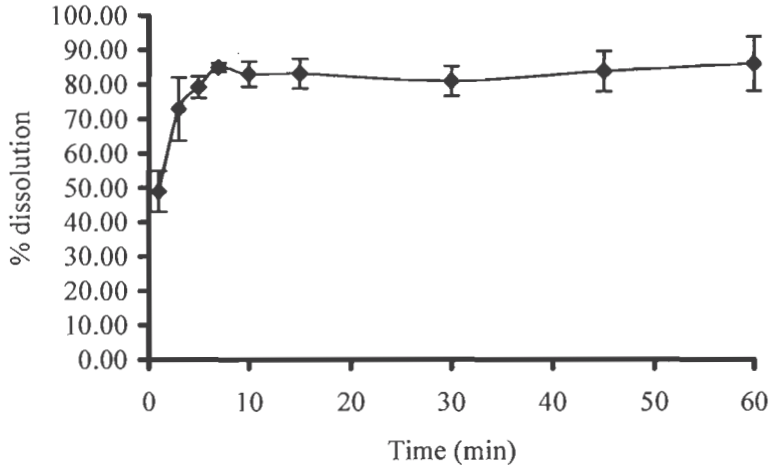



Figure 64 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (3:1)

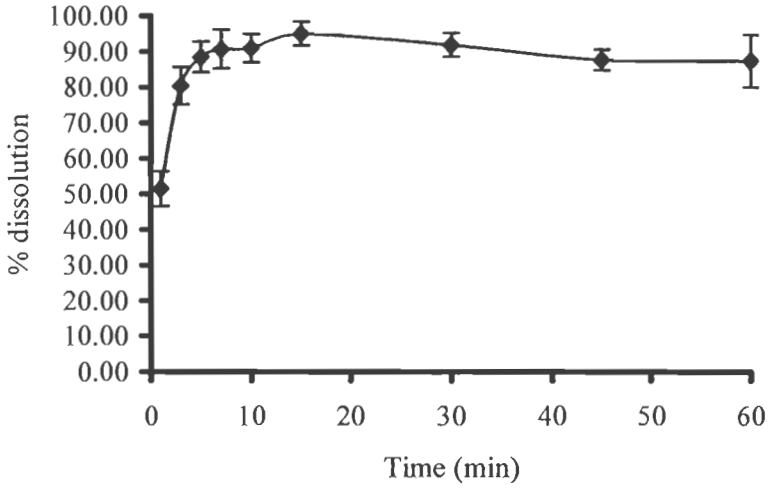



Figure 65 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (4:1)

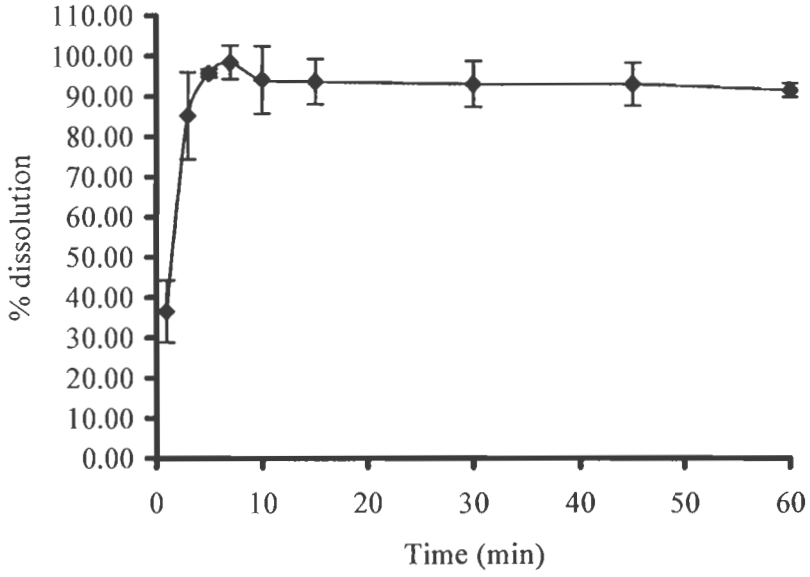



Figure 66 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (5:1)

From the result, it was found that mangostin rapidly released into the dissolution medium. About 80% of labeled amount was released within 3 minutes for E3HPC (2:1) and E3HPC (5:1) and within 5 and 7 minutes for E3HPC (4:1) and E3HPC (3:1), respectively. These findings may support the observation from scanning electron micrographs as shown previously that the fast dissolving films containing *Garcinia mangostana* extract with combination of HPMC 3 cps and HPC LV as film formers provided porous and spongy-like surface. Therefore, the high porosity of these films might affect the fast release rate.

In addition, the film formers, both HPMC 3 cps and HPC LV are hydrophilic polymers. They have ability to absorb water, thereby promoting the dissolution. Moreover,

the hydrophilic polymers would leach out and, hence, create more pores and channels for the drug to diffuse out of the patches (Wong, Yuen, and Peh, 1999).

Drug molecules are released from hydrocolloid matrices by the following 3 mechanisms:

1. Water induced relaxation of the polymer matrix
2. Erosion of the polymer gel layer surrounding the matrix dosage form
3. Diffusion of drug molecules through the swollen gel layer

The relative significance of these three mechanisms depends on the properties of the drugs and polymers. Drug release mechanism can generally be expressed by the following equation:

$$Q = kt^n$$

In this equation, $n$ is the diffusional exponent for the drug release, $k$ is the dissolution rate constant giving a measure of the velocity of drug release and $Q$ is the cumulative amount of drug release in a certain time interval (t).

The cellulose ether derivatives swell infinitely by absorbing water and concomitantly dissolve from the surface of the system. When polymer swelled the void spaces were increased with the polymer unfolded and the coil hydrated. The drug release from the systems occurs by diffusion of the molecules across these voids of the polymer matrix and also by the polymer from the surface. However, the viscosity of the gel determines the mechanism of the drug release from these systems. The high viscosity in the pores serves to retard the diffusion of the drug at the early stages of release. While hydration of the low molecular weight polymer occurred rapidly. After this period, significant erosion of the surface due to the convective movement of the solvent past the film was observed. The eroded particles exposed greater surface area for drug release, resulting in a progressive increase in release rate. Therefore, the release of drug from the low molecular weight and less viscous polymers (shorter chain length) is controlled by the dissolution of the polymer and follows zero order kinetics and the mechanism involved is erosion or relaxation controlled ($n$=1) (Lapidus and Lordi, 1968; Korsmeyer et al., 1983; Repka et al., 2005).

$$\text{Zero-order equation} : \quad Q_t = Q_0 + k_0 t$$

where $Q_t$ is the amount of drug dissolved in time t, $Q_0$ is the initial amount of drug in the solution (most times, $Q_0 = 0$) and $k_0$ is the zero order release constant.

Mockel and Lippold (1993) reported that drug release slowed down with increasing degree of viscosity of the gel forming polymer. The exponent $n$ decreased from 1 to approx. 0.5 with increasing solution viscosity. The release from low molecular weight polymers with a lower degree of viscosity proceeded according to zero-order kinetics,

whereas a higher viscosity resulted in drug release showing an approximately square root of time dependency. For the polymers with the same chemical structure, the dissolution rate decreased with rising molecular weight which can be accounted for by the increase in the thickness of the swollen layer.

This agreed with Taweekunthum (2001) who reported that the drugs would be trapped into more viscous hydrated films more than in lower viscous hydrated films resulting in reduction in drug release. Since the polymer systems utilized in this study were low viscous, which eroded easily in a few minutes so films exhibited an initial burst effect that would be advantageous for rapid onset of action.

In this study, the zero-order kinetics was applicable for the fast dissolving films containing *Garcinia mangostana* extract, as amount released versus time when plotted with the obtained dissolution data at initial part were linear. Although, the fastest and highest of mangostin release rate was observed in formulation E3HPC (5:1), the analysis of data showed that there was no statistically significant difference at initial stage (1 and 3 minutes) and overall of percentage of drug release in all formulations ($p$>0.05).

Table 33 Zero order kinetic parameters of mangostin release from orally fast dissolving films containing *Garcinia mangostana* extract

| Formulations | Zero-order plot | |
|---|---|---|
| | $k_0$ | $R^2$ |
| E3HPC (2:1) | 11.5440 | 0.8656 |
| E3HPC (3:1) | 7.5746 | 0.8985 |
| E3HPC (4:1) | 8.8800 | 0.9220 |
| E3HPC (5:1) | 14.8090 | 0.8786 |

## H. Antimicrobial Activity of Orally Fast Dissolving Films Containing *Garcinia mangostana* extract

All formulations of orally fast dissolving films containing *Garcinia mangostana* extract were tested against microorganisms commonly found in oro-dental infections namely *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$ using agar diffusion method.

### 1. Antimicrobial activity of dried films

The dried fast dissolving films containing *Garcinia mangostana* extract with diameters of 5.50 mm were place on Mueller Hinton agar plates that were swabbed with inoculum in concentration $10^8$ cells/ml. It was found that films disintegrated and spread around due to the films absorbed water in agar medium, therefore the clear zone was observed as shown in Figures 48-52 and Table 34. This result suggested that the film containing extract had antimicrobial activity against both test bacteria. However, the inhibition zone that exerted

from films containing *Garcinia mangostana* extract was less than from film containing cetylpyridinium chloride and chlorhexidine diacetate in the same concentration of extract and same formulation of film. These results may be due to the solubility of cetylpyridinium chloride and chlorhexidine diacetate in water were more than of extract. It was possible that cetylpyridinium chloride and chlorhexidine diacetate could more diffuse in agar medium than extract. The water solubility of cetylpyridinium chloride and chlorhexidine diacetate are 1 in 20 and 1 in 55 of water (Rowe, Sheskey, and Weller, 2003), whereas the solubility of extract is 0.028±0.0045 mg/ml (Hiranras, 2001). Additionally, cetylpyridinium chloride and chlorhexidine diacetate have much lower MIC value than that of extract.

From observation of commercial product strips A films, it was found that the area of films was less dense than that around on agar medium but it was not completely clear zone as shown in Figure 69(a). This might reveal that commercial product strips A had no antibacterial activity in this test.

Due to the flavoring agents of all formulas were menthol and eucalyptus oil, which has antimicrobial effect, especially menthol can inhibit both *Staphylococcus aureus* and showed weak activity against *Streptococcus mutans* at MIC value of 400 μg/ml (Iscan et al., 2002; Trivedi and Hotchandani, 2004). Therefore, the polymeric films (only film formers without any additives) and film bases (had additives) of all ratios of formulas were prepared and also test the antimicrobial activity for the control study. It was found that the polymeric film did not show any clear zone but the film base exhibited the reduction of growth of both bacteria due to dense of bacteria under film was less than around. This considerable experimental evidence suggested that was antimicrobial effect of additives in formulation such as menthol and eucalyptus oil.

To clarify the amount of extract in formulation that may affect *in vitro* antimicrobial activity test, the film containing extract in 2, 4, and 6 times of one of standard formula E3HPC (5:1) were prepared, but film that had 6 times of amount of extract was very brittle and easy to break. The analysis of antimicrobial activity results showed that there was no statistically significant difference between amount of mangostin in E3HPC (5:1) film ($p>0.05$).

Table 34 Antimicrobial activity of fast dissolving film using agar diffusion method (n=3)

| Film formulations | Inhibition zone (mm±SD) | |
|---|---|---|
| | *Staphylococcus aureus* ATCC 25923 | *Streptococcus mutans* $KPSK_2$ |
| Fast dissolving film containing *Garcinia mangostana* extract | | |
| E3HPC (2:1) | 8.23±1.36 | 6.67±0.29 |
| E3HPC (3:1) | 7.13±0.78 | 7.20±0.58 |
| E3HPC (4:1) | 7.55±0.60 | 6.17±0.45 |
| E3HPC (5:1) | 8.35±0.26 | 5.88±0.30 |
| E3HPC (5:1) extract x 2 | 7.02±0.63 | 5.85±0.33 |
| E3HPC (5:1) extract x 4 | 6.82±0.76 | 5.72±0.34 |
| E3HPC (5:1) extract x 6 | 7.52±0.60 | 5.85±0.25 |
| Commercial product strips A | 0.00 | 0.00 |
| Fast dissolving film containing cetylpyridinium chloride | | |
| E3HPC (2:1) | 35.93±6.82 | 32.65±2.41 |
| E3HPC (3:1) | 37.65±3.31 | 36.12±2.83 |
| E3HPC (4:1) | 35.92±1.26 | 30.25±7.97 |
| E3HPC (5:1) | 41.03±1.93 | 29.55±2.86 |
| Fast dissolving film containing chlorhexidine diacetate | | |
| E3HPC (2:1) | 24.40±1.19 | 27.97±0.65 |
| E3HPC (3:1) | 24.27±1.10 | 26.83±1.50 |
| E3HPC (4:1) | 24.47±3.07 | 29.08±0.38 |
| E3HPC (5:1) | 24.43±2.18 | 27.67±1.22 |

* Average of diameters of film sample were 5.50 mm.

**2. Antimicrobial activity of solution of fast dissolving film**

The solution of fast dissolving film consisted of a fast dissolving film completely dissolve or disintegtated in 2 ml of isotonic phosphate buffer pH 6.2 as simulated saliva. The film solution was dropped in sterile cup that place on Mueller Hinton agar swabbed with $10^8$ cells/ml of inoculum. The results found that no inhibition zone was observed in isotonic phosphate buffer pH 6.2, polymeric film and film base as negative control. Commercial product strips A film solution also showed no inhibition zone, while commercial mouth wash product B, consisted of high antiseptic volatile oil, and , commercial mouth wash product C

consisted of 0.12% w/v of chlorhexidine gluconate, exhibited antimicrobial activity against both types of bacteria (Table 34).

As the same result from dried films, the solution of fast dissolving film containing *Garcinia mangostana* extract had higher antimicrobial activity against *Staphylococcus aureus* ATCC 25923 than *Streptococcus mutans* ATCC $KPSK_2$. This was a inversely result that from in the study of cetylpyridinium chloride and chlorhexidine diacetate.

Table 35 Antimicrobial activity of solution of fast dissolving film using agar diffusion method (n=3)

| Film formulations | Inhibition zone (mm±SD) | |
|---|---|---|
| | *Staphylococcus aureus* ATCC 25923 | *Streptococcus mutans* $KPSK_2$ |
| Fast dissolving film containing *Garcinia mangostana* extract | | |
| E3HPC (2:1) | 8.43±0.58 | 6.82±0.33 |
| E3HPC (3:1) | 8.58±1.06 | 6.87±0.39 |
| E3HPC (4:1) | 9.13±0.13 | 8.03±2.30 |
| E3HPC (5:1) | 10.47±1.09 | 7.48±1.42 |
| E3HPC (5:1) extract x 2 | 12.13±2.66 | 7.78±1.56 |
| E3HPC (5:1) extract x 4 | 13.55±1.98 | 8.27±2.01 |
| E3HPC (5:1) extract x 6 | 12.33±0.19 | 9.55±3.18 |
| Commercial product strips A | 0.00 | 0.00 |
| Commercial mouth wash product B | 13.58±4.68 | 6.06±0.04 |
| Commercial mouth wash product C | 18.48±0.65 | 24.45±1.95 |
| Fast dissolving film containing cetylpyridinium chloride | | |
| E3HPC (2:1) | 10.55±0.78 | 15.47±1.00 |
| E3HPC (3:1) | 12.72±2.08 | 13.39±0.88 |
| E3HPC (4:1) | 15.02±4.32 | 16.97±0.98 |
| E3HPC (5:1) | 14.52±3.86 | 17.15±0.58 |
| Fast dissolving film containing chlorhexidine diacetate | | |
| E3HPC (2:1) | 18.70±0.40 | 20.90±2.68 |
| E3HPC (3:1) | 18.42±0.78 | 23.18±1.40 |
| E3HPC (4:1) | 17.90±0.17 | 24.17±1.07 |
| E3HPC (5:1) | 16.42±0.43 | 22.10±1.43 |

* Average diameters of sterile cups were 6.0 mm.

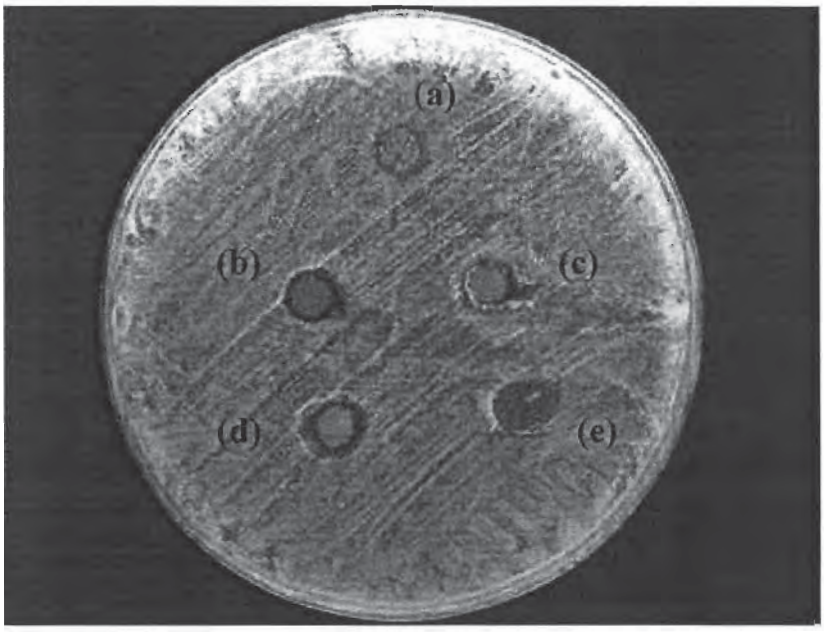



Figure 67 Agar diffusion test of fast dissolving film against *Staphylococcus aureus* ATCC 25923; (a) Commercial product strips A; (b) to (e) film containing extract in amount of 2, 4, 6 and 1 times, respectively

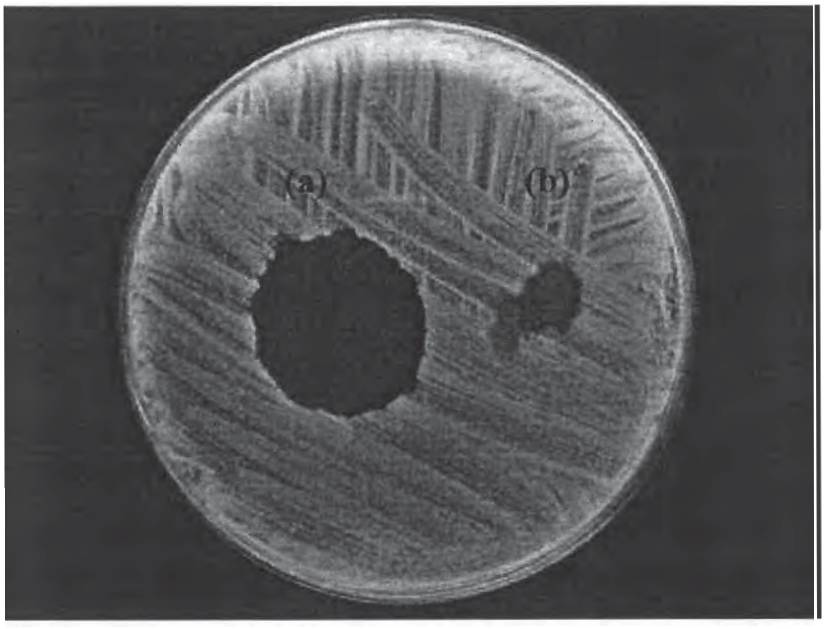



Figure 68 Agar diffusion test of fast dissolving film against *Staphylococcus aureus* ATCC 25923; (a) Chlorhexidine diacetate film; (b) film containing *Garcinia mangostana* extract

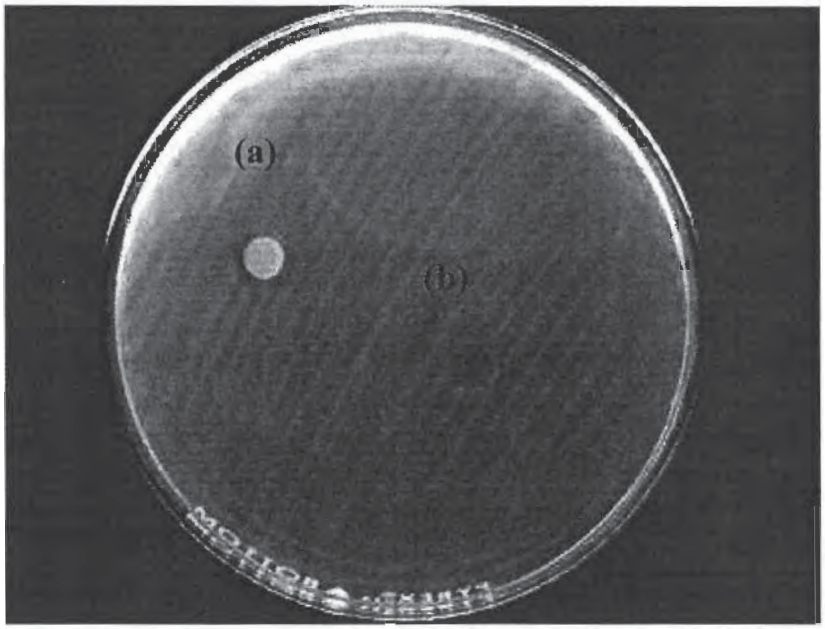



Figure 69 Agar diffusion test of fast dissolving film against *Streptococcus mutans* ATCC $KPSK_2$; (a) film with extract; (b) Commercial product strips A

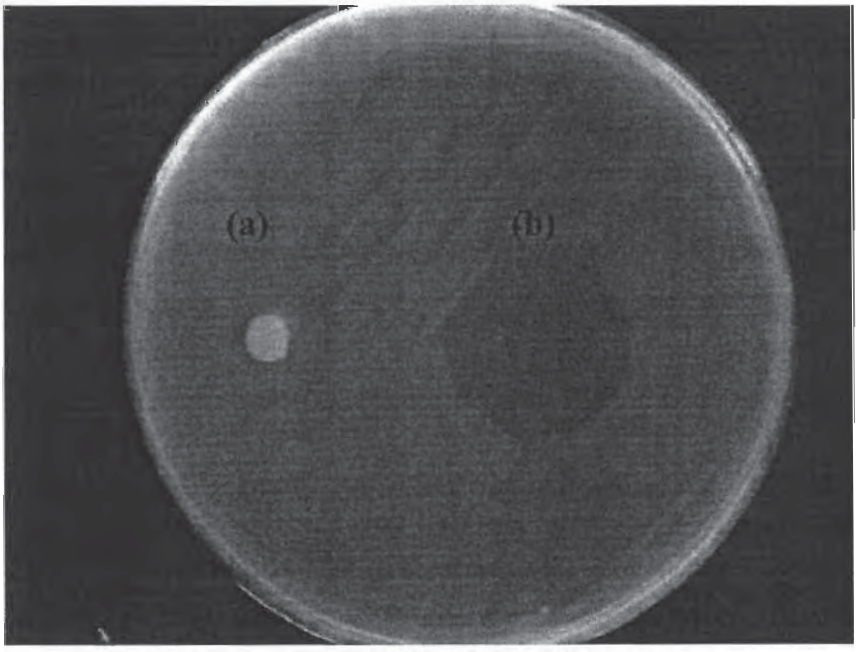



Figure 70 Agar diffusion test of fast dissolving film against *Streptococcus mutans* ATCC $KPSK_2$; (a) film with extract; (b) film with chlorhexidine diacetate

