DEVELOPMENT OF SHELLAC ESTERS AS ALTERNATIVE ENTERIC POLYMERS THROUGH SOLID-STATE REACTIONS

By

Danuch Panchapornpon

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

Program of Pharmaceutical Technology

Graduate School

SILPAKORN UNIVERSITY

2011

DEVELOPMENT OF SHELLAC ESTERS AS ALTERNATIVE ENTERIC POLYMERS THROUGH SOLID-STATE REACTIONS

By

Danuch Panchapornpon

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

Program of Pharmaceutical Technology

Graduate School

SILPAKORN UNIVERSITY

2011

การพัฒนาเชลแล็กเอสเทอร์เพื่อเป็นเอนเทอริกพอลิเมอร์ทางเลือก
โดยผ่านการทำปฏิกิริยาในสภาวะของแข็ง

โดย

นายดนุช ปัญจพรผล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีเภสัชกรรม
บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
ปีการศึกษา 2554

The Graduate School, Silpakorn University has approved and accredited the thesis title of “Development of Shellac Esters as Alternative Enteric Polymers Through Solid-State Reactions” submitted by Mr. Danuch Panchapornpon as a partial fulfillment of the requirements for the degree of Doctor of Pharmacy in Pharmaceutical Technology.

…….....

(Assistant Professor Panjai Tantatsanawong, Ph.D.)

Dean of Graduate School

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

The Thesis Advisors

1. Associate Professor Sontaya Limmatvapirat, Ph.D.
2. Professor Keiji Yamamoto, Ph.D.

The Thesis Examination Committee

……..…….. Chairman

(Associate Professor Pornsak Sriamornsak, Ph.D.)

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

..... Member

(Associate Professor Satit Puttipipatkhachorn, Ph.D.)

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

..... Member

(Associate Professor Jurairat Nuntanid, Ph.D.)

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

..... Member

(Associate Professor Sontaya Limmatvapirat, Ph.D.)

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

..... Member

(Professor Keiji Yamamoto, Ph.D.)

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

49353803 : MAJOR : PHARMACEUTICAL TECHNOLOGY
KEY WORDS : SHELLAC / SOLID-STATE REACTION / CYCLIC ANHYDRIDE / ENTERIC POLYMER
DANUCH PANCHAPORNPON : DEVELOPMENT OF SHELLAC ESTERS AS ALTERNATIVE ENTERIC POLYMERS THROUGH SOLID – STATE REACTIONS. THESIS ADVISORS : ASSOC. PROF. SONTAYA LIMMATVAPIRAT, Ph.D., AND PROF. KEIJI YAMAMOTO, Ph.D. 203 pp.

For pharmaceutical industry, shellac (SHL) has been used for moisture protection, glossing, while the use for enteric coating of pharmaceutical products has greatly declined. Severe problems associated with enteric properties are less solubility at pH of intestine and less stability of SHL. The objective of this research was to solve the problems by fabrication of ester derivatives of SHL through solid-state reaction. Cyclic anhydrides (CAHs), including succinic anhydride (SUCA), phthalic anhydride (PHTA), trimellitic anhydride (TMTA), were employed to esterify with SHL by grinding with heat treatment. The result showed that small molecule CAHs (SUCA) could easily esterify with SHL under low annealing temperature while the larger molecule CAHs (PHTA and TMTA) need higher annealing temperature for esterification. Acid value of all SHL esters was increased as prolonging of annealing time, especially shellac succinate (SHL-SUC) and shellac phthalate (SHL-PHT) while percent insoluble solid of SHL-SUC and SHL-PHT was lower than 2% w/w but that of shellac trimellitate (SHL-TMT) was higher than 35 % w/w, suggesting the better esterification of SUCA and PHTA while a failure of aging protection by TMTA. The formation of shellac esters was also confirmed by PXRD, DSC, FTIR and NMR spectroscopy. SHL-SUC and SHL-PHT were chosen to further investigate the enteric properties and stability of films and coated tablets. The result indicated that all shellac films demonstrated good gastric and moisture protection. In addition, the solubility of SHL-SUC and SHL-PHT films was increased in lower pH and the elasticity of these films was enhanced, as compared to SHL. The SHL-SUC and SHL-PHT coated tablets showed rapidly drug release. For stability test, SHL-PHT demonstrated better stability, as compared to SHL and SHL-SUC. The more steric effect of rigid aromatic ring of phthalic moiety might cause the separation of shellac chain and thus reduced the inter chain polymerization. In conclusion, the improved enteric properties and stability of modified shellac could be achieved under the concept of “green chemistry”.