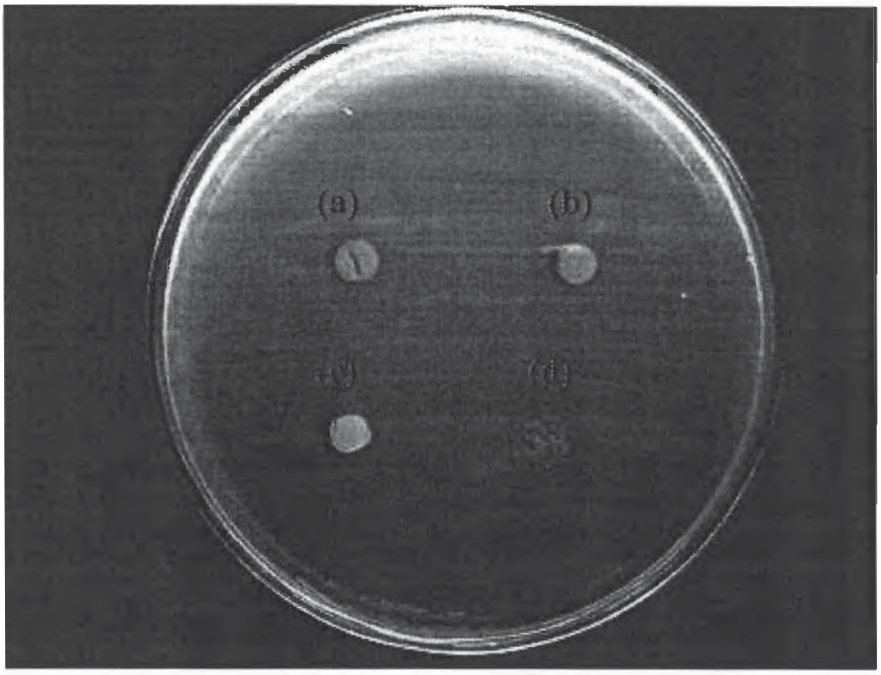



Figure 71 Agar diffusion test of fast dissolving film against *Streptococcus mutans* ATCC $KPSK_2$; (a) to (d) film containing extract in amount of 2, 4, 6 and 1 times, respectively

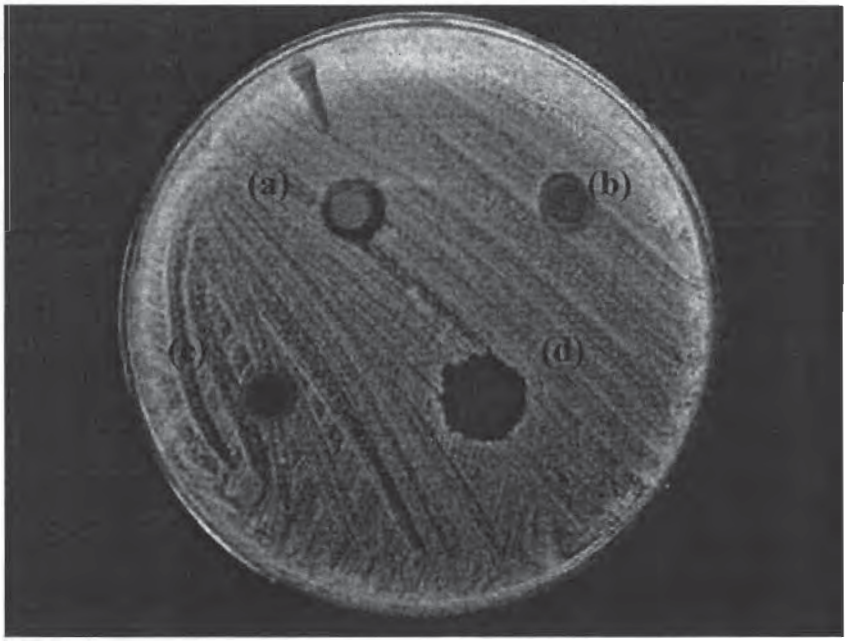



Figure 72 Agar diffusion test of fast dissolving film solution against *Staphylococcus aureus* ATCC 25923; (a) film with extract; (b) film base; (c) polymeric film without additives; (d) film containing cetylpyridinium chloride

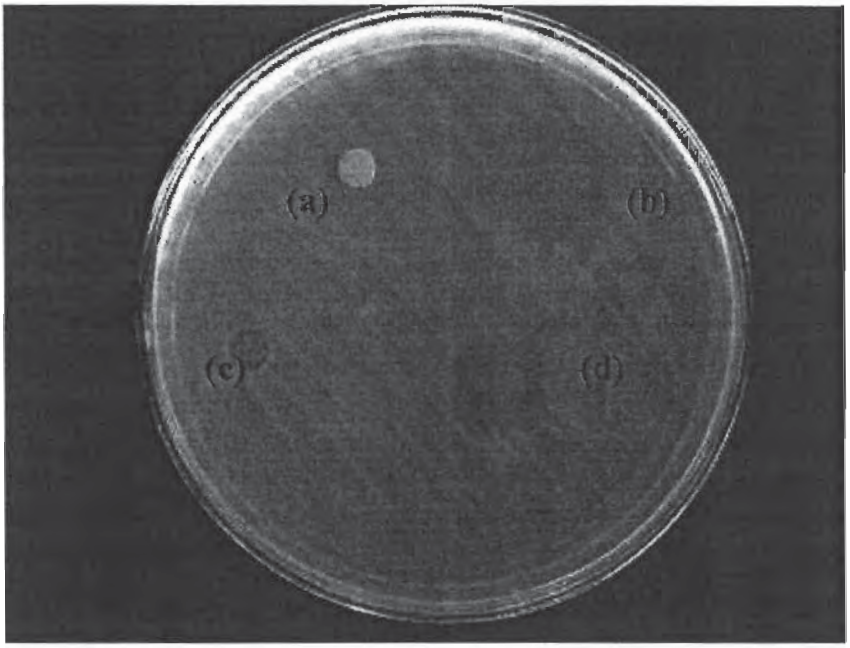



Figure 73 Agar diffusion test of fast dissolving film solution against *Streptococcus mutans* ATCC $KPSK_2$; (a) film with extract; (b) film base; (c) polymeric film without additives; (d) film containing cetylpyridinium chloride

**I. Stability Study of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract**

The stability study of *Garcinia mangostana* extract in orally fast dissolving films was performed by triplicate samples of four formulations. It individually packed in glass vials, which tightly sealed with rubber closures and aluminium caps and stored at 40 $^{\circ}$C, 75 %RH for three months. At the initial time, first, second and third month, the films were sampled and assayed for the remaining mangostin content. The physicochemical properties and the amount of mangostin of orally fast dissolving films in stability test were determined using differential scanning calorimetry (DSC) and high performance liquid chromatography (HPLC), respectively.

**1. Physicochemical stability study**

The orally fast dissolving films containing *Garcinia mangostana* extract change in the physicochemical properties of the film was observed from DSC thermograms.

As the results of the DSC thermograms of all formulations, no change in DSC thermograms between three months of stability study (at the initial time, first, second and third month) was observed. These results indicated that all formulations did not change in physicochemical properties after the stress condition for 3 months (Figure 55-58).

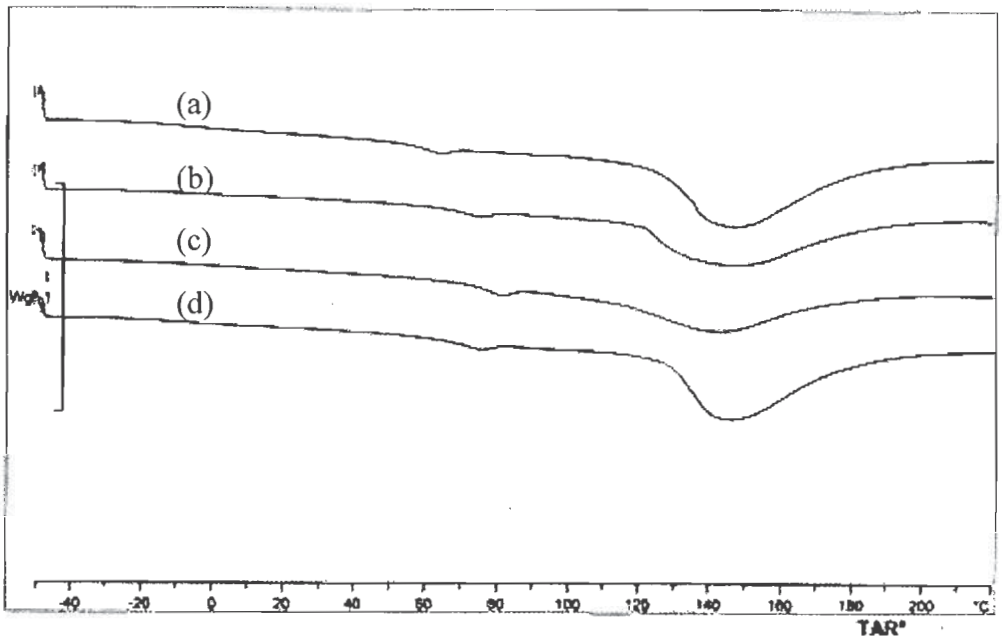



Figure 74 DSC thermograms of films in formulation E3HPC (2:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 4.96 mg); (b) at first month (sample weight 4.89 mg); (c) at second month (sample weight 5.04 mg); (d) at third month (sample weight 4.77 mg).

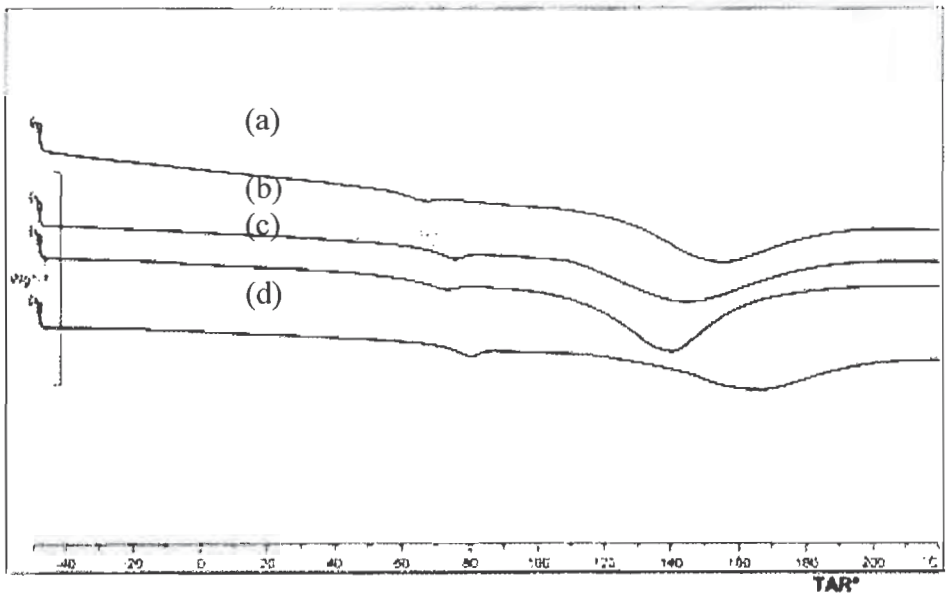



Figure 75 DSC thermograms of films in formulation E3HPC (3:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 5.00 mg); (b) at first month (sample weight 4.65 mg); (c) at second month (sample weight 4.98 mg); (d) at third month (sample weight 4.92 mg).

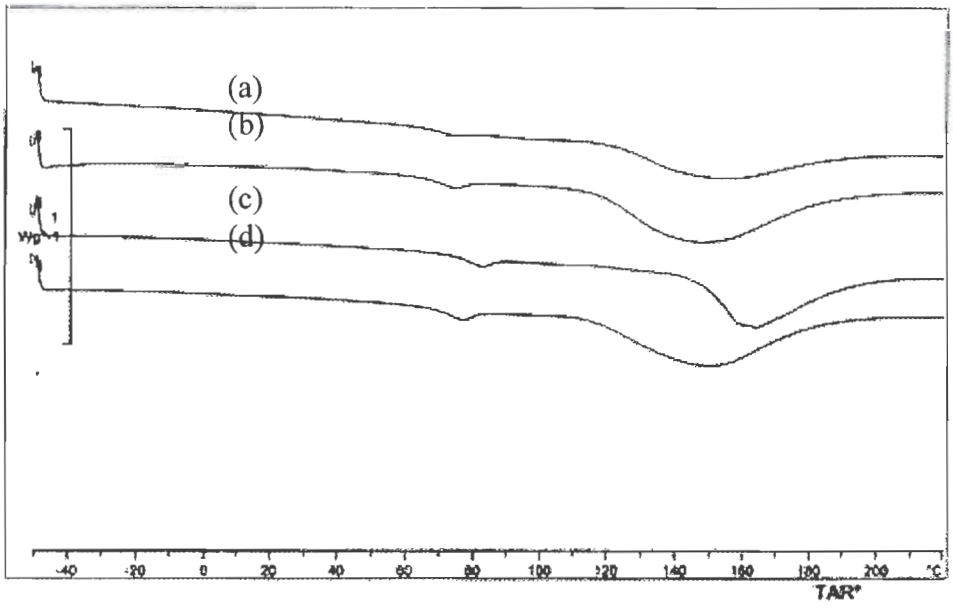



Figure 77 DSC thermograms of films in formulation E3HPC (4:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 5.32 mg); (b) at first month (sample weight 5.05 mg); (c) at second month (sample weight 5.02 mg); (d) at third month (sample weight 4.98 mg).

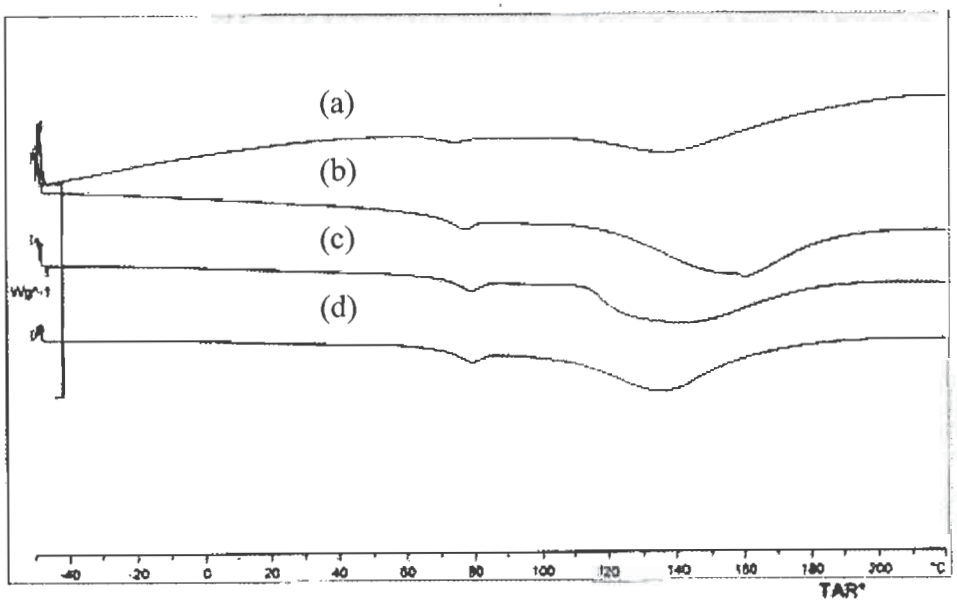



Figure 77 DSC thermograms of films in formulation E3HPC (5:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 5.30 mg); (b) at first month (sample weight 5.01 mg); (c) at second month (sample weight 5.05 mg); (d) at third month (sample weight 4.95 mg).

**2. Chemical stability study**

The amounts of mangostin containing in film formulations were assayed by HPLC at 0, 1, 2 and 3 month. The analytical method, which employed in this investigation was the HPLC method as previously described. In addition, the percentage loss of mangostin and percentage labeled amount after the exposure to heat and high humidity (40 $^{o}$C, 75 %RH) at each time interval and the end of storage were also calculated.

As the results, all orally fast dissolving films appeared to be stable due to their percentage loss of mangostin was less than 10 % of the initial value (Carstensen, 1990). It was found that E3HPC (4:1) degraded with the highest extent, however, its percentage loss of mangostin was only 8.00% at the end of storage, while E3HPC (2:1) degraded with the least extent at 3.70% (Tables 36 and 37). From the analysis of data, it indicated that the remaining amount and amount of drug loss of mangostin in the all formulations after stability study were no statistically significant difference ($p$>0.05).

Table 36 Amount of mangostin in four film formulations at initial time, first, second and third month after stability study

| formulation | Amount of mangostin ± SD (μg) | | | | Percentage loss of mangostin* |
|---|---|---|---|---|---|
| | initial | 1$^{st}$ month | 2$^{nd}$ month | 3$^{rd}$ month | |
| E3HPC (2:1) | 175.91±5.95 | 172.41±1.75 | 167.58±2.59 | 169.41±4.24 | 3.70 |
| E3HPC (3:1) | 195.83±4.50 | 194.37±2.86 | 185.93±1.92 | 180.92±6.51 | 7.61 |
| E3HPC (4:1) | 186.56±3.82 | 184.68±0.59 | 177.51±2.68 | 171.64±3.13 | 8.00 |
| E3HPC (5:1) | 186.31±3.03 | 187.01±5.38 | 182.37±6.56 | 173.22±8.83 | 7.03 |

Table 37 Percentage labeled amount of mangostin in film in stability test

| Formulation | % Labeled amount ± SD | | | | Percentage loss of mangostin* |
|---|---|---|---|---|---|
| | initial | 1$^{st}$ month | 2$^{nd}$ month | 3$^{rd}$ month | |
| E3HPC (2:1) | 96.86±3.28 | 94.93±0.96 | 92.28±1.43 | 93.28±2.34 | 3.70 |
| E3HPC (3:1) | 107.83±2.48 | 107.03±1.57 | 102.38±1.06 | 99.62±3.58 | 7.61 |
| E3HPC (4:1) | 102.73±2.10 | 101.69±0.32 | 97.74±1.47 | 94.51±1.72 | 8.00 |
| E3HPC (5:1) | 102.59±1.67 | 102.97±2.96 | 100.42±3.61 | 95.38±4.86 | 7.03 |

*Percentage loss of mangostin = $\frac{\text{Initial} - \text{Final \% labeled amount} \times 100}{\text{Initial \% labeled amount}}$

Since there was no the investigation on degradation kinetics of mangostin reported, the interpretation of the results is limited. Moreover, its degradation products have never been reported.

# PART III MONOGLYCERIDE-BASED DRUG DELIVERY SYSTEMS (LIQUID CRYSTALLINE) CONTAINING *Garcinia mangostana* EXTRACT

## J. Preparation of Monoglyceride-Based Drug Delivery System

### 1. Monoglyceride-Based Drug Delivery System

Monoglyceride-based drug delivery systems were developed by the ability of glyceryl monooleate and triglycerides to form liquid crystals in contact with water. Liquid crystalline phases were formed under the conditions used in this study. Partial phase diagrams of these liquid crystalline systems are displayed in Figures 59-61.