Program of Pharmaceutical Technology Graduate School, Silpakorn University Academic year 2011
Student’s signature ……..……..…
Thesis Advisors’ signature 1……..……..…… 2……..……..

49353803 : สาขาวิชาเทคโนโลยีเภสัชกรรม
คำสำคัญ : เชลแล็ก / ปฏิกิริยาในสภาวะของแข็ง / ไซคลิกแอนไฮไดรด์ / เอนเทอริกพอลิเมอร์

ดนุช ปัญจพรผล : การพัฒนาเชลแล็กเอสเทอร์ เพื่อเป็นเอนเทอริกพอลิเมอร์ทางเลือกโดยผ่านการทำปฏิกิริยาในสภาวะของแข็ง. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : รศ.ดร.สนทยา ลิ้มมัทวาภิรัติ์ และ Prof. Dr. Keiji Yamamoto. 203 หน้า.

เชลแล็กเป็นพอลิเมอร์ธรรมชาติที่มีความสามารถในการป้องกันความชื้นและมีความเงางาม จึงถูกประยุกต์ใช้อย่างแพร่หลาย แต่ในปัจจุบันการใช้เชลแล็กเป็นเอนเทอริกพอลิเมอร์ในอุตสาหกรรมยาลดลงอย่างมาก เนื่องจากปัญหาสำคัญสองประการ คือ การละลายน้อยที่พีเอชของน้ำย่อยในลำไส้เล็ก และปัญหาความคงตัวระหว่างการเก็บรักษา วัตถุประสงค์ของงานวิจัยนี้เพื่อแก้ปัญหาดังกล่าวของเชลแล็กโดยการปรับปรุงโครงสร้างโมเลกุลของเชลแล็กด้วยกระบวนการเอสเทอริฟิเคชันในสภาวะของแข็ง เชลแล็กเอสเทอร์เตรียมด้วยการบดผสมเชลแล็กกับไซคลิกแอนไฮไดรด์ เช่น ซักซินิกแอนไฮไดรด์ ทาลิกแอนไฮไดรด์ และไตรเมลลิติกแอนไฮไดรด์ร่วมกับการให้ความร้อน ผลการทดลองพบว่าไซคลิกแอนไฮไดรด์โมเลกุลเล็ก (ซักซินิกแอนไฮไดรด์) สามารถเกิดปฏิกิริยากับเชลแล็กได้อย่างมีประสิทธิภาพภายใต้อุณหภูมิที่ต่ำ ในขณะที่ไซคลิกแอนไฮไดรด์ขนาดโมเลกุลที่ใหญ่ขึ้น (ทาลิกแอนไฮไดรด์ และไตรเมลลิติกแอนไฮไดรด์) จำเป็นต้องใช้อุณหภูมิที่สูงขึ้นในการทำปฏิกิริยา ค่าความเป็นกรดของเชลแล็กเอสเทอร์เพิ่มสูงขึ้นเมื่อเพิ่มระยะเวลาในการอบโดยเฉพาะเชลแล็กซักซิเนตและเชลแล็กทาเลต ในขณะที่ปริมาณของแข็งที่ไม่ละลายของเชลแล็กซักซิเนตและเชลแล็กทาเลตมีค่าต่ำกว่าร้อยละ 2 โดยมวล แต่ของแข็งที่ไม่ละลายของเชลแล็กไตรเมลลิเตตมีค่าสูงกว่าร้อยละ 35 โดยมวล ซึ่งแสดงถึงการเกิดเอสเทอริฟิเคชันที่ดีกว่าของซักซินิกแอนไฮไดรด์และทาลิกแอนไฮไดรด์และความล้มเหลวของไตรเมลลิติกแอนไฮไดรด์ในการป้องกันการเสื่อมสภาพของเชลแล็ก การยืนยันการเกิดเชลแล็กเอสเทอร์ทำได้โดยใช้เครื่องมือหลายชนิด เช่น เครื่องทดสอบการเลี้ยวเบนรังสีเอกซ์ของผง ดิฟเฟอเรนเชียลสแกนนิงแคลอริมิเตอร์ อินฟราเรดและนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโคปี จากผลการทดลองข้างต้นได้เลือกเชลแล็กซักซิเนตและเชลแล็กทาเลตมาทดสอบต่อในส่วนคุณสมบัติของฟิล์มและยาเม็ดเพื่อประเมินคุณสมบัติความเป็นเอนเทอริกและความคงตัวต่อ ผลการศึกษาพบว่าฟิล์มเชลแล็กและเชลแล็กเอสเทอร์มีความสามารถในการป้องกันกรดและความชื้นได้ดี ในขณะที่ฟิล์มเชลแล็กเอสเทอร์สามารถละลายได้ดีในพีเอชที่ต่ำลงและมีความยืดหยุ่นที่ดีขึ้นเมื่อเปรียบเทียบกับฟิล์มเชลแล็ก นอกจากนี้ยาเม็ดเคลือบด้วยเชลแล็กซักซิเนตและเชลแล็กทาเลตสามารถละลายในพีเอชของลำไส้เล็กได้รวดเร็วยิ่งขึ้น จากการศึกษาความคงตัว พบว่าเชลแล็กทาเลตมีความคงตัวที่ดีเมื่อเปรียบเทียบกับเชลแล็กอื่น เนื่องจากผลสเตอริกของโครงสร้างอะโรมาติกจากโมเลกุลทาเลตที่สามารถแยกสายโซ่และลดปฏิกิริยาการก่อพอลิเมอร์ภายในโครงสร้างของเชลแล็กได้ กล่าวโดยสรุปคุณสมบัติทางเอนเทอริกและความคงตัวของเชลแล็กสามารถปรับปรุงโดยผ่านกระบวนการที่เป็นมิตรต่อสิ่งแวดล้อม

สาขาวิชาเทคโนโลยีเภสัชกรรม บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร ปีการศึกษา 2554

ลายมือชื่อนักศึกษา .....

ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ 1..... 2.....

ACKNOWLEDGEMENTS

The success of my graduate study would never have happened without support and invaluable advice from a number of persons who contributed to my valuable experience.

I would like to express my deep sense of gratitude and sincere appreciation to my advisor, Associate Professor Dr. Sontaya Limmatvapirat for his invaluable suggestion and guidance to adapt my ideas into the well scientific thought, for his assistance in the preparation of my thesis and research manuscript, his incredible creativity and interpretation skills have often inspired me, and especially for his tireless patience throughout my study.

I am very grateful to express my gratitude to Professor Dr. Keiji Yamamoto, my co-advisor, Associate Professor Dr. Kunikazu Moribe and Assistant Professor Dr. Kenjirou Higashi for their valuable guidances and helpful comments, for providing exceptional research facilities and their supports during entire study in Japan.

My great appreciation is acknowledged to Associate Professor Dr. Jurairat Nunthanid, Associate Professor Dr. Pornsak Sriamornsak, Associate Professor Dr. Chutima Limmatvapirat and Associate Professor Dr. Manee Luangtana-anan at the Department of Pharmaceutical Technology and Pharmaceutical Biopolymer group (PBiG), Faculty of pharmacy, Silpakorn University, for their esteem criticism, meaning advice, and discussion in my thesis. I also would like to thank Associate Professor Dr. Satit Puttipipatkhachorn from Faculty of Pharmacy, Mahidol University for his helpful suggestion and his valuable time being my thesis committee.