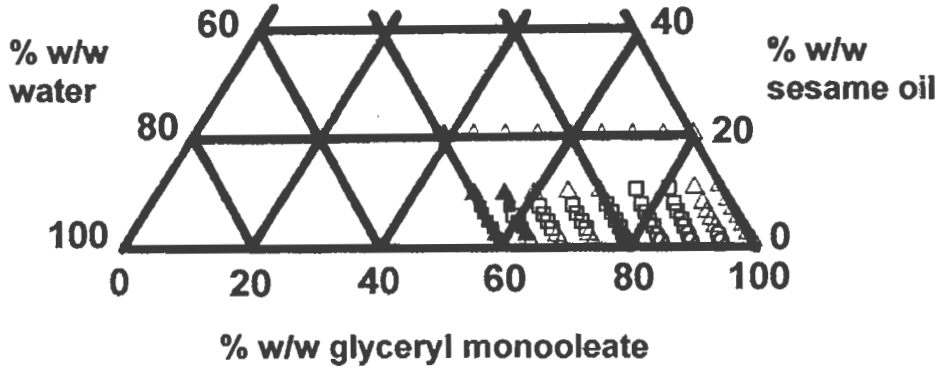



Figure 78 Ternary phase diagram of glyceryl monooleate-sesame oil-water system; (O) lamellar phase ($L_\alpha$); (□) reversed hexagonal phase ($H_{II}$); (■) reversed hexagonal phase ($H_{II}$) + aqueous; (△) isotropic, 1-phase; and (▲) isotropic, 2-phase

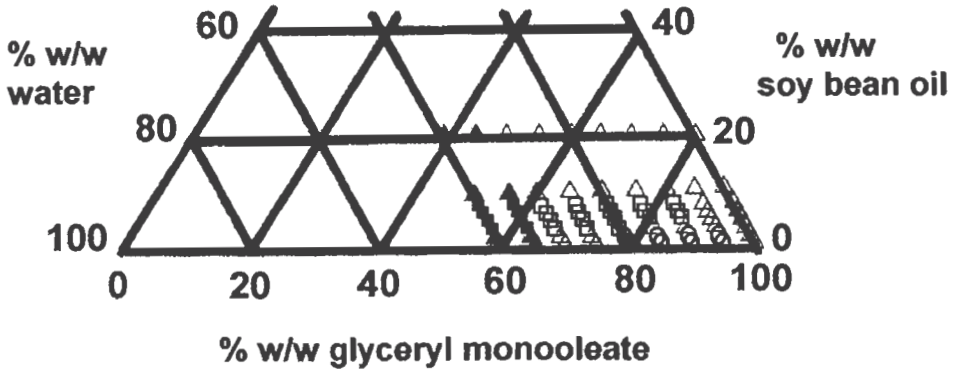



Figure 79 Ternary phase diagram of glyceryl monooleate-soybean oil-water system; (O) lamellar phase ($L_\alpha$); (□) reversed hexagonal phase ($H_{II}$); (■) reversed hexagonal phase ($H_{II}$) + aqueous; (△) isotropic, 1-phase; and (▲) isotropic, 2-phase

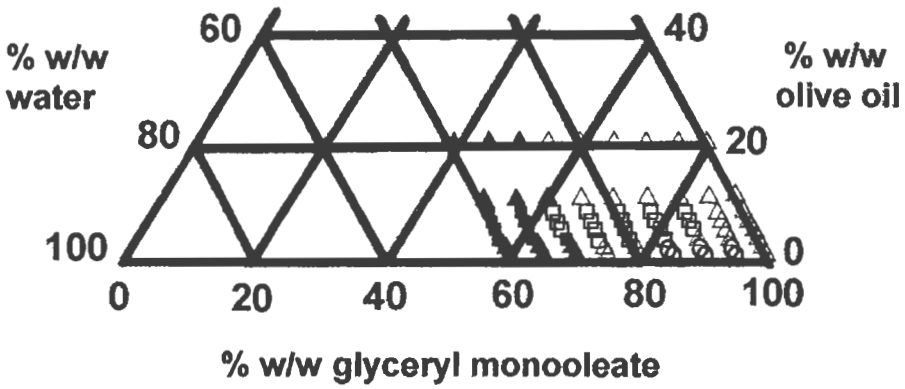


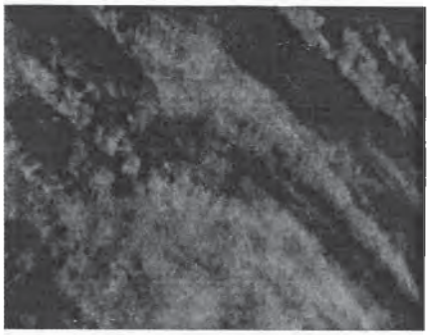

Figure 80 Ternary phase diagram of glyceryl monooleate-olive oil-water system; (O) lamellar phase ($L_\alpha$); (□) reversed hexagonal phase ($H_{II}$); (■) reversed hexagonal phase ($H_{II}$) + aqueous; (△) isotropic, 1-phase; and (▲) isotropic, 2-phase

In this study, partial phase diagrams were used because the outer area could not give the 1-phase liquid crystals. The ternary phase diagram of glyceryl monooleate-sesame oil-water system gave the area of one-phase liquid crystalline more than other two systems using soybean oil and olive oil. Although the differences in phase behavior were found by using various oils, these differences were generally small. The phase behavior of these systems is similar to the system (monoolein-sesame oil-water) studied by Norling et al. (1992). However, the precise locations of the phase boundaries in the diagram differed slightly.

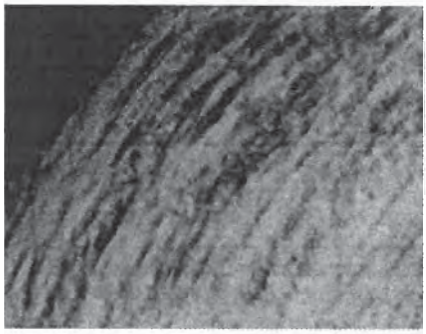
**2. Physical Characterization**

**2.1 Polarized Light Microscopy**

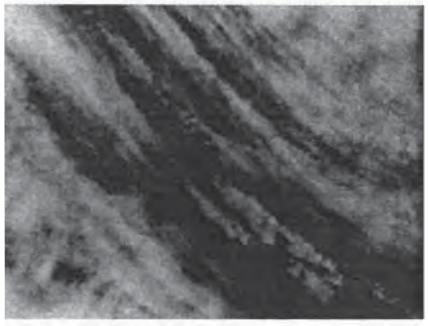
The formation and structure of liquid crystalline phases were identified under polarized light microscopy. Liquid crystals formed by glyceryl monooleate, water and triglycerides content not more than 4% had liquid crystal structures conformable to a lamellar structure established by Makai et al. (2003) as shown in Figure 62. When increasing the triglycerides content from 6% to 15%, liquid crystal structures were changed which structure similar to a reversed hexagonal phase observed by Geraghty et al. (1996) (Figure 63). The difference in triglycerides, sesame oil, soybean oil or olive oil, showed the same pattern of liquid crystalline phases under polarized light microscope. These results might be from the small amount of triglycerides compared to glyceryl monooleate which was the main component and had the ability to form liquid crystals. In this study, there had been a dark background under polarized light microscope which could not be identified to cubic phase or reversed micellar by this method. From the results, the formation and structure of liquid crystalline phases depended on the characteristic of the amphiphilic compounds and the ratio of the components in the phase diagram.



(a) system containing sesame oil



(b) system containing soybean oil



(c) system containing olive oil

Figure 81 Polarizing microscopic images of the lamellar phases; as observed at ×100 magnification

**2.2 Physical Stability**

The samples that could form reversed hexagonal phases were selected for this study due to their favorable sustained release (Norling et al., 1992). Under the heating-cooling cycle, the samples with water content lower than 30% did not show any changes in physical appearances. While the samples with water content 35% showed phase separation after the stress condition. The reason is that increasing water content, it tends to be two-phase separation according to the phase diagram.

(a) system containing sesame oil

(b) system containing soybean oil

(c) system containing olive oil

Figure 82 Polarizing microscopic images of the reversed hexagonal phases; as observed at ×100 magnification

**K. Formulation of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

The monoglyceride-based drug delivery systems were composed of mixtures of monoglyceride, triglycerides from vegetable oils and water. The relative amount of monoglyceride and vegetable oil in the composition for formulation of the reversed hexagonal liquid crystalline phase may vary over a considerable range. Generally, the vegetable oil is present in an amount sufficient to improve the flow characteristics of the composition upon release from the dosing device and induce the formation of a stable reversed hexagonal liquid crystalline phase upon contact with an aqueous liquid. Generally, the vegetable oil and monoglyceride are presented in a weight ratio of 1:99 to 30:70 vegetable oil to monoglyceride, most preferably from 5:95 to 15:85, based upon the combined weight of the monoglyceride and vegetable oil (Lading et al., 1992).

In this present work, monoglyceride-based drug delivery systems were constructed in the ratio of 8:62:30 and 12:58:30 (triglyceride: monoglyceride: water). Two percent of

mangostin extract was then incorporated into the samples. These ratios were chosen due to the water content of 30% gave the equilibrium water content and a separate water phase outside the liquid crystalline phase was not found. While the ratio of triglyceride between 8% and 12% resulted in the formation of the reversed hexagonal phases.

The differences in type and ratio of triglycerides were prepared to compare the effect of triglycerides on the physicochemical properties and *in vitro* drug release. The formulations used in this study are shown in Table 38. Formulations 1 to 3 and formulations 4 to 6 contained triglyceride: monoglyceride: water in the ratio of 8:62:30 and 12:58:30, respectively.

Table 38 Formulation of monoglyceride-based drug delivery system

| Composition | Formulation | | | | | |
|---|---|---|---|---|---|---|
| (% w/w) | 1 | 2 | 3 | 4 | 5 | 6 |
| GMO | 60.76 | 60.76 | 60.76 | 56.84 | 56.84 | 56.84 |
| Sesame oil | 7.84 | - | - | 11.76 | - | - |
| Soybean oil | - | 7.84 | - | - | 11.76 | - |
| Olive oil | - | - | 7.84 | - | - | 11.76 |
| Water | 29.40 | 29.40 | 29.40 | 29.40 | 29.40 | 29.40 |
| Mangostin | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |

## L. Characterization of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System

### 1. Determination of Physicochemical Properties

The physicochemical properties of formulations before and after incorporating the *Garcinia mangostana* extract were determined as follows:

#### 1.1 Physical Appearances

The physical appearances of formulations such as color, clarity and phase separation were observed. The color of monoglyceride-based drug delivery systems was light yellow, according to the color of glyceryl monooleate. After incorporation of mangostin extract, the color of formulations changed to dark yellow due to the deep yellow color of mangostin. The formulations before and after incorporation of mangostin extract were clear with some air bubbles due to the process of mixing, which disappeared when left to stand overnight. Phase separation and precipitation were not observed after incorporation of the mangostin extract. Different formulations showed the similar physical appearances.

#### 1.2 pH Measurement

The pH of monoglyceride-based drug delivery systems before and after incorporation of mangostin extract was in the range of 5.42-5.60 and 5.68-5.84, respectively (Table 39). These results indicated that after incorporation of mangostin extract the pH

values were slightly increased. The reason could not be clearly explained. Formulations 1 to 6 had the pH values in the same range.

Table 39 pH of formulation before and after incorporation of mangostin extract

| Formulation | pH | |
|---|---|---|
| | Base | With mangostin |
| 1 | 5.54±0.01 | 5.84±0.00 |
| 2 | 5.60±0.01 | 5.79±0.01 |
| 3 | 5.42±0.01 | 5.68±0.01 |
| 4 | 5.57±0.01 | 5.84±0.00 |
| 5 | 5.54±0.01 | 5.82±0.00 |
| 6 | 5.44±0.01 | 5.73±0.01 |

**1.3 Viscosity Measurement**

The viscosity values of monoglyceride-based drug delivery systems before and after incorporation of mangostin extract were in the range of 4758.94-5160.06 and 4824.34-5325.74 cps, respectively (Table 40). The viscosity of formulations with mangostin extract was slightly higher than that without the extract. The formulations with 8% triglyceride content (formulation 1-3) had higher viscosity than 12% triglyceride content (formulation 4-6). Analysis of data indicated that there was statistically significant difference between two groups ($P<0.05$). However, all of the formulations are high-viscous enough to sustain release of drug at the injection sites.

Table 40 Viscosity of formulation before and after incorporation of mangostin extract

| Formulation | Viscosity (cps) | |
|---|---|---|
| | Base | With mangostin |
| 1 | 5160.06±49.38 | 5236.36±29.49 |
| 2 | 5140.44±23.58 | 5325.74±54.46 |
| 3 | 4979.12±46.40 | 5075.04±63.07 |
| 4 | 4874.48±29.49 | 4918.08±47.16 |
| 5 | 4826.52±17.30 | 4887.56±49.52 |
| 6 | 4758.94±22.97 | 4824.34±38.32 |

**1.4 Polarized Light Microscopy**

Photomicrographs of monoglyceride-based drug delivery systems before and after incorporation of mangostin extract are shown in Figures 64-65. The polarizing microscopic images showed similar pattern of liquid crystalline phases which indicated that

mangostin extract did not affect the liquid crystal structure of the formulations. Different formulations also showed similar patterns of liquid crystalline phases.

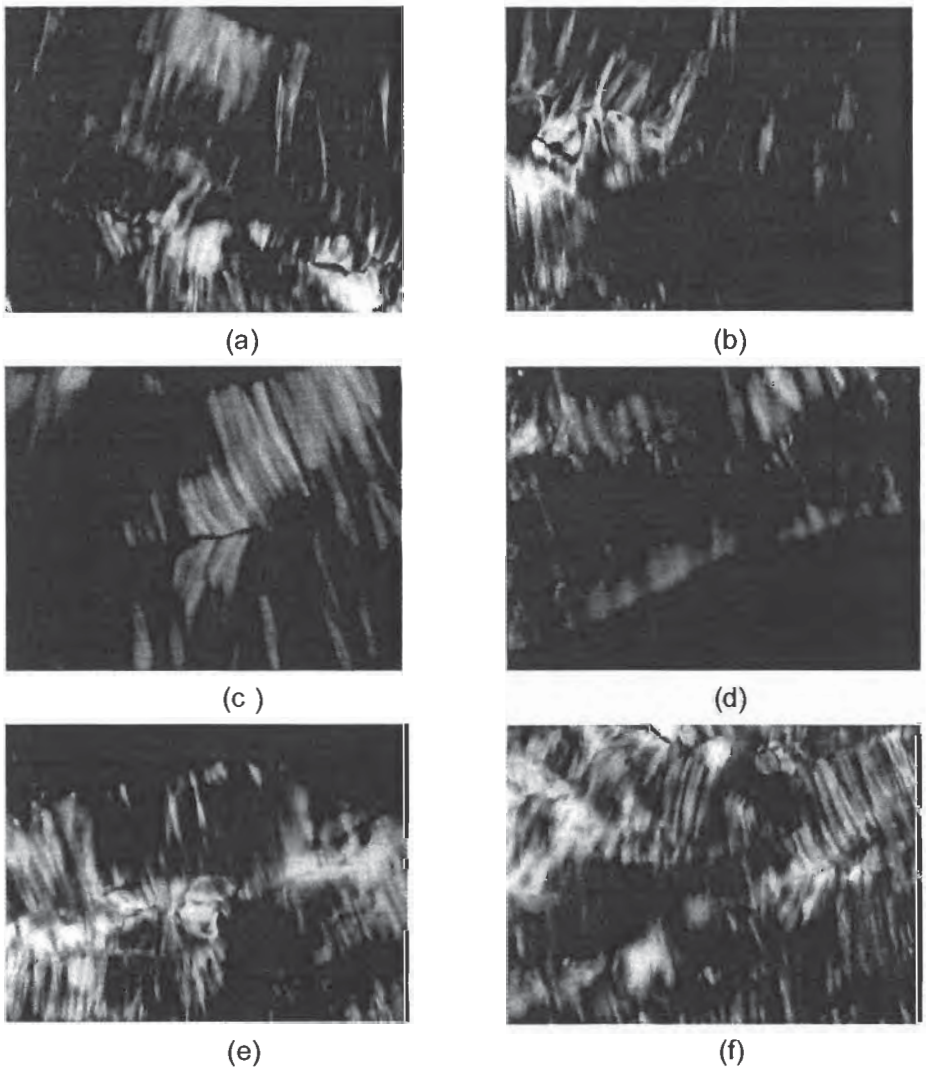



Figure 83 Polarizing microscopic images of formulation 1-3 before and after incorporation of mangostin extract; (a) and (b) formulation 1; (c) and (d) formulation 2; and (e) and (f) formulation 3, as observed at × 100 magnification

**2. Determination of Injectability through the Syringe**

Due to their high viscosity and stiffness, liquid crystalline phases are difficult to administer through a syringe. Therefore, the low-viscous states are used and triggered to the high-viscous states by dilution with fluid in the oral cavity.

In this study, the injectability of low-viscous state formulations was determined by administer through the syringe with 23-gauge tip needle. The viscosity values of the formulations are shown in Table 14. Triglycerides have been reported to lower the melting point and improve the flow properties of glyceryl monooleate (Norling et al., 1992; Okonogi et al., 2004). As a result, the formulations with 12% triglyceride content could reduce the viscosity and improve the flow properties of the formulation more than 8% triglyceride content. Analysis of data indicated that there was statistically significant difference in the

viscosity values between two groups ($P$<0.05). However, these viscosities were all low enough to be administered through the syringe with 23-gauge tip needle.

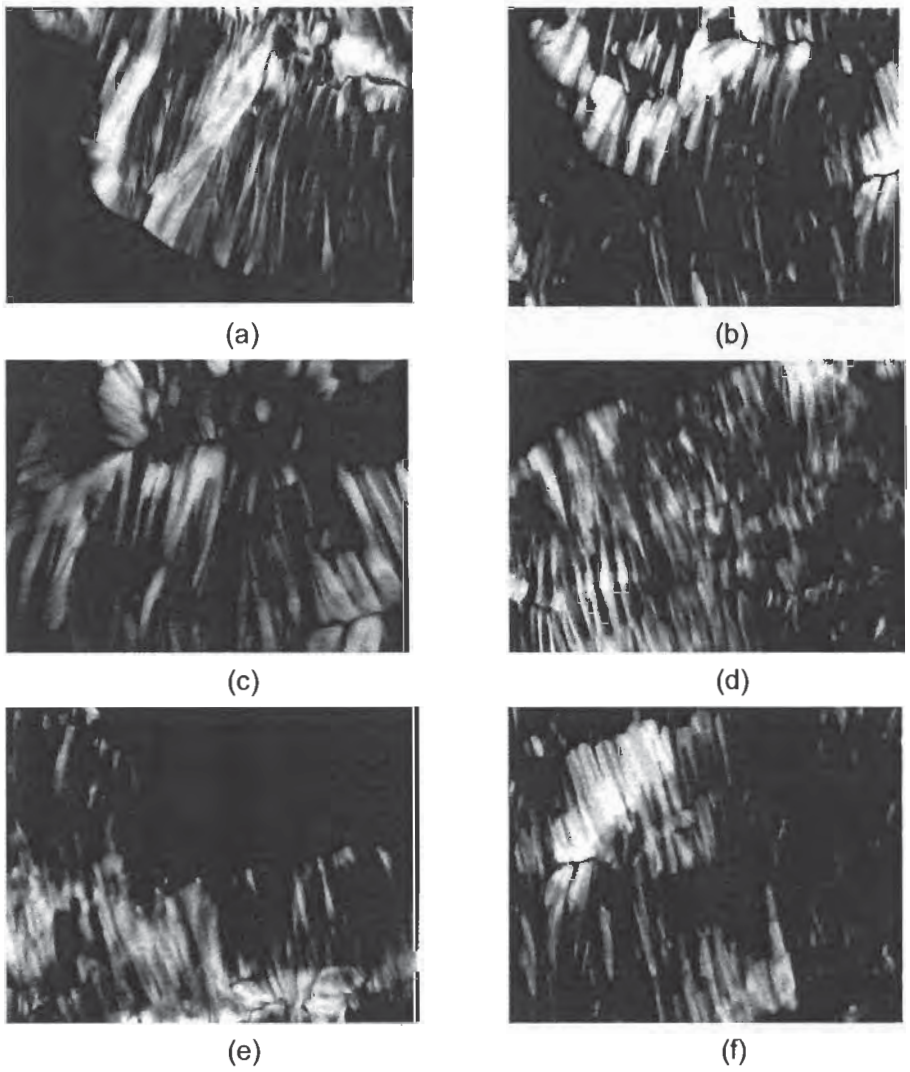



Figure 84 Polarizing microscopic images of formulation 4-6 before and after incorporation of mangostin extract; (a) and (b) formulation 4; (c) and (d) formulation 5; and (e) and (f) formulation 6, as observed at × 100 magnification

Table 41 Viscosity of low-viscous state formulation

| Formulation | Viscosity (cps) |
|---|---|
| 1 | 178.22±1.82 |
| 2 | 165.14±2.91 |
| 3 | 166.99±1.47 |
| 4 | 150.04±1.05 |
| 5 | 140.99±1.52 |
| 6 | 140.88±1.31 |

**3. *In vitro* Liquid Crystalline Phase Formation Study**

The *in situ* transition from a low-viscous state to the required high-viscous liquid crystalline phase after administration is important to the use of liquid crystalline phases for drug delivery. There are several parameters which may be used for triggering a transition *in situ* after administration. For glyceryl monooleate, the high-viscous liquid crystalline phases were formed upon contact with excess water.

This study was conducted with the low-viscous state formulations. After addition of the formulation into excess water, the liquid crystalline phase was detected by polarized light microscopy. As a result, all formulations could be transformed to the high-viscous liquid crystalline phase. The changes were found within 15 min after addition. Photographs from polarized light microscope showed the liquid crystals were formed and could be detected in 15 min (Figure 66). These results agreed with Scherlund et al. (2001), although there was a difference in liquid crystalline phase drug delivery system. Scherlund et al. suggested that in presence of a large amount of water, the samples take up the water immediately and form optically anisotropic phase, 30 min is enough to form the most of this phase.

The water uptake study of monoglyceride from Chang and Bodmeier (1997) showed that the water uptake initially increased rapidly and then leveled off and approached the equilibrium water content. The rapid swelling of the monoglyceride matrices indicated that the formation of the liquid crystalline phase was the fast process. These results were consistent with previous study. Geraghty et al. (1996) suggested that the rate of water uptake was inversely proportional to their initial water content. The hydration of monoglyceride containing 0% initial water content was observed within the first 15 min while the samples formulated with 40% water content did not hydrate or swell significantly as they already contained their equilibrium water content.

In conclusion, the *in vitro* study showed that low-viscous state formulations could be transformed to high-viscous liquid crystalline states upon dilution with water. Therefore it is possible to form this state after *in vivo* application.

**4. *In Vitro* Release Study**

**4.1 Solubility Study of the Receiver Fluid**

Although the pH of gingival crevice and periodontal pocket were reported in the mean of 6.92±0.03 (Eggert et al., 1991), the most of *in vitro* release studies of drugs used in periodontal pocket used pH 7.4 phosphate buffer for the medium (Roskos et al., 1995; Esposito et al., 1996; Jones et al., 1997; Sendil et al., 1999; Schwach-Abdellaoui et al., 2001, 2002). Therefore, pH 7.4 phosphate buffer was used in this study. Because of the poor solubility of mangostin in this medium, ethanol was added into the medium to enhance drug solubility. In this present work, ethanol ranging from 0-35% was added into the receiver fluid and examined the solubility of mangostin. The results showed that the solubility of mangostin was increased as a function of ethanol concentrations (Table 42).