I would like to acknowledge the Graduate School, Silpakorn University for scholarship to do a part of my research and the Thailand Research Fund (TRF) through the Royal Golden Jubilee (RGJ.) Ph.D. Program (Grant No. PHD/0166/2548) for finance support during my study in Thailand and Japan.

I also record my special appreciation for the kind assistance, support and friendship granted me by all my friends in both Thailand and Japan. Finally, I wish to declare my infinite gratitude to my dearest parents and my sisters for their endless love, encouragement, care and precious spiritual support throughout my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Shellac is a natural polymer of animal origin secreted by lac insect (*Laccifer Lacca*), which live on trees called lac host tree in Thailand, India, Burma and to a minor extent in other Asian countries (Brydson 1999: 867-869). Shellac is widely used in food industry, to some extent still in pharmaceutical industry. Shellac can be used as a moisture barrier for food and nutritional/health supplements. Further applications of shellac are enteric coating for food, retard coating for food and it also can be used as sub-coat or gloss coat. Shellac is special interesting because it is one of the few excipients allowed for these coating purposes in food. In addition, it is natural material from renewable resources (Krause and Muller 2001: 89-92). In pharmaceutical industry, shellac has excellent film forming and protective properties. It has been used for sealing, glossing and enteric coating of pharmaceutical products, while the use of shellac as an enteric coating material has fallen into disfavor for several reasons. Problems associated with shellac are less solubility in pH of intestine and less stability, comparing to synthetic and semi-synthetic enteric polymer e.g., polyacrylate, cellulose derivatives (Tarcha 1999: 39-66).

Shellac structure comprises mainly of hydroxyl and carboxyl groups (Brydson 1999: 867-869; Sontaya Limmatvapirat et al. 2004: 41-49). The low amount of carboxylic acid group per shellac molecule and the high pK_a lead to the poor solubility. Shellac is not dissolved in the solution at pH below 7. However, the pH in proximal region of small intestine is between 3.8-6.9, failure of shellac coated tablets

to disintegrate is still major problem. The other problem is that shellac undergoes an “aging effect” upon storage. The reduced solubility of shellac on storage is attributed to polymerization resulting from trans-esterification of the hydroxyl group of one shelloic or aleuritic acid molecule with the carboxyl group of another of the hydroxyl containing carboxylic acids (Wruble 1930: 318). The disintegration time of shellac-coated tablets has been shown to increase dramatically, even when stored at room temperature or elevated temperatures (>70ºC) (Lee and Mukherjee 1992: 48-49; Brydson 1999: 867-869). Therefore several works have attempted to solve these problems. The addition of pore former, such as organic acid or hydrophilic polymer, was a simplified approach to improve the solubility of shellac. The organic acids and hydrophilic polymer performed as plasticizer, reducing glass transition temperature, which significantly decreased the disintegration time of shellac-coated soft gelatin capsules in simulated intestinal fluid while retained the gastric resistance in 0.1 N hydrochloric acid (Nantarat Pearnchob et al. 2004: 313-321). However, this method did not directly modify at the structure of shellac and need some specific reagents that might later affect the properties of shellac. As shown in Figure 1, shellac composed of polyester that should be modified by hydrolysis. By hydrolysis treatment with 2.0% NaOH, acid value of hydrolyzed shellac increased with prolonged alkali-treatment time. The acid value was influenced by the amount of free carboxylic group and the ionization constant (pK_a). However the pK_a of hydrolyzed shellac was not changed (6.5-6.8), so the increment of acid value should be affected by an increase in free carboxyl groups. As compared to the other enteric polymers, shellac showed the higher pK_a (Davis et al. 1986: 157-166; Sontaya Limmatvapirat et al. 2004: 41-49). These explained the lower aqueous solubility of shellac. The results demonstrated

the solubility of hydrolyzed shellac which was increased with increasing hydrolysis time. The improved solubility was well corresponded with the increasing acid value, suggesting the solubility increment by ionization of more carboxylic groups. Although the partially hydrolyzed shellac showed better solubility property (Sontaya Limmatvapirat et al. 2004: 41-49), the stability problem was not yet solved. The polymerization effect still occurred during storage (Nantarat Pearnchob et al. 2004: 313-321). Furthermore, the improved enteric properties of shellac by forming salt with 2-amino-2-methyl-1-propanol (AMP) and ammonium hydroxide (AMN) were investigated. The results showed that solubility of the shellac salts was enhanced as the ratio of AMP:AMN increased. The increasing absorbance ratio of the FTIR peaks assigned to carbonyl stretching of carboxylate and carboxylic acid (ABS_{1556}/ABS_{1716}) was observed with the increment of the AMP fraction, indicating that the solubility improvement was due to more ionization of AMP salts. The AMN salts also demonstrated increased solubility and stability. However, insoluble solid which was a parameter of the instability of shellac, was increased, the percent dissolved was significantly decreased and the value of ABS_{1556}/ABS_{1716} was also rapidly decreased. For AMP salts system, although the absorbance ratio was gradually declined, the insoluble solid and the percent dissolved were not changed, confirming that the polymerization might not occur during storage up to 6 months. The proposed diagram of salt formation and aging mechanism (polymerization) of various forms of shellac is shown in Figure 2. The increase of percentage of insoluble solid and the reduction of aqueous solubility and acid value of the acid form of shellac were due to the polymerization among hydroxyl and carboxyl groups of polymer chain of shellac after storage. Since the carboxylic acid was conversed to carboxylate by salts formation

with AMP or AMN, the solubility and stability (the polymerization could be protected) of shellac were increased. AMP salts might more strongly interact with the carboxylate and demonstrated the hydrogen bonds formation between hydroxyl groups of AMP and shellac, resulted in more ionization, stability and plasticization. In addition, the polymer chains of shellac were separated by the steric effect of the large molecule of AMP leading to the prevention of polymerization among polymer chains. While the stability of AMN salts was decreased by losing ammonium ion from carboxylic group during storage time. The conversion of carboxylate to carboxylic acid and the polymerization among polymer chains should be the other reasons for the aging effect. In conclusion, The AMP salts demonstrated better solubility and stability than AMN salts, whereas film prepared from the AMP salt showed lower moisture protection and film strength as compared to that prepared from AMN salt (Sontaya Limmatvapirat et al. 2007: 690-698). However, the structure modification of shellac has been only focused on carboxylic acid while modification of hydroxyl group has not been reported. Kokubo et al. (Kokubo et al. 1997: 1350-1353) developed the cellulose-derived polymer by using trimellitic and maleic anhydride as modifying agents. The number of carboxyl groups in the cellulose structure could be increased, and hence the solubility of the modified cellulose was improved at lower pH value. Since shellac consists of a large amount of hydroxyl groups (Figure 1), esterification with cyclic anhydrides, may be an alternative way for increasing a number of carboxylic groups.

Most studies of chemical synthesis were achieved with organic solvents; however the toxic organic waste was occurred from the reaction. Solid state reaction, a chemical reaction without the presence of a solvent, has been recently introduced for

solving the problem. The solid state reaction has several advantages, including waste minimization, efficient and economical technology (green chemical); safety, easier product work-up, reduced cost, increased reactivity and selectivity. The reaction can conduct by mechanochemical process; involving with mixing and grinding of substances with, or without thermal activation (Kidwai 2001: 147-151; Strauss 2002: 400-401; Toda 2002). The research works including nitrones synthesis (Colacinoa E. et al. 2008: 5569-5576), amorphization (Mallick et al. 2008: 346-351; Mallick et al. 2008: 726-734), methylation (Ohashi et al. 1988: 733-739), amide synthesis (Fukuoka 1994: 1342-1344), and eutectic mixture formation, have been reported (Sakata et al. 2007: 12-19). However the formation of shellac derivatives by mechanochemical process is not yet investigated.