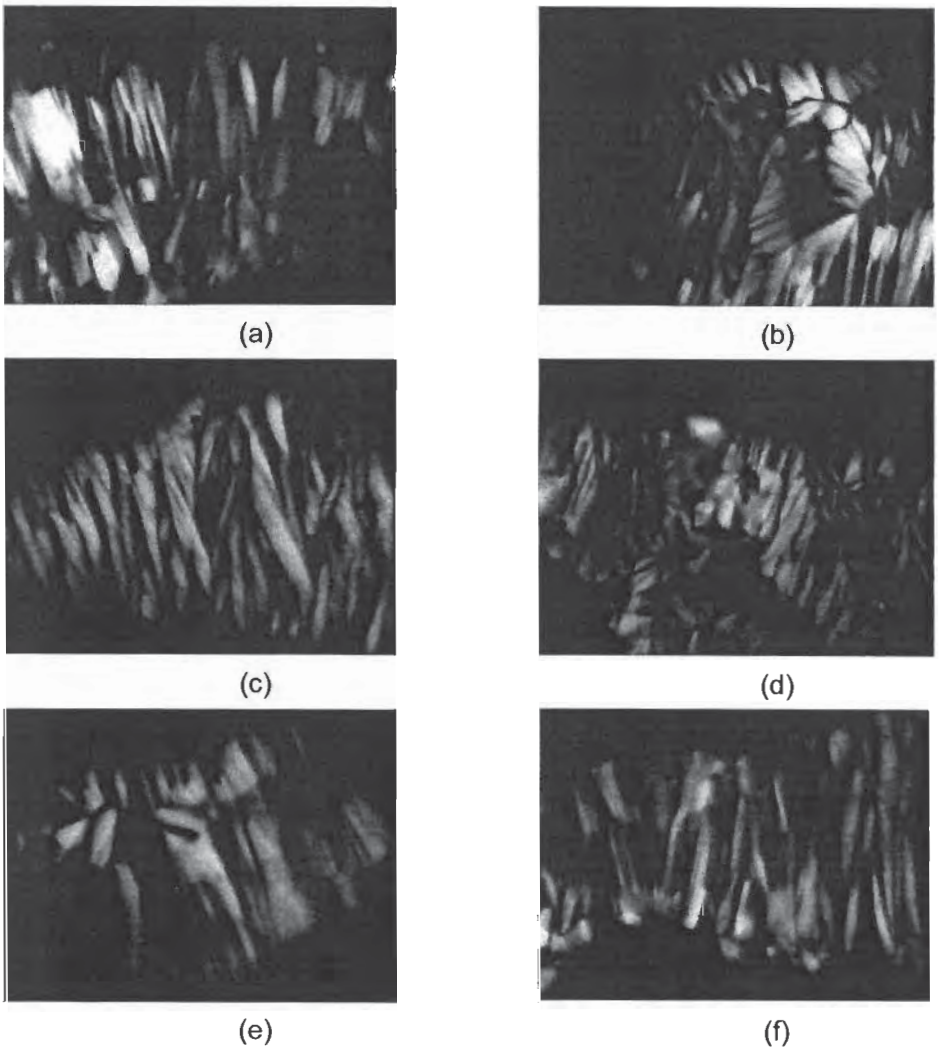



Figure 85 Polarizing microscopic images of *in vitro* liquid crystalline phase formation after dilution with water; (a) formulation 1; (b) formulation 2; (c) formulation 3; (d) formulation 4; (e) formulation 5; and (f) formulation 6, as observed at × 100 magnification

The solubility of mangostin in pH 7.4 phosphate buffer was 0.0234±0.0030 mg/ml and in the present of 35% ethanol the solubility was increased to 1.1277±0.0886 mg/ml, which was able to maintain the sink condition in the release study.

Table 42 Solubility of mangostin in pH 7.4 phosphate buffer and various concentrations of ethanol in pH 7.4 phosphate buffer

| Solvent | Solubility (mg/ml) |
|---|---|
| pH 7.4 phosphate buffer | 0.0234±0.0030 |
| pH 7.4 phosphate buffer+10% ethanol | 0.0559±0.0047 |
| pH 7.4 phosphate buffer+20% ethanol | 0.1008±0.0109 |
| pH 7.4 phosphate buffer+25% ethanol | 0.1965±0.0230 |
| pH 7.4 phosphate buffer+30% ethanol | 0.4550±0.0568 |
| pH 7.4 phosphate buffer+35% ethanol | 1.1277±0.0886 |

### 4.2 *In vitro* Release Study

*In vitro* drug release methods are frequently used to gain information about the release profiles of active ingredients in the formulation development. In the present work, two compartment Franz diffusion cells were used for the experiments. The amounts of drug release were analyzed using UV spectrophotometer. The validation methods are shown in the following part.

The release profiles were plotted between the cumulative amounts of drug release versus time as shown in Figures 40-41. The release data are presented in Appendix B. All formulations could sustain the release of drug over a period of 48 hr. These results were consistent with previous reports. Norling et al. (1992) reported that the reversed hexagonal form of monoglyceride-based drug delivery system could give a sustained release of drug over a period of 48 hr while the cubic form showed a complete release in 24 hr. Because of the diffusion pathway is more obstructed in the reversed hexagonal form than in the cubic one, which has connected water channels. The closed water channels of the reversed hexagonal phase slow down the diffusion of dissolved drug through the matrix. *In vivo* results from Esposito et al. (1996) showed that monoglyceride-based drug delivery system was persistent in the periodontal pockets with 80% of the initial level after 8 hr of application.

In the present study, various oils showed similar release profiles but there was a slight difference in the percentages of drug release. The highest drug release was obtained from the formulations with sesame oil, followed by soybean oil and olive oil, respectively. However, analysis of data indicated that there was no statistically significant difference ($P>0.05$) in overall percentages of drug release. The formulations with 8% triglycerides content showed less percentage of drug release at the beginning of the release profiles than the formulations with 12% triglycerides content but overall percentages of drug release were similar in the range of 91.97-94.46%. In addition, the formulations with 8% triglycerides content did not show more prolonged release over a period of study. These data indicated that increasing triglycerides content of the formulations could improve the flow properties and injectability of the formulation into the periodontal pockets but did not affect the release-controlling mechanism of the drug delivery system.

Several mathematical models have been used to describe the release of the drug. The release data obtained from this study were plotted according to the following models to describe the mechanism of drug release: zero-order kinetics, first-order kinetics, and Higuchi diffusion model where the cumulative amount of drug release per unit surface area is directly proportional to the square root of time.

Zero-order equation: $Q_t = Q_0 - k_0 t$

First order equation: $ln\ Q_t = ln\ Q_0 - k_1 t$

Higuchi's equation: $Q_t = k_H t^{1/2}$

where $Q_t$ is the amount of drug release at time t, $k_0$ is the zero-order release rate constant, $k_1$ is the first-order release rate constant, and $k_H$ is the diffusion rate constant.

The kinetic parameters for zero-order, first-order and Higuchi model during the initial 24 hr were calculated and are presented in Table 16. The zero-order plot, the first-order plot and the Higuchi plot are shown in Figures 40-41, Figures 42-43 and Figures 44-45, respectively. The release data of mangostin tend to follow Higuchi model rather than the other two models, because the highest coefficient of determination ($R^2$) was obtained with the Higuchi model. Analysis of data showed that there was statistically significant difference ($P<0.05$) in coefficients of determination between the three models. Moreover, analysis of variance by regression showed a significant difference ($P<0.05$) in coefficients of determination of the Higuchi model, which indicated the correlation of % cumulative release and square root of time. The highest Higuchi release rate constant was obtained from the formulations with olive oil. Analysis of data indicated that the Higuchi release rate constant of the formulations with olive oil was statistically significant difference ($P<0.05$) than the formulations with sesame oil and soybean oil but there was no statistically significant difference ($P>0.05$) in the release rate constant between the formulations with sesame oil and soybean oil.

The mechanism of diffusion controlled release (Higuchi, 1961, 1963) was dominated by the penetration of the medium into the drug matrix through the channels of the liquid crystalline phase, and then the drug was presumed to leach out by gradually dissolving into the permeating medium and diffusing from the matrix along the channels filled with the extracting medium. Thus, the release behavior of drug was expected to be governed by the solubility and diffusion coefficient of the drug in the liquid crystalline phase. This mechanism was explained by Higuchi's equation as follows:

$$Q = \left[\frac{D\varepsilon}{\tau}(2A - \varepsilon C_s) C_s t\right]^{1/2}$$

where Q is the amount of drug release after time t per unit exposed area, D is the diffusion coefficient of the drug in the matrix, $\varepsilon$ is the porosity of the matrix, $\tau$ is the tortuosity factor of the matrix, A is the total amount of drug in the matrix per unit volume, and $C_s$ is the solubility of drug in the matrix.

The result from this study conformed to many reports which suggested that the release mechanism from monoglyceride-based drug delivery system followed square

root of time kinetics indicating that the rate of release was diffusion controlled (Geraghty et al., 1996; Chang and Bodmeier, 1997; Komsri, 1997; Helledi and Schubert, 2001). However, other studies showed that monoglyceride-based drug delivery system could be followed zero-order and first-order kinetics (Burrows, Collett and Attwood, 1994). These data indicated that the similar liquid crystalline system could demonstrate different release profiles. These depend on several factors including a wide range of drug, solubility and concentration of the incorporated drug substance.

Table 43 Kinetic parameters of mangostin release from monoglyceride-based drug delivery system

| Formulation | Zero-order plot | | First-order plot | | Higuchi plot | |
|---|---|---|---|---|---|---|
| | $k_0$ | $R^2$ | $k_1$ | $R^2$ | $k_H$ | $R^2$ |
| 1 | 2.5407 | 0.8806 | 0.0488 | 0.7528 | 16.1968 | 0.9791 |
| 2 | 2.6269 | 0.8845 | 0.0541 | 0.7572 | 16.6843 | 0.9761 |
| 3 | 2.8727 | 0.8570 | 0.0667 | 0.6816 | 18.4496 | 0.9671 |
| 4 | 2.6607 | 0.9046 | 0.0473 | 0.7977 | 16.7773 | 0.9840 |
| 5 | 2.7437 | 0.9068 | 0.0504 | 0.7908 | 17.2936 | 0.9856 |
| 6 | 3.0679 | 0.8834 | 0.0647 | 0.7337 | 19.4843 | 0.9748 |

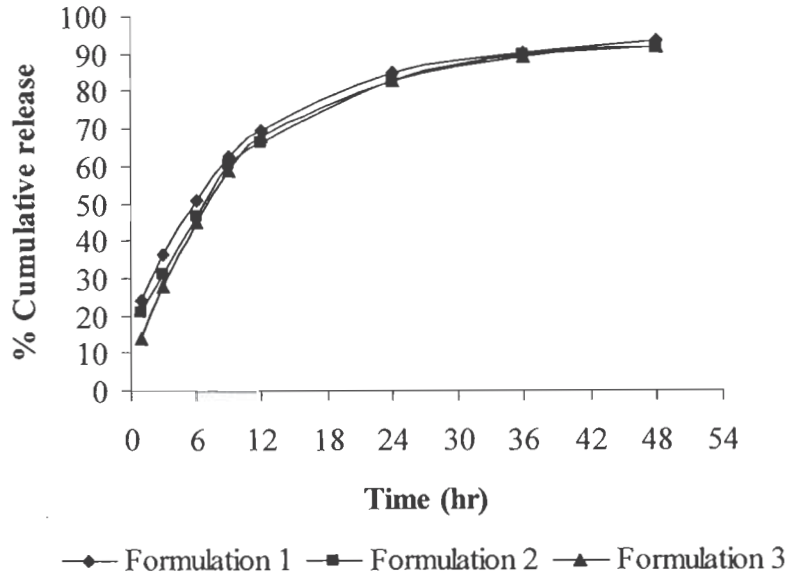



Figure 86 Release profiles of mangostin from monoglyceride-based drug delivery system containing triglyceride: monoglyceride: water in the ratio of 8:62:30

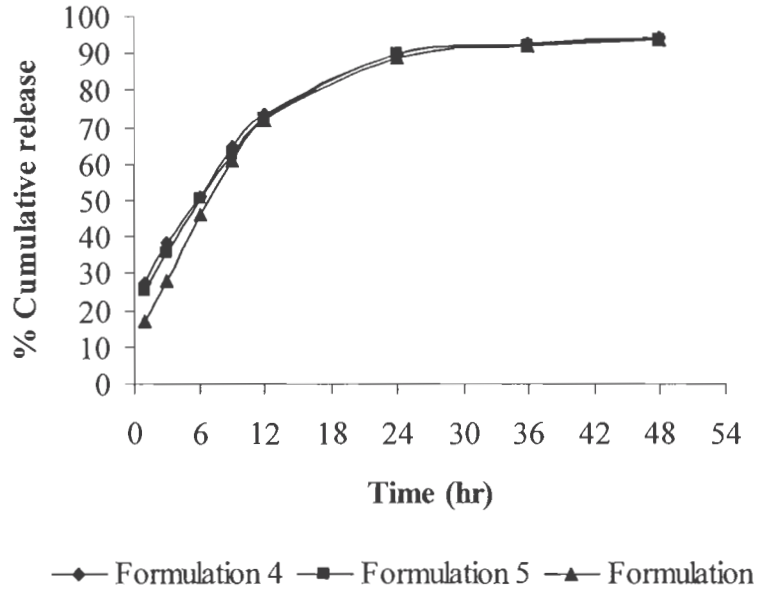



Figure 87 Release profiles of mangostin from monoglyceride-based drug delivery system containing triglyceride: monoglyceride: water in the ratio of 12:58:30

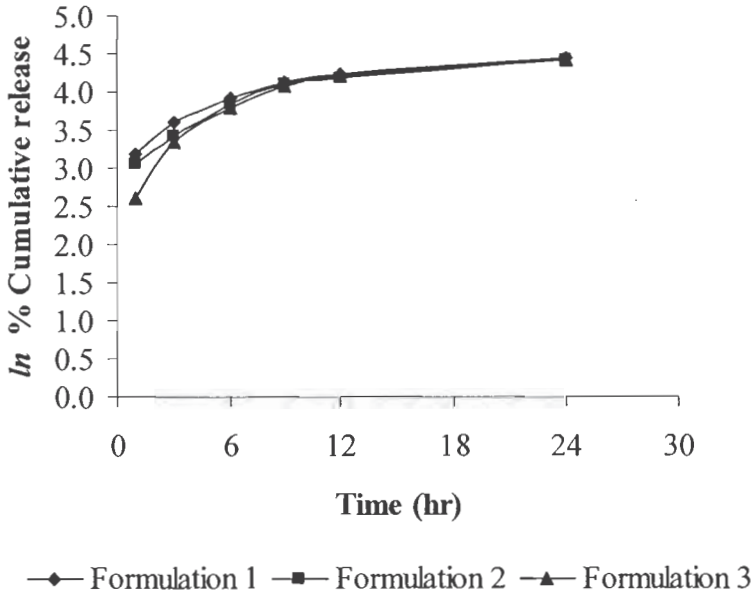



Figure 88 First-order plot of mangostin from monoglyceride-based drug delivery system containing triglyceride: monoglyceride: water in the ratio of 8:62:30

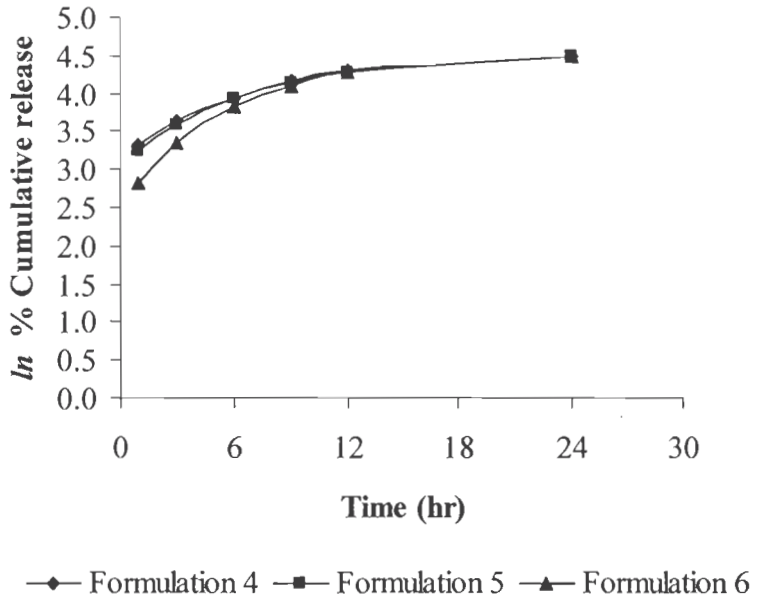



Figure 89 First-order plot of mangostin from monoglyceride-based drug delivery system containing triglyceride: monoglyceride: water in the ratio of 12:58:30

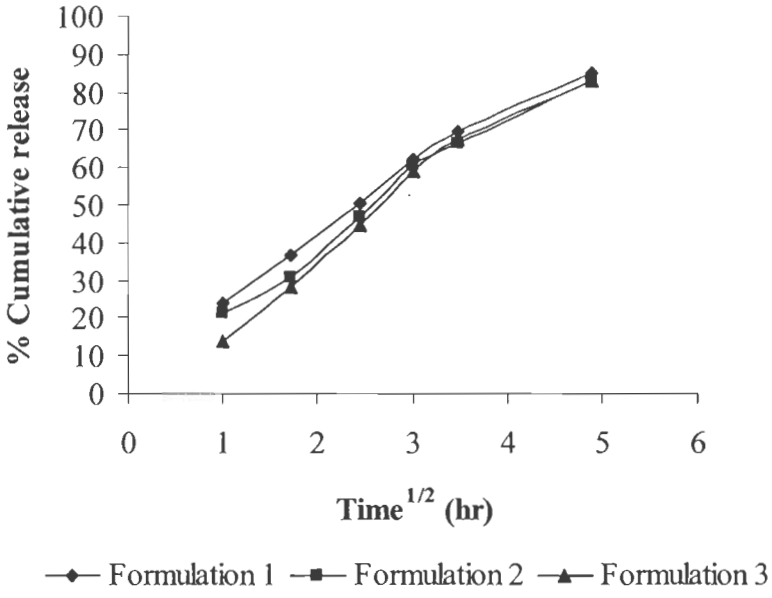



Figure 90 Higuchi plot of mangostin from monoglyceride-based drug delivery system containing triglyceride: monoglyceride: water in the ratio of 8:62:30

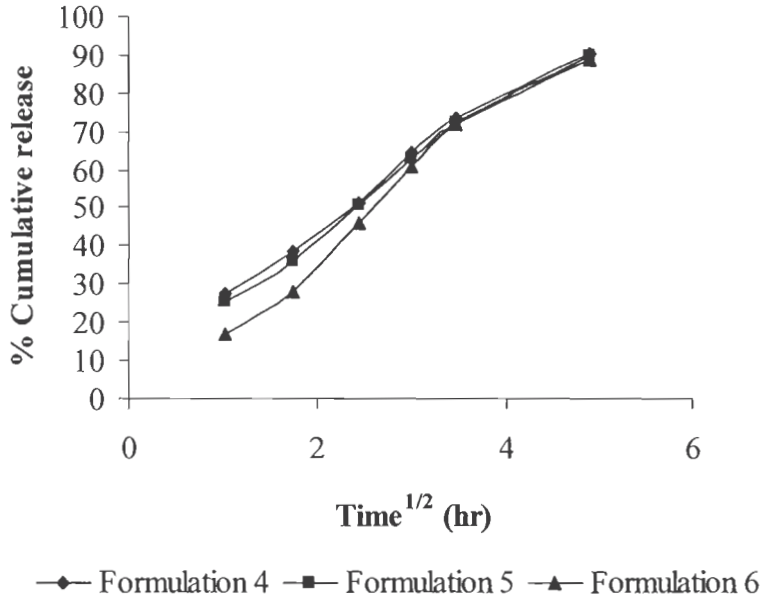



Figure 91 Higuchi plot of mangostin from monoglyceride-based drug delivery system containing triglyceride: monoglyceride: water in the ratio of 12:58:30

**5. Differential Scanning Calorimetric (DSC) Method**

DSC is a thermal analysis method for detecting changes in physical or chemical properties of materials as a function of temperature. The hydrocarbon chains of amphiphilic molecules are subjected to undergoing a transformation from an order (gel) state to a more disorder (liquid crystalline) state. These changes have been characterized by DSC which requires the input of additional thermal energy show up as endothermic peaks on heating.

In the temperature range of -20 to 60 °C, DSC thermograms of liquid crystalline state showed broad endothermic peaks at temperature range of -10 to 0 °C (Figures 73-74). Both samples with and without mangostin showed similarly in pattern of the peaks and temperature range. No other endothermic peak was observed within the studied temperature range. The presence of these peaks was possible that a phase transition occurred. In this study, DSC thermograms presented two endothermic peaks which might be correspond to the gel to liquid crystal transition and the pretransition peaks, which was due to a rearrangement of the molecules in the structure (Koyama et al., 1999). Chang and Bodmeier (1996) reported that the phase transitions of glyceryl monooleate-water mixtures were below room temperature. This suggested that the liquid crystalline phase should remain physically stable in the studied temperature range without phase transformation. Therefore, they could be stored at room temperature. In addition, the study showed the transition temperature decreased from 25 °C to 7 °C when the water content increased from 0% to 30%. Helledi and Schubert (2001) reported that no phase transition of the liquid crystalline phase from glyceryl monooleate was detected in the temperature range of 20 to 70 °C both samples with and without addition of drug. These results demonstrated that the drug can be incorporated into the liquid crystalline phase without causing phase transition.

In the temperature range of -20 to 250 °C, the DSC thermograms of liquid crystalline state both formulations with and without mangostin showed endothermic peaks at temperature range of 123.94 to 133.45 °C (Figures 52-57), while the DSC thermogram of pure compounds used in this study (Figure 49) did not present the peaks in that temperature range. The presence of these peaks was possible that the water was desolvated from the bound water in liquid crystalline state. In the samples containing mangostin, the melting peak of mangostin in the temperature range of 179 to 180 °C as described in Figure 23 was not found. The possible explanation is that mangostin was incorporated into the liquid crystal structures and could not be detected.

In conclusion, the liquid crystalline phase remained physically stable in the studied temperature range without phase transformation. In addition, mangostin does not influence the transition behavior and can be incorporated into the liquid crystalline phase without causing phase transition.

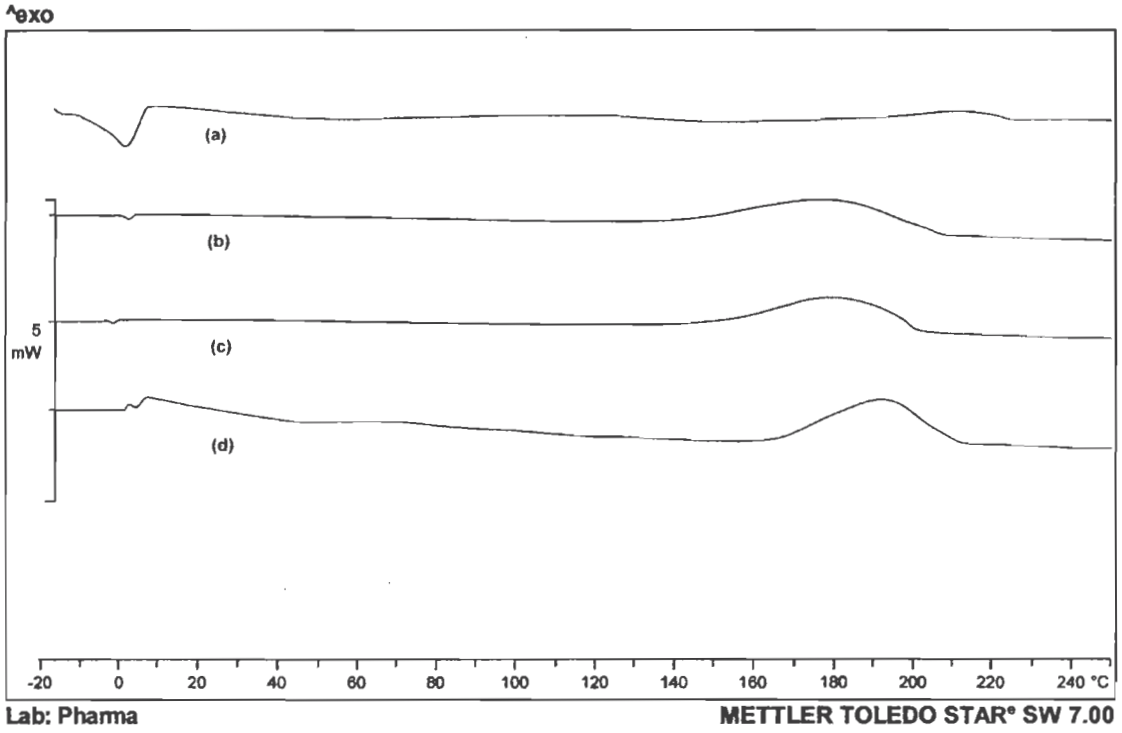



Figure 92 DSC thermogram of pure compounds in the temperature range of -20 to 250 °C; (a) glyceryl monooleate (sample weight 4.36 mg); (b) sesame oil (sample weight 3.76 mg); (c) soybean oil (sample weight 3.34 mg); and (d) olive oil (sample weight 3.58 mg)

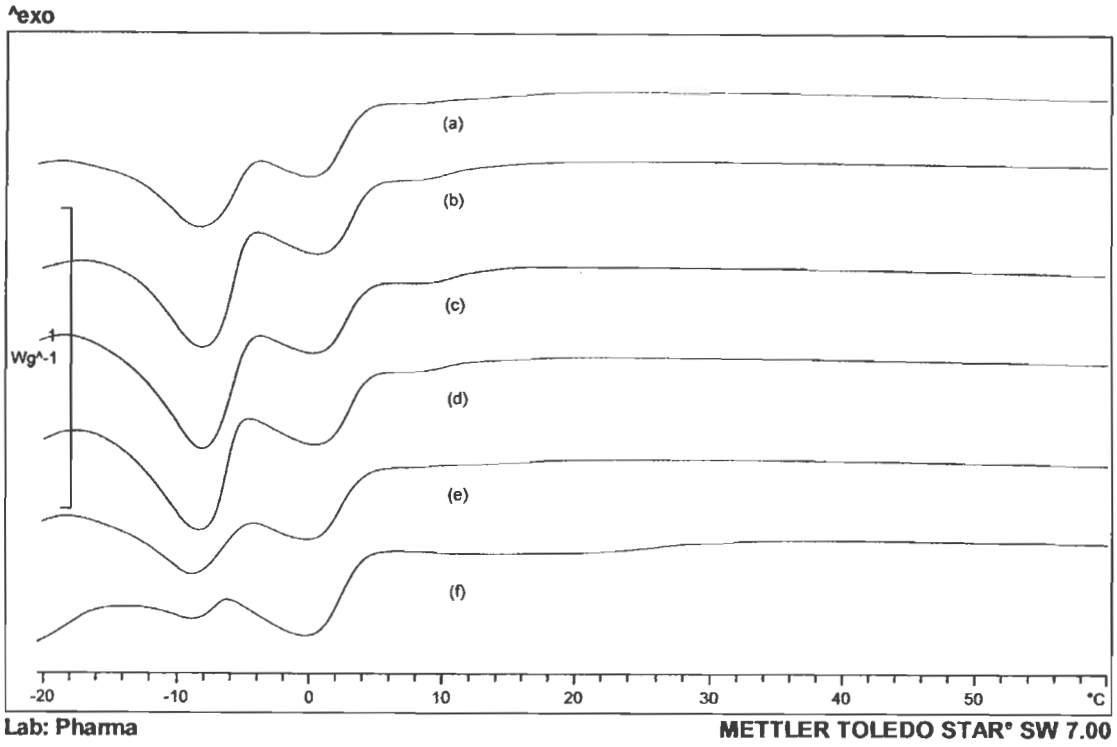



Figure 93 DSC thermogram of formulation 1-3 in the temperature range of -20 to 60 °C; (a) formulation 1 with mangostin (sample weight 4.00 mg); (b) formulation 1 without mangostin (sample weight 4.04 mg); (c) formulation 2 with mangostin (sample weight 4.12 mg); (d) formulation 2 without mangostin (sample weight 4.11 mg); (e) formulation 3 with mangostin (sample weight 4.08 mg); and (f) formulation 3 without mangostin (sample weight 4.20 mg)

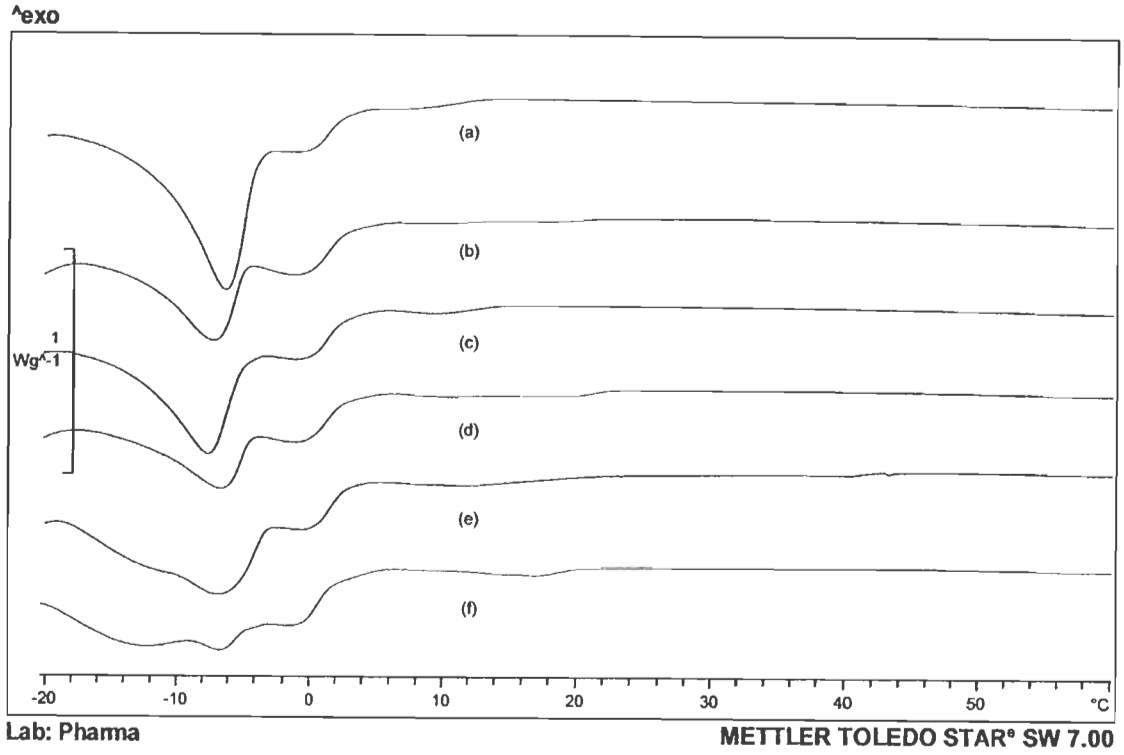



Figure 94 DSC thermogram of formulation 4-6 in the temperature range of -20 to 60 °C; (a) formulation 4 with mangostin (sample weight 4.04 mg); (b) formulation 4 without mangostin (sample weight 4.03 mg); (c) formulation 5 with mangostin (sample weight 4.17 mg); (d) formulation 5 without mangostin (sample weight 4.12 mg); (e) formulation 6 with mangostin (sample weight 4.07 mg); and (f) formulation 6 without mangostin (sample weight 4.08 mg)

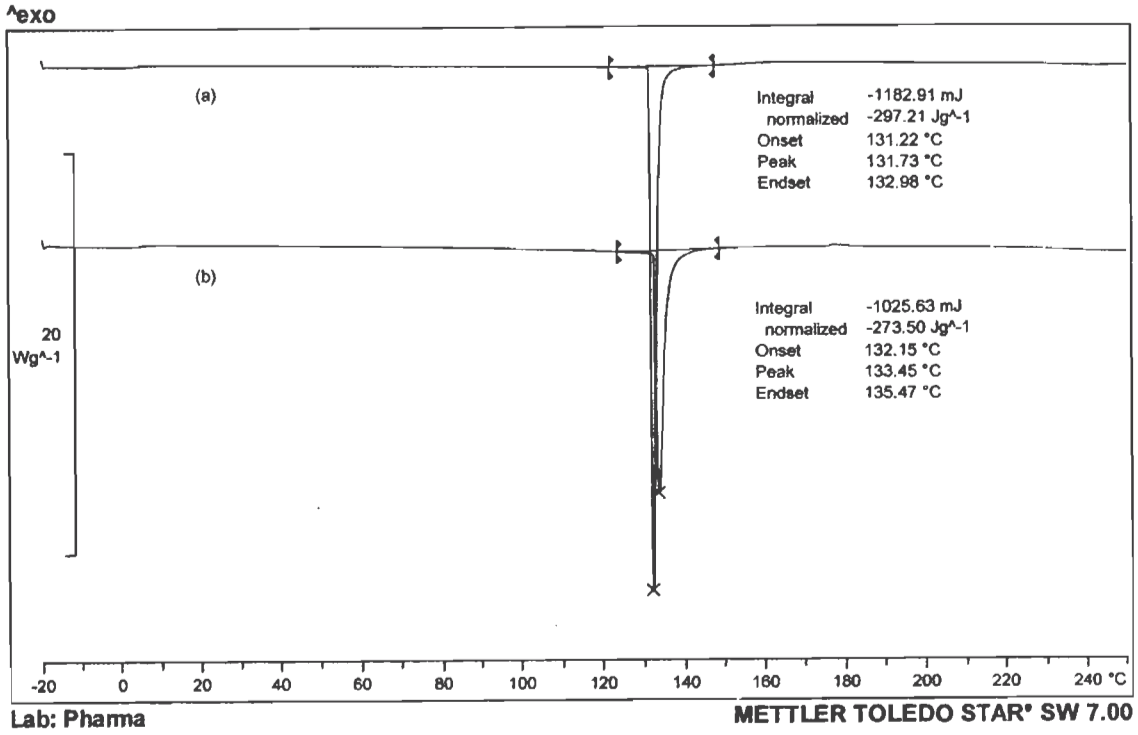



Figure 95 DSC thermogram of formulation 1 in the temperature range of -20 to 250 °C; (a) formulation 1 with mangostin (sample weight 4.00 mg); and (b) formulation 1 without mangostin (sample weight 4.03 mg)

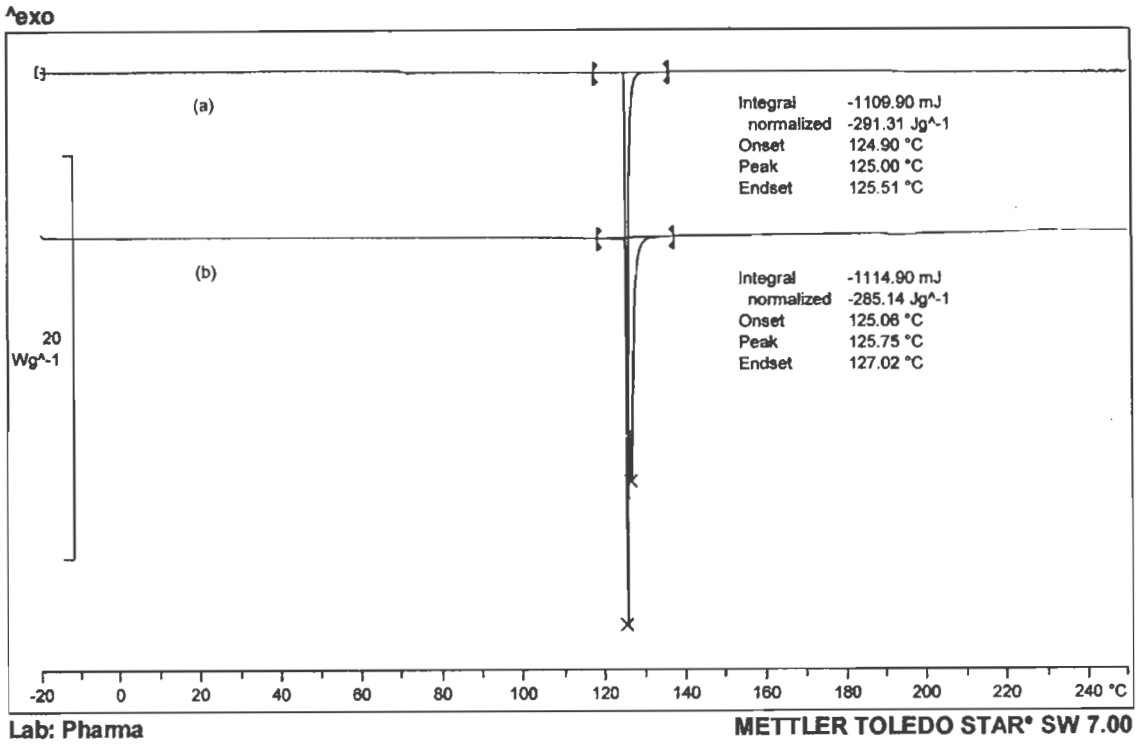



Figure 96 DSC thermogram of formulation 2 in the temperature range of -20 to 250 °C; (a) formulation 2 with mangostin (sample weight 3.81 mg); and (b) formulation 2 without mangostin (sample weight 3.91 mg)

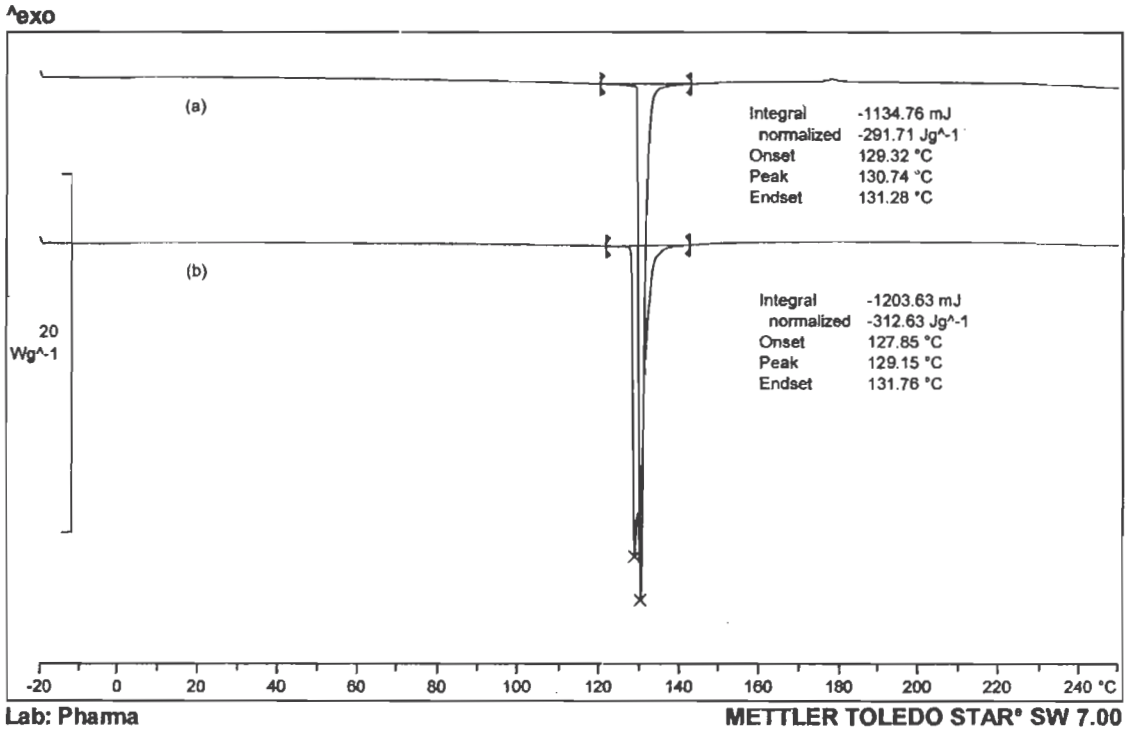



Figure 97 DSC thermogram of formulation 3 in the temperature range of -20 to 250 °C; (a) formulation 3 with mangostin (sample weight 3.89 mg); and (b) formulation 3 without mangostin (sample weight 3.85 mg)

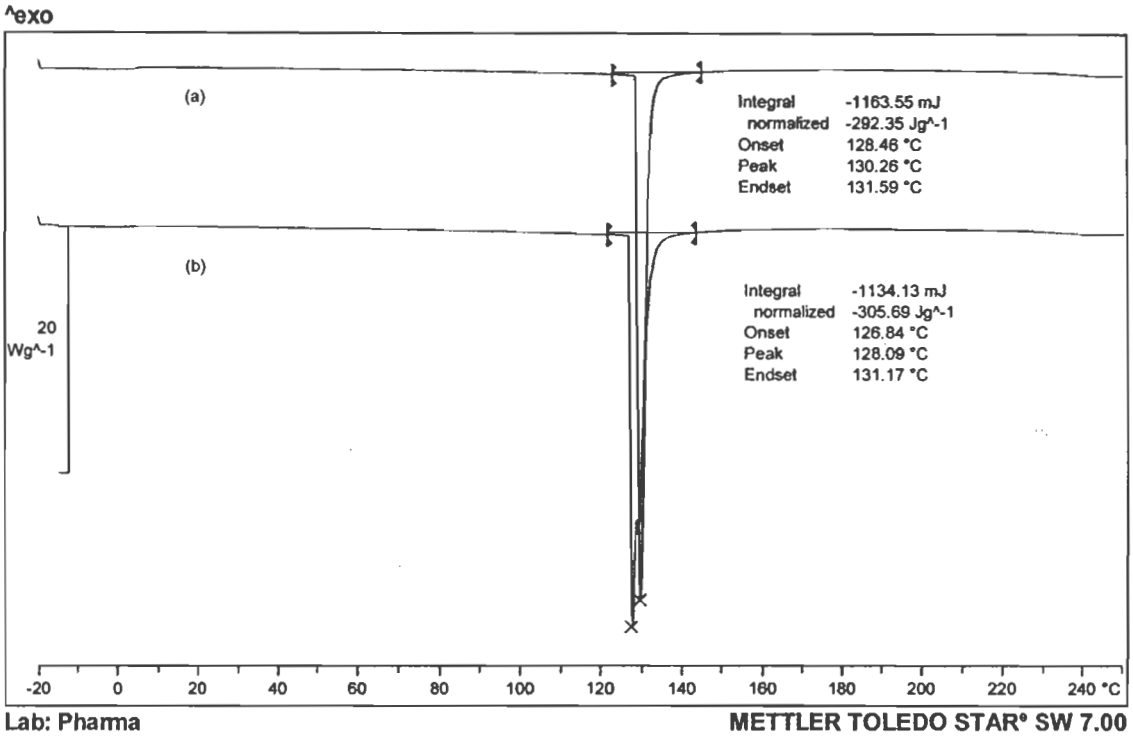



Figure 98 DSC thermogram of formulation 4 in the temperature range of -20 to 250 °C; (a) formulation 4 with mangostin (sample weight 3.71 mg); and (b) formulation 4 without mangostin (sample weight 3.98 mg)

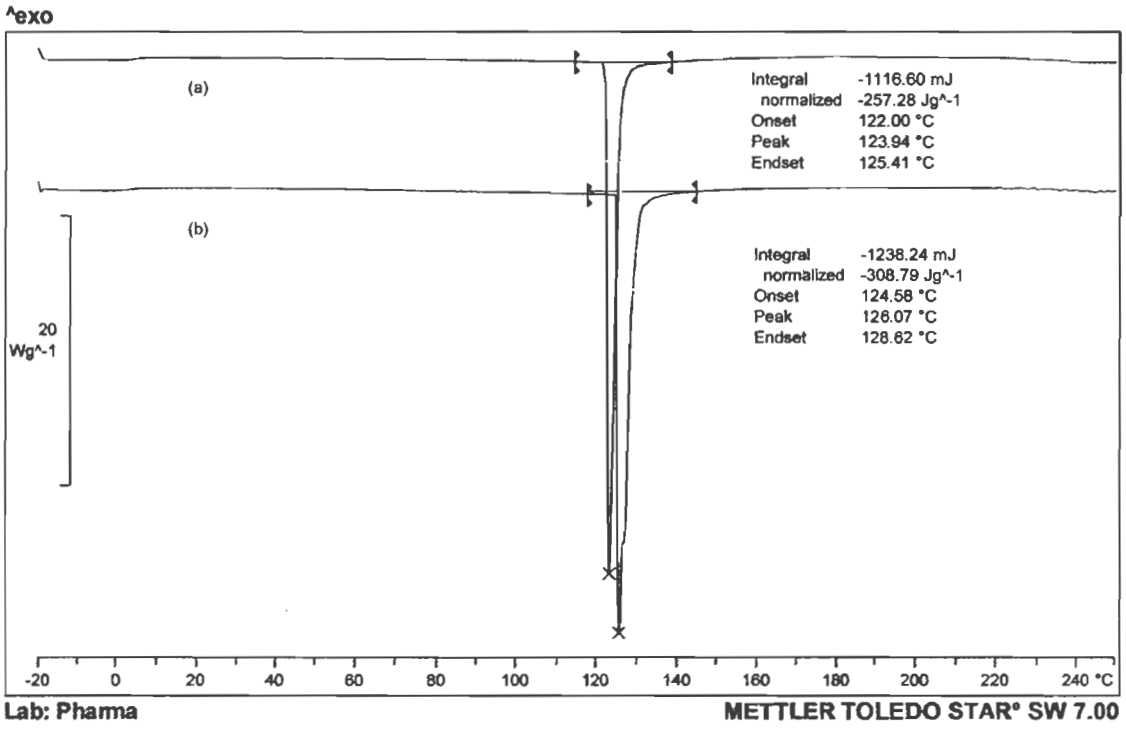



Figure 99 DSC thermogram of formulation 5 in the temperature range of -20 to 250 °C; (a) formulation 5 with mangostin (sample weight 3.90 mg); and (b) formulation 5 without mangostin (sample weight 4.01 mg)

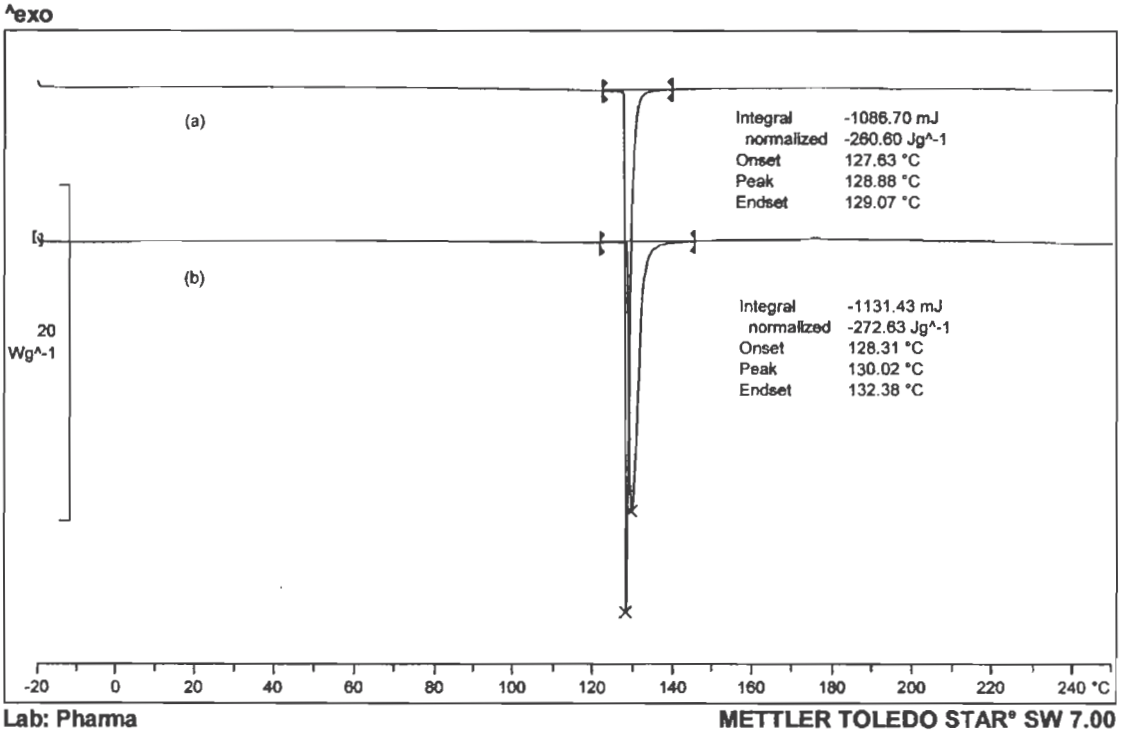



Figure 100 DSC thermogram of formulation 6 in the temperature range of -20 to 250 °C; (a) formulation 6 with mangostin (sample weight 4.17 mg); and (b) formulation 6 without mangostin (sample weight 4.15 mg)

**6. Determination of Antimicrobial Activity of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

It is well documented that dental caries and the progression to periodontal diseases begin with an accumulation of bacteria in dental plaque. The main microorganism linked with dental plaque seems to be *Streptococcus mutans*.

From previous unpublished data performed by Torrungruang, K., and Vichienroj, P., Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, it was found that *Garcinia mangostana* extract derived from this study exhibited antimicrobial activity against *Streptococcus mutans* $KPSK_2$ and *Porphyromonas gingivalis* W50, the microbial species associated with dental caries and periodontal diseases, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Streptococcus mutans* $KPSK_2$ and *Porphyromonas gingivalis* W50 are shown in Table 22.

Table 44 MIC and MBC of *Garcinia mangostana* extract on *Streptococcus mutans* $KPSK_2$ and *Porphyromonas gingivalis* W50

| Types of bacteria | MIC (µg/ml) | MBC (µg/ml) |
|---|---|---|
| *Streptococcus mutans* $KPSK_2$ | 0.625 | 1.250 |
| *Porphyromonas gingivalis* W50 | 20 | 40 |

The agar diffusion method is the method for determining the antimicrobial susceptibility pattern of a bacterial strain. A filter paper disk, a hole, a porous cup or an open-ended cylinder containing measured quantities of drug is placed on a solid medium that has been heavily seeded with the test organisms. In this study, the antimicrobial activity against *Streptococcus mutans* $KPSK_2$ was examined by using a hole for placed the samples. When bacterial multiplication proceeds more rapidly than the drug can diffuse, the bacterial cells that are not inhibited by the antimicrobial will continue to multiply until a lawn of growth is visible and no zone of inhibition appears around the hole. When the antimicrobial is present in inhibitory concentrations, no growth will appear in the zone around the hole.

As a result, all formulations with and without mangostin showed no inhibition zone. The result obtained was quite unexpected. The question was whether mangostin extract had lost the antimicrobial activity or mangostin extract could not release from the formulation. Therefore, mangostin extract was tested for antimicrobial activity against *Streptococcus mutans* $KPSK_2$ by agar diffusion method using the same condition. Two percent of mangostin extract in 95% ethanol, equivalent to the formulations, was used in this study. The result showed that mangostin had an inhibition zone with a diameter of $15.33\pm0.29$ mm, which indicated that mangostin had an antimicrobial activity against *Streptococcus mutans* $KPSK_2$. From visual inspection, all formulations with and without mangostin could not diffuse into the surrounding medium, they still contained in the hole. In contrast, mangostin extract in 95% ethanol diffused into the medium and presented an empty hole with inhibition zone. The probable explanation is that these formulations are oily semi-solid, which is immiscible to aqueous solid medium. Since, mangostin extract is lipophilicity (from the following study) therefore it likes the more lipophilic region and does not diffuse into the medium. To solve this problem, the formulation with more hydrophilicity should be prepared. Increasing the water content in the formulation can increase the hydrophilicity. However, the result from the previous study indicated that the higher water content than 30% could not make the high-viscous liquid crystalline phase, it appears to be two-phase separation. Therefore, another aqueous formulation preparing from poloxamer 407 was used in this study.

Poloxamer 407 is a series of closely related block copolymers of ethylene oxide and propylene oxide. Poloxamer 407 displays a temperature-induced thickening and acting as an *in situ* forming drug delivery system. In this study, 2% mangostin was incorporated into poloxamer 407 and evaluated for antimicrobial activity against *Streptococcus mutans* $KPSK_2$ comparing to blank formulation, mangostin extract, 95% ethanol and 0.2% chlorhexidine.

After incubation, areas of inhibition zone were presented in all tested compounds except blank formulation. The diameters of inhibition zone are shown in Table 45. It was found that 0.2% chlorhexidine had the most inhibitory effect on *Streptococcus mutans* $KPSK_2$ and mangostin extract had a slightly higher effect than the formulation. While 95%

ethanol had a little effect on the antimicrobial activity which indicated that the inhibitory effect of mangostin came from itself. Blank formulation did not show zone of inhibition which meant there was no antimicrobial activity of poloxamer 407. These results indicated that mangostin incorporated into the formulation can diffuse into the medium at sufficient concentration for inhibition of the bacterial growth.

Table 45 Inhibition zone diameters of the test compounds on *Streptococcus mutans* $KPSK_2$

| Test compounds | Inhibition zone diameter (mm) |
|---|---|
| Monoglyceride-based drug delivery system with mangostin | no |
| Monoglyceride-based drug delivery system without mangostin | no |
| Poloxamer 407 formulation with mangostin | 13.33±0.29 |
| Poloxamer 407 formulation without mangostin | no |
| Mangostin extract in 95% ethanol | 15.33±0.29 |
| 0.2% chlorhexidine | 24.00±0.00 |
| 95% ethanol | 6.75±0.25 |

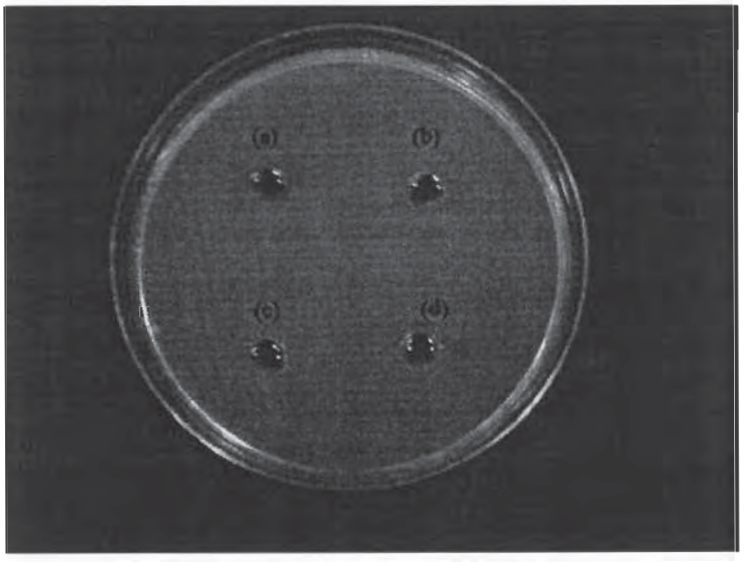



Figure 101 Agar diffusion test of monoglyceride-based drug delivery system; (a) and (b) monoglyceride-based drug delivery system with mangostin; (c) and (d) monoglyceride-based drug delivery system without mangostin

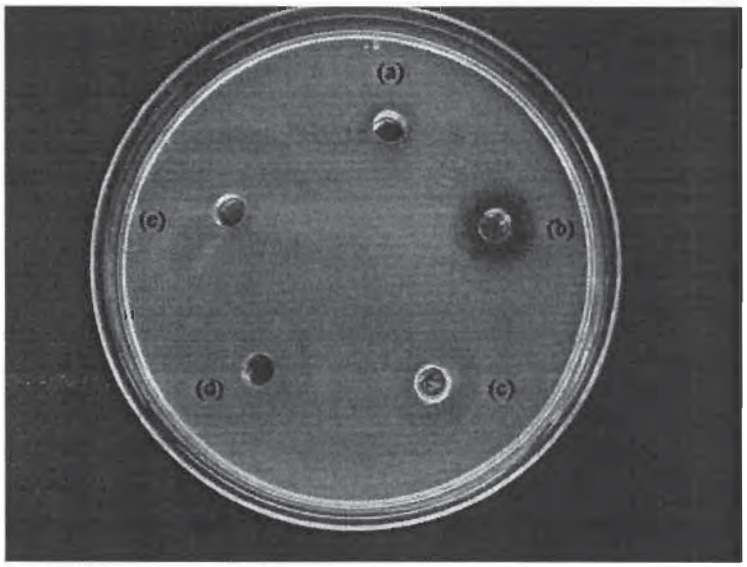



Figure 102 Agar diffusion test of various compounds; (a) poloxamer 407 formulation without mangostin; (b) poloxamer 407 formulation with mangostin; (c) mangostin extract; (d) 95% ethanol; and (e) 0.2% chlorhexidine

### 7. Determination of Partition Coefficient of *Garcinia mangostana* Extract

The lipophilicity of an organic compound is usually described in terms of a partition coefficient (P), which can be defined as the ratio of the equilibrium concentrations of a dissolved substance between organic and aqueous phases. There are many different techniques which can be used to solve this problem. The most common method is the shake flask method, the thorough mixing of the two phases followed by their separation in order to determine the equilibrium concentration for the substance being examined. The n-octanol-water system has been widely adopted as a model of the lipid phase. Lipophilic species dissolve in the aliphatic regions of the octanol, whilst hydrophilic species are drawn to the polar regions. The partition coefficient is usually given in a logarithmic scale, therefore, a log P = 0 means that the compound is equally soluble in water and in the partitioning solvent. If the compound has a log P = 5, then the compound is 100,000 times more soluble in the partitioning solvent. A log P = -2 means that the compound is 100 times more soluble in water, i.e., it is quite hydrophilic.

In this study, the determination of partition coefficient was performed by the shake flask method. *Garcinia mangostana* extract was shaken between n-octanol and water layers, and then the adequate volume was withdrawn and analyzed by using UV spectrophotometry. As a result, the concentrations of mangostin in n-octanol and water phases were 4.7547 $\pm$ 0.3031 mg/ml and 0.0038 $\pm$ 0.0006 mg/ml, respectively. The partition coefficient is the quotient of two concentrations and is given in the form of its logarithm to base ten (log P). Therefore, the partition coefficient and log P of mangostin were 1251.24 and 3.10, respectively. These data indicated that the compound was 1251.24 times more soluble in n-octanol than in water and it was quite lipophilic.

From the high partition coefficient of mangostin obtained from this study, it confirmed the result of the agar diffusion method (topic E6). Mangostin is highly lipophilic that the diffusion into the aqueous solid medium is negligible. Thus, the antimicrobial activity of monoglyceride-based drug delivery system with mangostin could not be demonstrated.

**L. Stability Study of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System**

The stability study of *Garcinia mangostana* extract in monoglyceride-based drug delivery systems was performed by the stress condition using the heating-cooling cycle. The physicochemical properties and the amount of mangostin were determined at the initial time and after the stability study. The analytical method which employed in this investigation was the HPLC method as previously described.

As a result by visual inspection, the color change, phase separation and precipitation of all formulations were not observed after the heating-cooling cycle. The pH of formulations before and after the heating-cooling cycle were similar in the range of 5.64-5.85 and 5.70-5.83, respectively. The viscosity values of formulations were slightly decreased compared to those before the heat-cooling cycle. The polarizing microscopic images at the initial time and after the stability study were not different (Figures 84-85).

Table 46 pH and viscosity of formulation before and after stability study

| Formulation | Before stability study | | After stability study | |
|---|---|---|---|---|
| | pH | Viscosity (cps) | pH | Viscosity (cps) |
| 1 | 5.78 | 5203.66 | 5.83 | 5055.42 |
| 2 | 5.79 | 5262.52 | 5.81 | 5007.46 |
| 3 | 5.64 | 5109.92 | 5.70 | 4944.24 |
| 4 | 5.85 | 4937.70 | 5.83 | 4796.00 |
| 5 | 5.81 | 4933.34 | 5.81 | 4743.68 |
| 6 | 5.64 | 4830.88 | 5.74 | 4691.36 |

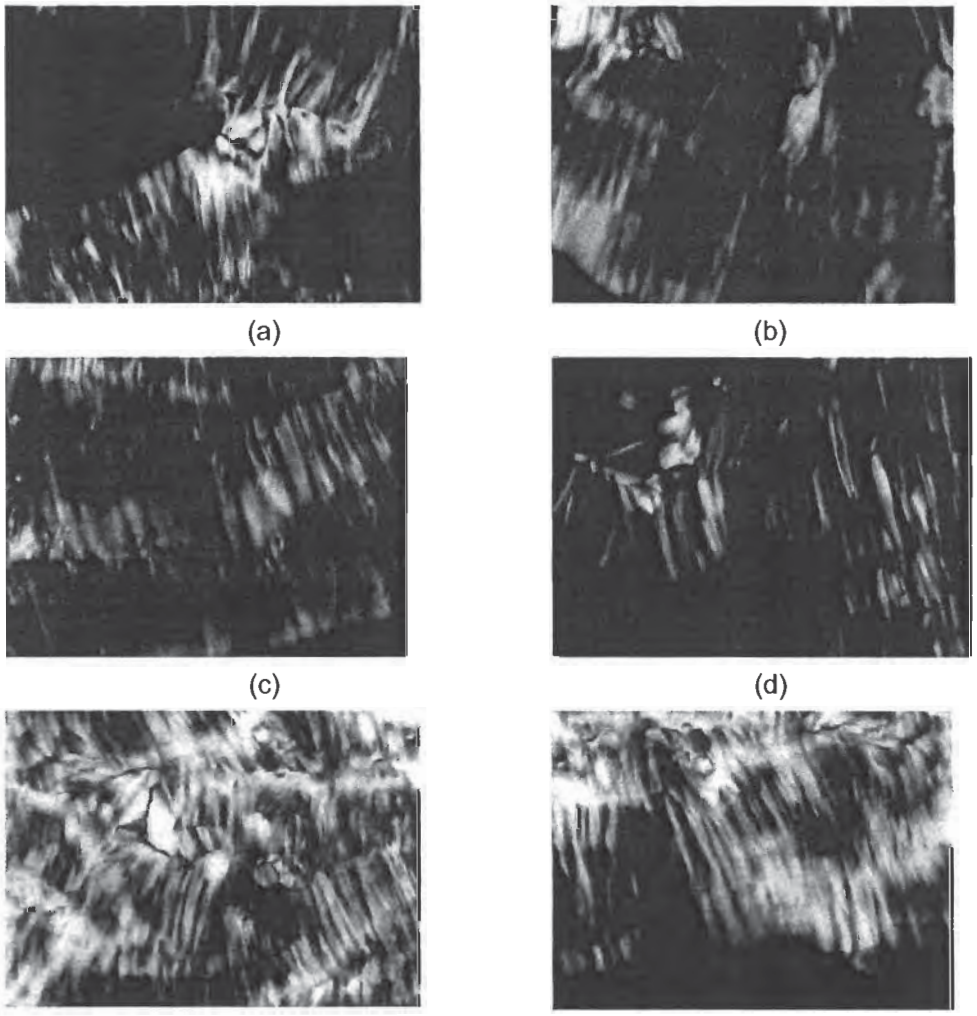



Figure 103 Polarizing microscopic images of formulation 1-3 before and after the stability study; (a) and (b) formulation 1; (c) and (d) formulation 2; and (e) and (f) formulation 3, as observed at ×100 magnification

Since these formulations were expected to act as an *in situ* forming drug delivery system, therefore the low-viscous state formulations should be considered. In this study, the formulations both low-viscous state and high-viscous liquid crystalline phase were analyzed the amount of mangostin and calculated the percent remaining and percent loss of mangostin in monoglyceride-based drug delivery systems (Tables 25-26). Analysis of data indicated that the amount of mangostin in the formulations before and after the stability study were significantly different ($P<0.05$) in both low-viscous state and high-viscous liquid crystalline phase, however all formulations appeared to be stable due to their percentage loss after storage was less than 10% of the initial value. In addition, there was no statistically significant difference ($P>0.05$) in amount of mangostin remaining between six formulations both low-viscous state and high-viscous liquid crystalline phase.

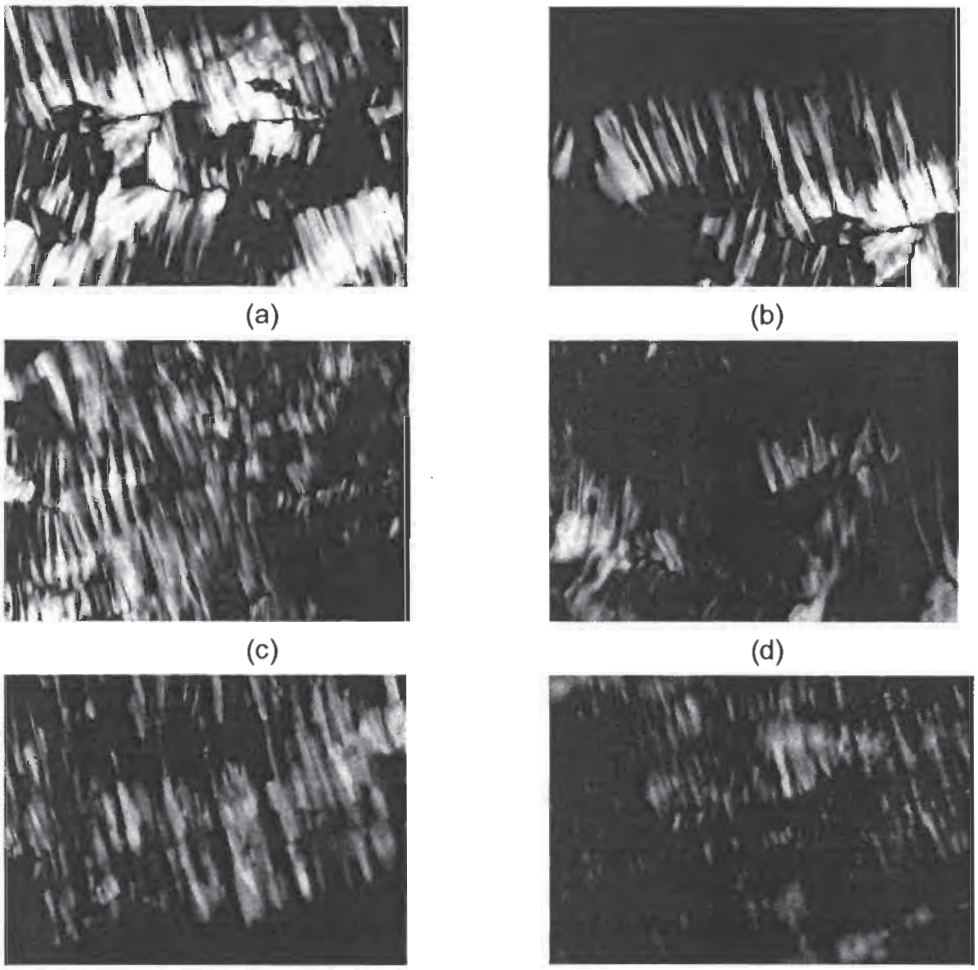



Figure 104 Polarizing microscopic images of formulation 4-6 before and after the stability study; (a) and (b) formulation 4; (c) and (d) formulation 5; and (e) and (f) formulation 6, as observed at ×100 magnification

Table 47 Amount of mangostin in low-viscous state formulation before and after stability study

| Formulation | Amount of mangostin (mg) | | Percent remaining of mangostin | Percent loss of mangostin |
|---|---|---|---|---|
| | Before | After | | |
| 1 | 19.92±0.24 | 19.68±0.28 | 98.80±1.39 | 1.20 |
| 2 | 19.93±0.19 | 19.66±0.15 | 98.65±0.73 | 1.35 |
| 3 | 20.02±0.26 | 19.78±0.16 | 98.81±0.82 | 1.19 |
| 4 | 19.85±0.29 | 19.65±0.21 | 99.00±1.07 | 1.00 |
| 5 | 20.01±0.20 | 19.71±0.18 | 98.48±0.92 | 1.52 |
| 6 | 19.95±0.25 | 19.78±0.23 | 99.18±1.15 | 0.82 |

Table 48 Amount of mangostin in high-viscous liquid crystalline phase formulation before and after stability study

| Formulation | Amount of mangostin (mg) | | Percent remaining of mangostin | Percent loss of mangostin |
|---|---|---|---|---|
| | Before | After | | |
| 1 | 20.01±0.17 | 19.73±0.19 | 98.59±0.95 | 1.41 |
| 2 | 19.93±0.24 | 19.68±0.35 | 98.73±1.76 | 1.27 |
| 3 | 19.98±0.20 | 19.67±0.50 | 98.45±2.48 | 1.55 |
| 4 | 19.97±0.30 | 19.67±0.47 | 98.54±2.33 | 1.46 |
| 5 | 19.90±0.35 | 19.73±0.55 | 99.12±2.77 | 0.88 |
| 6 | 20.03±0.42 | 19.60±0.47 | 97.81±2.33 | 2.19 |

# CHAPTER V

# CONCLUSIONS

## PART I: Extraction, Identification, Quanlitative and Quantitative Analysis And Antibacterial Activity of *Garcinia mangostana* Husk Extract

1. The crude extract of *Garcinia mangostana* could be prepared by maceration of the dried fruit hull powder with ethyl acetate as yellow crystalline powder of mangostin. The yield was calculated as 7.47%.

2. The identification of mangostin was carried out by TLC and DSC. The TLC qualitative analysis of mangostin was done by using the alumina sheet and ethyl acetate: hexane (3:1) as mobile phase. The Rf value as compared with standard mangostin was 0.60. The DSC thermogram showed the melting point of the crude extract at 165.04-166.80 $^{o}$C.

3. The quantitative analyses of mangostin were developed using the HPLC and UV spectrophotometric methods. The HPLC system using methanol: water (87:13) as mobile phase, clotrimazole as internal standard and using UV detector at 243 nm. The UV spectrophotometric method was carried out using the UV spectrophotometer at 243 nm. The validation of both systems gave high specificity, linearity, accuracy and precision.

4. The solubility study of mangostin showed the low water insolubility. The water solubility was improving with increasing ethanol content.

5. The microbiological activity of mangostin was studied. The MIC of the extract 3 μg/ml for *Staphylococcus aureus* ATCC 25923 and 1.5 μg/ml for *Streptococcus mutans* ATCC $KPSK_2$. The MBC of the extract was 4 μg/ml for *Staphylococcus aureus* ATCC 25923 and 3 μg/ml for *Streptococcus mutans* ATCC $KPSK_2$

## PART II Formulation of fast dissolving oral strips containing *Garcinia mangostana* husk exract

The fast dissolving films could open a new horizon in drug delivery systems. These thin and flexible films rapidly dissolved in oral cavity for reasonable improved patient compliance and convenience for use all the times without need of water.

In this study, orally fast dissolving films containing *Garcinia mangostana* husk extract were developed using by solvent casting method. The effect of type and ratio of polymer on mechanical properties, dissolution time and antimicrobial activity of formulations were investigated. The results of this study can be concluded as follows:

1. The yellow powder of *Garcinia mangostana* ectract was obtained by macerating method with ethyl acetate. The quantititive analysis of mangostin in extract by HPLC method was 55.86% w/w.

2. *Garcinia mangostana* extract showed antimicrobial activity against *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* $KPSK_2$ with the minimum inhibitory concentration

(MIC) of 3 and 1.5 μg/ml, respectively, and minimum bactericidal concentration (MBC) of 4 and 3 μg/ml, respectively.

3. From the results of mechanical properties of film bases, it was found that HPC LV gave the most soft and weak film and combination with HPMC in various ratios provided the stronger film. When increased HPMC 3 cps ratio in the combined polymers of HPMC 3 and 5 cps showed markedly decreasing the tensile strength, percentage of elongation and work of failure. It indicated that these films were more brittle and softer than HPMC 5 cps films alone. The effect of plasticizers on mechanical properties of HPMC 3 cps films were also investigated. It was found that glycerin increased toughness of the film, while combination of three plasticizers, PEG 400, PG and glycerin made the film too soft, moist and easy to break off.

4. The dissolution time of all film bases were observed. The results showed that the films with combination of HPMC 3 cps and HPC LV at all ratios had no statistically significant difference from a commercial product strips A ($p$>0.05). Therefore, these formulations were developed to the fast dissolving film containing *Garcinia mangostana* extract.

5. The study of surface morphology using by scanning electron microscope (SEM) and physicochemical properties by differential scanning calorimeter (DSC) of fast dissolving incorporated *Garcinia mangostana* extract found that the film with extract were more porous and rougher than its film bases. From DSC thermograms of film with extract, it was found that no peak of crystalline form of substances. This result indicated that the extract and other ingredients changed to either molecular dispersed or amorphous form.

6. According to the results of dissolution profiles, all formulations showed the rapid release of mangostin in simulated saliva fluid. About 80% of labeled amount of drug was released within 3-7 minutes. These revealed that the prepared fast dissolving films containing *Garcinia mangostana* extract had high efficacy of the films for rapid drug release.

7. Determination of antimicrobial activity of the films were performed and compared with commercial product strips A strips using agar diffusion method. The results found that film with extract exhibited antimicrobial effect against both *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* $KPSK_2$, while commercial product strips A strips did not show this effect.

8. The fast dissolving films in the presence of *Garcinia mangostana* extract were stable under stress condition (40 $^{o}$C, 75%RH) both chemical and physicochemical properties.

From the results obtained in this study, the fast dissolving films containing *Garcinia mangostana* extract that had *in vitro* antimicrobial activity against the oro-dental bacteria could be developed. The prepared films had good physical appearance and were stable. However, the further study of these films for *in vitro* killing time and *in vivo* antimicrobial activity is necessary. That will provide a useful information to develop these fast dissolving films to the commercialized product in the future.

## PART III Formulation of monoglyceride-based drug delivery systems (liquid crystalline) containing *Garcinia mangostana* husk extract

In this study monoglyceride-based drug delivery systems containing *Garcinia mangostana* extract were prepared. The effects of type and ratio of triglycerides on the ternary phase diagram, physicochemical properties and antimicrobial activity of the formulations were investigated. The results of this study can be concluded as follows:

1. *Garcinia mangostana* extract was obtained by maceration method with ethyl acetate. The melting point of the extract was conformed to the melting point of mangostin. In addition, TLC and HPLC chromatogram of the extract were similar to standard mangostin. Based on these data, the extract was corresponded to mangostin.

2. From the ternary phase diagram, glyceryl monooleate-sesame oil-water system showed more area of one-phase liquid crystals than systems containing soybean oil and olive oil; however, the differences were slight. The structures of liquid crystalline phases under polarized light microscope were not influenced from using different oils while the ratio of oils affected these liquid crystals.

3. Monoglyceride-based drug delivery systems could be prepared from the ratio of triglyceride: monoglyceride: water as 8:62:30 and 12:58:30 with good physical appearances. After incorporating *Garcinia mangostana* extract, the physicochemical properties were not changed.

4. Monoglyceride-based drug delivery systems have the potential of acting as *in situ* liquid crystalline forming drug delivery systems due to their viscosity which was initially low enough to be administered through the syringe with 23-gauge tip needle. The systems could be transformed to the high-viscous liquid crystalline phase upon contact with excess water.

5. From the release profiles, all of these systems could sustain the release of mangostin over a period of 48 hr. The release kinetics followed Higuchi's equation indicating that the release of mangostin from these systems was diffusion controlled.

6. Various oils showed similar release profiles but different in the percentages of drug release. The highest percentage of release was obtained from the system containing sesame oil, followed by soybean oil and olive oil, respectively. Increasing triglycerides content into the formulations did not affect the release of the delivery systems.

7. The DSC thermograms demonstrated that *Garcinia mangostana* extract can be incorporated into the liquid crystalline phase of monoglyceride-based drug delivery systems without causing phase transition.

8. Although, *Garcinia mangostana* extract exhibited antimicrobial activity against *Streptococcus mutans* $KPSK_2$, monoglyceride-based drug delivery systems containing the extract did not showed the clear inhibition zone in the agar diffusion method. The probable reason is that mangostin is a lipophilic compound and does not diffuse into the medium.

9. Monoglyceride-based drug delivery systems in the presence of *Garcinia mangostana* extract both low-viscous state and high-viscous liquid crystalline phase were stable under the heating-cooling cycle.

From this investigation, the stable formulation of monoglyceride-based drug delivery systems containing *Garcinia mangostana* extract could be developed. The *in situ* liquid crystalline phase could be formed from the injectable low viscosity formulation upon contact with water. Since this study used agar diffusion method for determination of antimicrobial activity, the solid agar medium could not simulate the condition of fluid in the oral cavity. Therefore, the further *in vivo* study should be performed to investigate the efficacy of monoglyceride-based drug delivery systems containing *Garcinia mangostana* extract.

## REFERENCES


Agarwal, R.K., Robinson, D.H., Maze, G.I., and Reinhardt, R.A. 1993. Development and characterization of tetracycline-poly(lactide/glycolide) films for the treatment of periodontitis. J. Control. Release 23(2): 137-146.

Ainamo, J., Lie, T., Ellingsen, B.H., Hansen, B.F., Johansson, L.A., Karring, T., Kisch, J., Paunio, K., and Stoltze, K. 1992. Clinical responses to subgingival application of a metronidazole 25% gel compared to the effect of subgingival scaling in adult periodontitis. J. Clin. Periodontol. 19(9): 723-729.

Ansel, H.C., Allen, L.V., and Popovich, N.G. 1999. Pharmaceutical dosage forms and drug delivery systems. 7th ed. Philadelphia: Lippincott Williams and Wilkins.

Asai, F., Tosa, H., Tanaka, T., and Iinuma, M. 1995. A xanthone from pericarps of *Garcinia mangostana*. Phytochemistry 39(4): 943-944.

Aulton, M.E., and Abdul-Razzak, M.H. 1981. The mechanical properties of hydroxypropylmethylcellulose films derived from aqueous systems: part 1 the influence of plasticisers. Drug Dev. Ind. Phar. 7(6): 649-668.

Balasubramanian, K., and Rajagopalan, K. 1988. Novel xanthones from Garcinia mangostana, structures of BR-xanthone-A and BR-xanthone-B. Phytochemistry 27(5): 1552-1554.

Barauskas, J., Švedaitė, I., Butkus, E., Razumas, V., Larsson, K., and Tiberg, F. 2005. Synthesis and aqueous phase behavior of 1-glyceryl monooleyl ether. Colloids Surf. B: Biointerfaces 41(1): 49-53.

Bollen, C.M.L., and Quirynen, M. 1996. Microbiological response to mechanical treatment in combination with adjunctive therapy. A review of the literature. J. Periodontol. 67(11): 1143-1158.

Borsadia, S.B., Halloran, D., and Osborne, J.L. 2004. Quick-dissolving films: a novel approach to drug delivery. http://www.drugdeliverytechnology.com.

Brittain, H.G. 1995. Physical characterization of pharmaceutical solids. New York: Marcel Dekker.

Bruneton, J. 1995. Pharmacognosy and phytochemistry medicinal plants. Paris: Lavoisier.

Budavari, S., ed. 2001. The Merck Index: An encyclopedia of chemicals, drugs, and biologicals. 13th ed. New Jersey: Merck & Co.

Burrows, R., Collett, J.H., and Attwood, D. 1994. The release of drugs from monoglyceride-water liquid crystalline phases. Int. J. Pharm. 111(3): 283-293.

Carstensen, J.T. 1990. Drug stability. New York: Marcel Dekker.

Cawson, R.A., and Odell, E.W. 2002. Cawson's essentials of oral pathology and oral medicine. Edinburgh: Churchill Livingstone

Chairungsrilerd, N., Furukawa, K., Ohta, T., Nozoe, S., and Ohizumi, Y. 1996a. Histaminergic and Serotonic receptor blocking substances from the medicinal plant *Garcinia mangostana*. Planta Med. 62(5): 471-472.

Chairungsrilerd, N., Furukawa, K., Ohta, T., Nozoe, S., and Ohizumi, Y. 1996b. Pharmacological properties of α-mangostin, a novel histamine $H_1$ receptor antagonist. Eur. J. Pharmacol. 314(3): 351-356.

Chairungsrilerd, N., Takeuchi, K., Ohizumi, Y., Nozoe, S., and Ohta, T. 1996. Mangostanol, a prenyl xanthone from *Garcinia mangostana*. Phytochemistry 43(5): 1099-1102.

Chanarat, P., Chanarat, N., Fujihara, M., and Nagumo, T. 1997. Immunopharmacological activity of polysaccharide from the pericarp of mangosteen garcinia: phagocytic intracellular killing activities. J. Med. Assoc. Thai. 80(1): S149-154.

Chang, C.M., and Bodmeier, R. 1997. Swelling of and drug release from monoglyceride-based drug delivery systems. J. Pharm. Sci. 86(6): 747-752.

Chang, C.M., and Bodmeier, R. 1998. Low viscosity monoglyceride-based drug delivery systems transforming into a highly viscous cubic phase. Int. J. Pharm. 173(1-2): 51-60.

Chen, S.X., Wan, M., and Loh, B.N. 1996. Active constituents against HIV-1 protease from *Garcinia mangostana*. Planta Med. 62(4): 381-382.

Chiang, L.C., Cheng, H.Y., Liu, M.C., Chiang, W., and Lin, C.C. 2004. In vitro evaluation of antileukemic activity of 17 commonly used fruits and vegetables in Taiwan. Lebensm.-Wiss. u.-Technol. 37(5): 539-544.

Costa, P., Lobo, J.M.S. 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13: 123-133.

Deasy, P.B., Collins, A.E.M., Maccarthy, D.J., and Russell, R.J. 1989. Use of strips containing tetracycline hydrochloride or metronidazole for the treatment of advanced periodontal disease. J. Pharm. Pharmacol. 41(10): 694-699.

Denyer, S.P., Hodges, N.A., and Gorman, S.P. 2004. Hugo & Russell's pharmaceutical microbiology. 7th ed. Oxford: Blackwell Publishing.

Drisko, C.L., Cobb, C.M., Killoy, W.J., Michalowicz, B.S., Pihlstrom, B.L., Lowenguth, R.A., Caton, J.G., Encarnacion, M., Knowles, M., and Goodson, J.M. 1995. Evaluation of periodontal treatments using controlled-release tetracycline fibers: Clinical response. J. Periodontol. 66(8): 692-699.

Eggert, F.M., Drewell, L., Bigelow, J.A., Speck, J.E., and Goldner, M. 1991. The pH of gingival crevices and periodontal pockets in children, teenagers and adults. Archs. oral Biol. 36(3): 233-238.

Engström, S., and Engström, L. 1992. Phase behavior of the lidocaine-monoolein-water system. Int. J. Pharm. 79(1-3): 113-122.

Esposito, E., Carotta, V., Scabbia, A., Trombelli, L., D'Antona, P., Menegatti, E., and Nastruzzi, C. 1996. Comparative analysis of tetracycline-containing dental gels: poloxamer- and monoglyceride-based formulations. Int. J. Pharm. 142(1): 9-23.

Farnsworth, N.R. and Bunyapraphatsara, N. 1992. Thai medicinal plants. Bangkok: Medicinal Plant Information Center, Faculty of Pharmacy, Mahidol University.

Fejerskov, O., and Kidd, E. 2003. Dental caries: The disease and its clinical management. Malden, MA: Blackwell Munksgaard.

Ganem-Quintanar, A., Quintanar-Guerrero, D., and Buri, P. 2000. Monoolein: A review of the pharmaceutical applications. Drug Dev. Ind. Pharm. 26(8): 809-820.

Garrett, S., Adams, D.F., Bogle, G., Donly, K., Drisko, C.H., Hallmon, W.W., Hancock, E.B., Hanes, P., Hawley, C.E., Johnson, L., Kiger, R., Killoy, W., Mellonig, J.T., Raab, F.J., Ryder, M., Stoller, N., Polson, A., Wang, H.L., Wolinsky, L.E., Yukna, R.A., Harrold, C.Q., Hill, M., Johnson, V.B., and Southard, G.L. 2000. The effect of locally delivered controlled-release doxycycline or scaling and root planing on periodontal maintenance patients over 9 months. J. Periodontol. 71(1): 22-30.

Garrett, S., Johnson, L., Drisko, C.H., Adams, D.F., Bandt, C., Beiswanger, B., Bogle, G., Donly, K., Hallmon, W.W., Hancock, E.B., Hanes, P., Hawley, C.E., Kiger, R., Killoy, W., Mellonig, J.T., Polson, A., Raab, F.J., Ryder, M., Stoller, N.H., Wang, H.L., Wolinsky, L.E., Evans, G.H., Harrold, C.Q., Arnold, R.M., and Southard, G.L. 1999. Two multi-center studies evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene, and scaling and root planing in the treatment of periodontitis. J. Periodontol. 70(5): 490-503.

Geraghty, P.B., Attwood, D., Collett, J.H., and Dandiker, Y. 1996. The *in vitro* release of some antimuscarinic drugs from monoolein/water lyotropic liquid crystalline gels. Pharm. Res. 13(8): 1265-1271.

Goldmann, D., Janietz, D., Schmidt, C., and Wendorff, J.H. 2004. Columnar liquid crystalline phases through hydrogen bonding and nanoscale segregation. J. Mater. Chem. 14(10): 1521-1525.

Goodson, J.M., Haffajee, A., and Socransky, S.S. 1979. Periodontal therapy by local delivery of tetracycline. J. Clin. Periodontol. 6(2): 83-92.

Goodson, J.M., Holborow, D., Dunn, R.L., Hogan, P., and Dunham, S. 1983. Monolithic tetracycline-containing fibers for controlled delivery to periodontal pockets. J. Periodontol. 54(10): 575-579.

Gopalakrishnan, G., and Balaganesan, B. 2000. Two novel xanthones from *Garcinia mangostana*. Fitoterapia 71(5): 607-609.

Gopalakrishnan, G., Banumathi, B., and Suresh, G. 1997. Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. J. Nat. Prod. 60(5): 519-524.

Gorbach, S.L., Bartlett, J.G., and Blacklow, N.R. 2004. Infectious diseases. 3rd ed. Philadelphia: Lippincott Williams and Wilkins.

Griffiths, G.S., Smart, G.J., Bulman, J.S., Weiss, G., Schrowder, J., and Newman, H.N. 2000. Comparison of clinical outcomes following treatment of chronic adult periodontitis with subgingival scaling or subgingival scaling plus metronidazole gel. J. Clin. Periodontol. 27(12): 910-917.

Gritsanapan, W., Rojanapanthu, P., Amornchat, C., Manakit, C., and Wiwego, C. (2002). Antibacterial activity of Thai herbal mouthwash. Proceedings of the 5th Scientific Conference of the Asian Societies of Cosmetic Scientists, 2001. Mahidol University Annual Research Abstracts 29: Abstract No. 510.

Haffajee, A.D., and Socransky, S.S. 1994. Microbial etiological agents of destructive periodontal diseases. Periodontol. 2000 5: 78-111.

Hamada, M., Iikubo, K., Ishikawa, Y., Ikeda, A., Umezawa, K., and Nishiyama, S. 2003. Biological activities of α-mangostin derivatives against acidic sphingomyelinase. Bioorganic and Medicinal Chemistry Letters 13:3151-3153.

Hatefi, A., and Amsden, B. 2002. Biodegradable injectable *in situ* forming drug delivery systems. J. Control. Release 80(1): 9-28.

Helledi, L.S., and Schubert, L. 2001. Release kinetics of acyclovir from a suspension of acyclovir incorporated in a cubic phase delivery system. Drug Dev. Ind. Pharm. 27(10): 1073-1081.

Higuchi, T. 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50(10): 874-875.

Higuchi, T. 1963. Mechanism of sustained-action medication. J. Pharm. Sci. 52(12): 1145-1149.

Hiranras, P. 2001. Formulation of *Garcinia mangostana* Linn. extract buccal mucoadhesive film. Master's Thesis, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Ho, C.K., Huang, Y.L., and Chen, C.C. 2002. Garcinone E, a xanthone derivative, has potent cytotoxic effect against hepatocellular carcinoma cell lines. Planta Med. 68(11): 975-979.

Huang, Y.L., Chen, C.C., Chen, Y.J., Huang, R.L., and Shieh, B.J. 2001. Three xanthones and a benzophenone from *Garcinia mangostana*. J. Nat. Prod. 64(7): 903-906.

Iikubo, K., Idshikawa, Y., Ando, N., Umezawa, K., and Nishiyama, S. 2002. The first direct synthesis of α-mangostin, apotent inhibitor of the acidic sphingomyelinase. Tetrahedron Letters 43: 291-293.

Iinuma, M., Tosa, H., Tanaka, T., Asai, F., Kobayashi, Y., Shimano, R., and Miyauchi, K. 1996. Antibacterial activity of xanthones from Guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. J. Pharm. Pharmacol. 48(8): 861-865.

Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K.H., and Demirci, F. 2002. Antimicrobial screening of Mentha piperita essential oils. J. Agric. Food Chem. 50(14): 3943-3946.

Jeffcoat, M.K., Bray, K.S., Ciancio, S.G., Dentino, A.R., Fine, D.H., Gordon, J.M., Gunsolley, J.C., Killoy, W.J., Lowenguth, R.A., Magnusson, N.I., Offenbacher, S., Palcanis, K.G., Proskin, H.M., Finkelman, R.D., and Flashner, M. 1998. Adjunctive use of a subgingival controlled-release chlorhexidine chip reduces probing depth and improves attachment level compared with scaling and root planing alone. J. Periodontol. 69(9): 989-997.

Jones, D.S., Woolfson, A.D., Brown, A.F., and O'Neill, M.J. 1997. Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: *in vitro* release kinetics, syringeability, mechanical and mucoadhesive properties. J. Control. Release 49(1): 71-79.

Kaldahl, W.B., Kalkwarf, K.L., and Patil, K.D. 1993. A review of longitudinal studies that compared periodontal therapies. J. Periodontol. 64(4): 243-253.

Kibbe, A.H., ed. 2000. Handbook of pharmaceutical excipients. 3rd ed. Washington, DC: American Pharmaceutical Association.

Killoy, W.J. 1998. The use of locally-delivered chlorhexidine in the treatment of periodontitis. Clinical results. J. Clin. Periodontol. 25(11): 953-958.

Kimura, S., Toda, H., Shimabukuro, Y., Kitamura, M., Fujimoto, N., Miki, Y., and Okada, H. 1991. Topical chemotherapy in human periodontitis using a new controlled-release insert containing ofloxacin. I. Microbiological observation. J. Periodontol. Res. 26(1): 33-41.

Kinane, D.F. 2001. Causation and pathogenesis of periodontal disease. Periodontol. 2000 25: 8-20.

Klinge, B., Attstrom, R., Karring, T., Kisch, J., Lewin, B., and Stoltze, K. 1992. 3 regimens of topical metronidazole compared with subgingival scaling on periodontal pathology in adults. J. Clin. Periodontol. 19(9): 708-714.

Komsri, R. 1997. The development of *Andrographis paniculata* extract gel. Master's Thesis, Faculty of Pharmacy, Mahidol University.

Komwatchara, T. 1996. The development of *Andrographis paniculata* extract gel for microbial inhibition in adult periodontitis. Master's Thesis, Faculty of Pharmacy, Mahidol University.

Kornman, K.S. 1993. Controlled-release local delivery antimicrobials in periodontics: Prospects for the future. J. Periodontol. 64(8): 782-791.

Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., and Peppas, N.A. 1983. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 15: 25-35.

Koyama, T.M., Stevens, C.R., Borda, E.J., Grobe, K.J., and Cleary, D.A. 1999. Characterizing the gel to liquid crystal transition in lipid-bilayer model systems. Chem. Educator 4(1): 12-15.

Kuchekar, B.S., Bhisa, S.B., and Arumugam, V. 2001. Design of fast dissolving tablets. http://www.ijpe.org.

Kulkarni, N., Kumar, L.D., and Sorg, A. 2003. Fast dissolving orally consumable films containing a sweetener. US. patent 20030211136.

Kumjorn, K. 2003. A comparison study of the foot ulcer healing between dressing with mangostin cream and normal saline wet dressing in diabetic patient. Master's Thesis, Faculty of Graduate studies, Mahidol University.

Lading, P., Lundsholm, Y., and Norling, T. 1992. Method and composition for controlled delivery of biologically active agents. United States Patent: 5,143,934.

Lapidus, H., and Lordi, N.G. 1968. Drug release from compressed hydrophilic matrices. J. Pharm. Sci. 57(8): 1292-1301.

Lee, J., and Kellaway, I.W. 2000. Buccal permeation of [D-Ala$^{2}$, D-Leu$^{5}$]enkephalin from liquid crystalline phases of glyceryl monooleate. Int. J. Pharm. 195(1-2): 35-38.

Leslie, S.B., Puvvada, S., Ratna, B.R., and Rudolph, A.S. 1996. Encapsulation of hemoglobin in a bicontinuous cubic phase lipid. Biochim. Biophys. Acta 1285(2): 246-254.

Leung, S.H.S., Leone, R.S., and Kumar, L.D. 2003. Fast dissolving orally consumable films. US. Patent 20036596298.

Lindhe, J., Heijl, L., Goodson, J.M., and Socransky, S.S. 1979. Local tetracycline delivery using hollow fiber devices in periodontal therapy. J. Clin. Periodontol. 6(3): 141-149.

Loesche, W.J. 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. 50(4): 353-380.

Loesche, W.J., Giordano, J., Soehren, S., Hutchinson, R., Rau, C.F., Walsh, L., and Schork, M.A. 1996. Nonsurgical treatment of patients with periodontal disease. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 81(5): 533-543.

Lorian, V., 1991. Antibiotics in laboratory medicine. 3$^{rd}$ ed. New York: Williams and Wilkins.

Mahabusarakam, W., Phongpaichit, S., Jansakul, C., and Wiriyachitra, P. 1983. Screening of antibacterial activity of chemicals from *Garcinia mangostana*. Songklanakarin J. Sci. Technol. 5(4): 337-339.

Mahabusarakam, W., Phongpaichit, S., and Wiriyachitra, P. 1983. Screening of antifungal activity of chemicals from *Garcinia mangostana*. Songklanakarin J. Sci. Technol. 5(4): 341-342.

Mahabusarakam, W., Proudfoot, J., and Croft, K. 2000. Inhibition of lipoprotein oxidation by prenylated xanthones derived from mangostin. Free Radic. Res. 33(5): 643-659.

Mahabusarakam, W., Wiriyachitra, P., and Taylor, W.C. 1987. Chemical constituents of *Garcinia mangostana*. J. Nat. Prod. 50(3): 474-478.

Mahon, C.R., and Manuselis, G. 2000. Textbook of diagnostic microbiology. 2nd ed. Philadelphia: W.B. Saunders.

Makai, M., Csányi, E., Németh, Zs., Pálinkás, J., and ErŐs, I. 2003. Structure and drug release of lamellar liquid crystals containing glycerol. Int. J. Pharm. 256(1-2): 95-107.

Malmsten, M. 2002. Surfactants and polymers in drug delivery. New York: Marcel Dekker.

Mandell, G.L., Bennett, J.E., Dolin, R. 2000. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 5th ed. Vol 1. Philadelphia: Churchill livingstone.

Marco, P., Fausto, P. 2004. Quick water-dissolving film containing cosmetic, aromatic, pharmaceutical or food substances. US. patent 20040086539.

Martin, A, Swarbrick, J, and Cammarata, A. 1983. Physical pharmacy. 3rd ed. Philadelphia: Lea and Febiger.

Mashru, R.C., Sutariya, V.B., Sankalia, M.G., and Parikh, P.P. 2005. Development and evaluation of fast-dissolving film of salbutamol sulfate. Drug Dev. Ind. Phar. 1:25-34.

Matsumoto, K., Akao, Y., Kobayashi, E., Ohguchi, K., Ito, T., Tanaka, T., Iinuma, M., and Nozawa, Y. 2003. Induction of apoptosis by xanthones from mangosteen in human leukemia cell lines. J. Nat. Prod. 66(8): 1124-1127.

Matsumoto, K., Akao, Y., Yi, H., Ohigachi, K., Ito, T., Tanaka, T., Kobayashi, E., Iinuma, M., and Nozawa, Y. 2004. Preferential target is mitochondria on α-mangostin-incuced apoptosis in human leukemia HL60 cells. Bioorganic and Medicinal Chemistry 12: 5799-5806.

Maxwell, J.R., and Greenberg, M.J. 2004a. Breath freshening and oral cleansing product with cinnamaldehyde. World Intellectual Property Organization 2004000234.

Maxwell, J.R., and Greenberg, M.J. 2004b. Breath freshening and oral cleansing product with magnolia bark extract. US. patent 20040081713.

Mcgregor, R.A., Homan, H.D., and Gravina, S.A. 2004. Fast dissolving film delivery of nucleotides that inhibit the unpleasant taste of tasting medications. World Intellectual Property Organization 2004019885.

Mertin, D. 1998. Rapifilm: rapid release dosage form. Available from: http://www.pharmaforum.com[2004,July 30]

Mims, C.A., Dimmock, N.J., Nash, A., and Stephen, J. 1995. Mims' pathogenesis of infectious disease. 4th ed. Great Britain: WBC Book manufacturing.

Mockel, J.E., and Lippold, B.C. 1993. Zero-order drug release from hydrocolloid matrices. Pharm. Res. 10(7): 1066-1070.

Moongkarndi, P., Kosem, N., Kaslungka, S., Luanratana, O., Pongpan, N., and Neungton, N. 2004. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia*

*mangostana* (mangosteen) on SKBR3 human breast cancer cell line. J. Ethnopharmacol. 90(1): 161-166.

Moongkarndi, P., Kosem, N., Luanratana, O., Jongsomboonkusol, S., and Pongpan, N. 2004. Antiproliferative activity of Thai medicinal plant extracts on human breast adenocarcinoma cell line. Fitoterapia. 75(3-4): 375-377.

Morton, J.F. 1987. Fruits of warm climates. Miami, FL: Creative Resource Systems.

Nabandith, V., Suzui, M., Morioka, T., Kaneshiro, T., Kinjo, T., Matsumoto, K., Akao, Y., Iinuma, M., and Yoshimi, N. 2004. Inhibitory effects of crude α-mangostin, a xanthone derivative, on two different categories of colon preneoplastic lesions induced by 1,2-dimethylhydrazine in the rat. Asian Pacific J. Cancer Prev. 5(4): 433-438.

Nakatani, K., Atsumi, M., Arakawa, T., Oosawa, K., Shimura, S., Nakahata, N., and Ohizumi, Y. 2002. Inhibitions of histamine release and prostaglandin $E_2$ synthesis by mangosteen, a Thai medicinal plant. Biol. Pharm. Bull. 25(9): 1137-1141.

Nakatani, K., Nakahata, N., Arakawa, T., Yasuda, H., and Ohizumi, Y. 2002. Inhibition of cyclooxygenase and prostaglandin $E_2$ synthesis by γ-mangostin, a xanthone derivative in mangosteen, in C6 rat glioma cells. Biochem. Pharmacol. 63(1): 73-79.

Nilar and Harrison, L.J. 2002. Xanthones from the heartwood of *Garcinia mangostana.* Phytochemistry 60(5): 541-548.

Norling, T., Lading, P., Engström, S., Larsson, K., Krog, N., and Nissen, S.S. 1992. Formulation of a drug delivery system based on a mixture of monoglycerides and triglycerides for use in the treatment of periodontal disease. J. Clin. Periodontol. 19(9): 687-692.

Noyan, Ü., Yilmaz, S., Kuru, B., Kadir, T., Acar, O., and Büget, E. 1997. A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult periodontitis patients. J. Clin. Periodontol. 24(3): 158-165.

Okonogi, S., Khongkhunthain, S., Bunyaratavej, P., Thusaphorn, T., and Umpriwan, R. 2004. Development of local injectable dental gel: The influence of certain additives on physicochemical properties of glycerylmonooleate-based formulations. Drug Dev. Ind. Pharm. 30(4): 347-357.

Organization for Economic Cooperation and Development, 1995. Guidelines for the testing of chemicals, OECD 107, Partition coefficient (n-octanol/water): Shake flask method. Paris: Organization for Economic Cooperation and Development.

Panchinda, W. 1992. A follow up study of chronic ulcer healing dressing with 1.5 percent mangostin cream. Master's Thesis, Faculty of Nursing, Mahidol University.

Pader, M. 1988. Cosmetic science and technology series: oral hygiene products and practice. Vol 6. New York: Marcel Dekker.

Panchinda, W. 1992. A follow up study of chronic ulcer healing dressing with 1.5 percent mangostin cream. Master's Thesis, Faculty of Nursing Sciences, Mahidol University.

Paquette, D., Oringer, R., Lessem, J., Offenbacher, S., Genco, R., Persson, G.R., Santucci, E.A., and Williams, R.C. 2003. Locally delivered minocycline microspheres for the treatment of periodontitis in smokers. J. Clin. Periodontol. 30(9): 787-794.

Parveen, M., Khan, N.U., Achari, B., and Dutta, P.K. 1991. A triterpene from *Garcinia mangostana*. Phytochemistry 30(1): 361-362.

Pedrazzoli, V., Kilian, M., and Karring, T. 1992. Comparative clinical and microbiological effects of topical subgingival application of metronidazole 25% dental gel and scaling in the treatment of adult periodontitis. J. Clin. Periodontol. 19(9): 715-722.

Pearce, T.M., and Pearce, T.V. 2003. Snacks of orally soluble edible films. US. patent 20030224090.

Peh, K.K., and Wong, F.C. 1999. Polymeric films as vehicle for buccal delivery: Swelling, mechanical, and bioadhesive properties. J. Pharm. Pharmaceut. Sci. 2(2): 53-61.

Perry, L.M., and Metzger, J. 1980. Medicinal plants of East and Southeast Asia. London: MIT Press.

Phongpaichit, S., Ongsakul, M., Nilrat, L., Tharavichitkul, P., Bunchoo, S., Chuaprapaisilp, T., and Wiriyachitra, P. 1994. Antibacterial activities of extracts from *Garcinia mangostana* pericarps on methicillin-resistant *Staphylococcus aureus* and *Enterococcus* species. Songklanakarin J. Sci. Technol. 16(4): 399-405.

Pramyothin, P., Sapwarobol, S., and Ruangrungsi, N. 2003. Hepatotoxic effects of xanthones extracted from rind of *Garcinia mangostana* in isolated rat hepatocytes. Thai. J. Pharm. Sci. 27(3-4): 123-129.

Rathbone, M.J. 1996. Oral mucosal drug delivery. Vol 74. New York: Marcel Dekker.

Rathbone, M.J., Hadgraft, J., and Roberts, M.S. 2003. Modified-release drug delivery technology. Vol. 126. New York: Marcel Dekker.

Repka, A.M., Gutta, K., Prodduturi, S., Munjal, M., Stodghill, P.S. 2005. Charaterization of cellulosic hot-melt extruded films containing lidocaine. Eur. J. Pharm. Biopharm. 59: 189-196.

Rojanapanthu, P., Gritsanapan, W., Amornchat, C., Pornsawadeechai, K., and Ngamgosol, K. (2002). The development of medicinal plant toothpaste. Proceedings of the 5th Scientific Conference of the Asian Societies of Cosmetic Scientists, 2001. Mahidol University Annual Research Abstracts 29: Abstract No. 509.

Rosan, B., and Lamont, R.J. 2000. Dental plaque formation. Microbes Infect. 2(13): 1599-1607.

Roskos, K.V., Fritzinger, B.K., Rao, S.S., Armitage, G.C., and Heller, J. 1995. Development of a drug delivery system for the treatment of periodontal disease based on bioerodible poly(ortho esters). Biomaterials 16(4): 313-317.

Rowe, R.C., Sheskey, P.J., and Weller, P.J. 2003. Handbook of pharmaceutical excipients. 4th ed. Washinton: pharmaceutical press.

Sadhale, Y., and Shah, J.C. 1999. Biological activity of insulin in GMO gels and the effect of agitation. Int. J. Pharm. 191(1): 65-74.

Sakagami, Y., Iinuma, M., Piyasena, K.G.N.P., and Dharmaratne, H.R.W. 2005. Antibacterial activity of α-mangostin against vancomycin resistant *Entericocci* (VRE) and synergism with antibiotics. Phytomedicine 12(3)203-208.

Sallam, A.S., Khalil, E., Ibrahim, H., and Freij, I. 2002. Formulation of an oral dosage form utilizing the properties of cubic liquid crystalline phases of glyceryl monooleate. Eur. J. Pharm. Biopharm. 53(3): 343-352.

Sapwarobol, S. 1997. Effects of xanthones extracted from rind of *Garcinia mangostana* in isolated rat hepatocytes. Master's Thesis, Inter-Department of Pharmacology, Graduate School, Chulalongkorn University.

Sato, A., Fujiwara, H., Oku, H., Ishiguro, K., and Ohizumi, Y. 2004. α-mangostin induces $Ca^{2+}$-ATPase-dependent apoptosis via mitochondrial pathway in PC12 cells. J. Pharmacol. Sci. 95: 33-40.

Scherlund, M. 2000. *In situ* gelling drug delivery systems for periodontal anaesthesia. Doctoral dissertation, Faculty of Pharmacy, Uppsala University.

Scherlund, M., Welin-Berger, K., Brodin, A., and Malmsten, M. 2001. Local anaesthetic block copolymer system undergoing phase transition on dilution with water. Eur. J. Pharm. Sci. 14(1): 53-61.

Schwach-Abdellaoui, K., Loup, P.J., Vivien-Castioni, N., Mombelli, A., Baehni, P., Barr, J., Heller, J., and Gurny, R. 2002. Bioerodible injectable poly(ortho ester) for tetracycline controlled delivery to periodontal pockets: Preliminary trial in humans. AAPS PharmSci. 4(4): article 20.

Schwach-Abdellaoui, K., Monti, A., Barr, J., Heller, J., and Gurny, R. 2001. Optimization of a novel bioerodible device based on auto-catalyzed poly(ortho esters) for controlled delivery of tetracycline to periodontal pocket. Biomaterials 22(12): 1659-1666.

Schwach-Abdellaoui, K., Vivien-Castioni, N., and Gurny, R. 2000. Local delivery of antimicrobial agents for the treatment of periodontal diseases. Eur. J. Pharm. Biopharm. 50(1): 83-99.

Schwarzwälder, C., and Meier, W. 1997. New nonionic head type polysoaps: A way to reversed liquid crystalline phases. Macromolecules 30(16): 4601-4607.

Sebti, I., Broughton, J.D., and Coma, V. 2003. Physicochemical properties and bioactivity of nisin-containing cross-linked hydroxypropylmethylcellulose films. J. Agric. Food Chem. 51: 6468-6474.

Sen, A.K., Sarkar, K.K., Mazumder, P.C., Banerji, N., Uusvuori, R., and Hase, T.A. 1980. A xanthone from *Garcinia mangostana*. Phytochemistry 19(10): 2223-2225.

Sen, A.K., Sarkar, K.K., Mazumder, P.C., and Banerji, N. 1981. Minor xanthones of *Garcinia mangostana*. Phytochemistry 20(1): 183-185.

Sendil, D., Gürsel, I., Wise, D.L., and Hasırcı, V. 1999. Antibiotic release from biodegradable PHBV microparticles. J. Control. Release 59(2): 207-217.

Shah, J.C., Sadhale, Y., and Chilukuri, D.M. 2001. Cubic phase gels as drug delivery systems. Adv. Drug Deliv. Rev. 47(2-3): 229-250.

Shankaranarayan, D., Gopalakrishnan, C., and Kasemswaran, L. 1979. Pharmacological profile of mangostin and its derivatives. Arch. Int. Pharmacodyn. Ther. 239(2): 257-269.

Sindermsuk, J., and Deekijsermphong, S. 1989. The antibacterial activities of crude extract from the fruit hull of *Garcinia mangostana* on enteric pathogens and intestinal commensal. Bull. Dept. Med. Serv. 14(6): 421-426.

Sornprasit, A., Sripiyarattanakul, K., Chuay-Yim, P., and Tanakittithum, P. 1987. Preliminary toxicological study of mangostin. Songklanakarin J. Sci. Technol. 9(1): 51-57.

Soskolone, W.A., Chajek, T., Flashner, M., Landau, I., Stabholtz, A., Kolatch, B., and Lerner, E.I. 1998. An *in vivo* study of the chlorhexidine release profile of the PerioChip™ in the gingival crevicular fluid, plasma and urine. J. Clin. Periodontol. 25(12): 1017-1021.

Southard, G.L., and Godowski, K.C. 1998. Subgingival controlled release of antimicrobial agents in the treatment of periodontal disease. Int. J. Antimicrob. Agents. 9(4): 239-253.

Stoller, N.H., Johnson, L.R., Trapnell, S., Harrold, C.Q., and Garrett, S. 1998. The pharmacokinetic profile of a biodegradable controlled-release delivery system containing doxycycline compared to systemically delivered doxycycline in gingival crevicular fluid, saliva, and serum. J. Periodontol. 69(10): 1085-1091.

Stoltze, K. 1992. Concentration of metronidazole in periodontal pockets after application of a metronidazole 25% dental gel. J. Clin. Periodontol. 19(9): 698-701.

Stoltze, K. 1995. Elimination of Elyzol 25% Dentalgel matrix from periodontal pockets. J. Clin. Periodontol. 22(3): 185-187.

Suksamrarn, S., Suwannapoch, N., Ratananukul, P., Aroonlerk, N., and Suksamrarn, A. 2002. Xanthones from the green fruit hulls of *Garcinia mangostana*. J. Nat. Prod. 65(5): 761-763.

Suksamrarn, S., Suwannapoch, N., Phakhodee, W., Thanuhiranlert, J., Ratananukul, P., Chimnoi, N., and Suksamrarm, A. 2003. Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. Chem. Pharm. Bull. 51(7):857-859.

Sundaram, B.M., Gopalakrishnan, C., Subramanian, S., shankaranarayanan, D., and Kameswaran, L. 1983. Antimicrobial activities of *Garcinia mangostana*. Planta Med. 48(1): 59-60.

Suomi, J.D., West, T.D., Chang, J.J., and McClendon, B.J. 1971. The effect of controlled oral hygiene procedures on the progression of periodontal disease in adults: radiographic findings. J. Periodontol. 42(9): 562-564.

Szeles, L.H., Mohseni, S., Xu, G., Williams, M., Viscio, D.B. , and Masters, J.G. 2004. Enzyme enhanced breath freshening film. US. patent 20040018156.

Taweekunthum, R. 2001. Preparation and in vitro evaluation of lidocaine hydrochloride oral mucoadhesive film. Master's Thesis, Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Tan, V. 2004. Formulation of monoglyceride-based drug delivery systems containing *Garcinia mangostana* extract. Master's Thesis, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Trivedi, N.A., and Hotchandani, S.C. 2004. A study of the antimicrobial activity of oil of eucalyptus. Indian J. Pharmacol. 36(2): 93-94.

The United States Pharmacopeil Convention, Inc. 2003. The United States Pharmacopeia 26/21 The National Formulary 21: USP26/NF21 Philadelphia: National Publishing.

Umbach, W. 1991. Cosmetics and toiletries: Development, production and use. New York: Ellis Horwood.

Vandekerckhove, B.N.A., Quirynen, M., and Steenberghe, D.V. 1997. The use of tetracycline-containing controlled-release fibers in the treatment of refractory periodontitis. J. Periodontol. 68(4): 353-361.

Voravuthikunchai, S.P., and Kitpipat, L. 2005. Activity of medicinal plant extracts against hospital isolates of methicillin-resistant *Staphylococcus aureus*. Clinical Microbiology and Infection 11(6): 510-512.

Willett, N.P., Rosen, S., and White, R.R. 1991. Essential dental microbiology. Norwalk, CT: Appleton & Lange.

Williams, P., Ongsakul, M., Proudfoot, J., Croft, K., and Beilin, L. 1995. Mangostin inhibits the oxidative modification of human low density lipoprotein. Free Radic. Res. 23(2): 175-184.

Williams, R.C., Paquette, D.W., Offenbacher, S., Adams, D.F., Armitage, G.C., Bray, K., Caton, J., Cochran, D.L., Drisko, C.H., Fiorellini, J.P., Giannobile, W.V., Grossi, S., Guerrero, D.M., Johnson, G.K., Lamster, I.B., Magnusson, I., Oringer, R.J., Persson,

G.R., Van Dyke, T.E., Wolff, L.F., Santucci, E.A., Rodda, B.E., and Lessem, J. 2001. Treatment of periodontitis by local administration of minocycline microspheres: A controlled trial. J. Periodontol. 72(11): 1535-1544.

Wilson, T.G., and Kornman, K.S. 2003. Fundamentals of periodontics. 2nd ed. Chicago: Quintessence Publishing.

Wong, F.C., Yuen, H.K., and Peh, K.K. 1999. Formulation and evaluation of controlled release eudragit buccal patches. Int. J. Pharm. 178: 11-22.

Wong. F.K. 2002. Medicinal plants of southeast asia. 2nd ed. PA: Pearson Malaysia.

Wyatt, D.M., and Dorschel, D.A. 1992. A cubic-phase delivery system composed of glyceryl monooleate and water for sustained release of water-soluble drugs. Pharm. Technol. 16(10): 116-130.

Xu, G., Reci, M., Blackwell, B.L., Robinson, R.S., Viscio, D.B., and Curtis, J.P. 2002. Breath freshening film. US. patent 20026419903.

Yoshikawa, M., Harada, E., Miki, A., Tsukamoto, K., Liang, S.Q., Yamahara, J., and Murakami, N. 1994. Antioxidant constituents from the fruit hulls of mangosteen (*Garcinia mangostana* L.) originating in Vietnam. Yakugaku Zasshi 114(2): 129-133.

Zerbe, H.G., Al-Khalil, F. 2003. Flavored film. US. patent 20030053962