The purpose of this study was to investigate the formation of shellac esters through solid state reaction. Shellac and cyclic anhydrides (Figure 3) such as succinic anhydride, phthalic anhydride, and trimellitic anhydride are co-ground and thermally activated. Formation of shellac esters was characterized by acid value, FTIR spectroscopy, nuclear magnetic resonance (NMR), and powder X-ray diffraction (PXRD). The film and coated tablets prepared by shellac and shellac esters was comparatively evaluated.

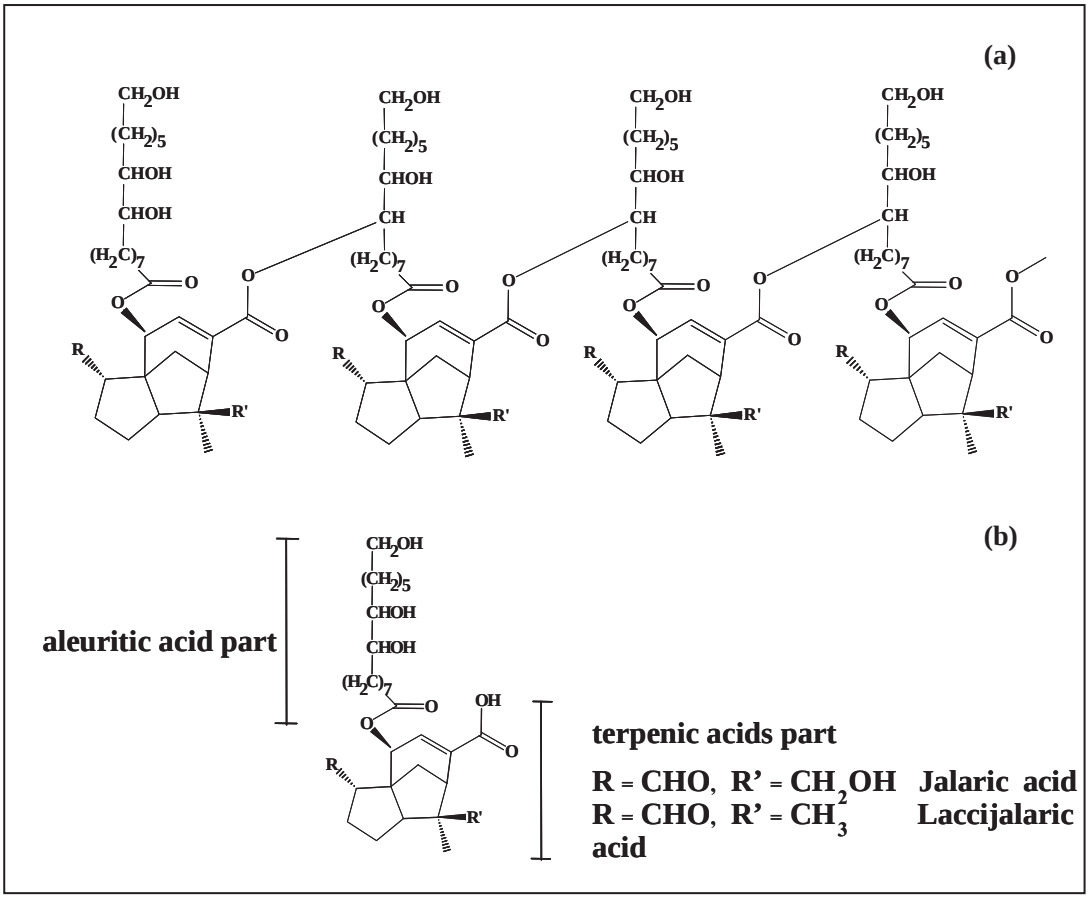


Figure 1 Chemical structure of shellac; polyester (a) and single ester (b)

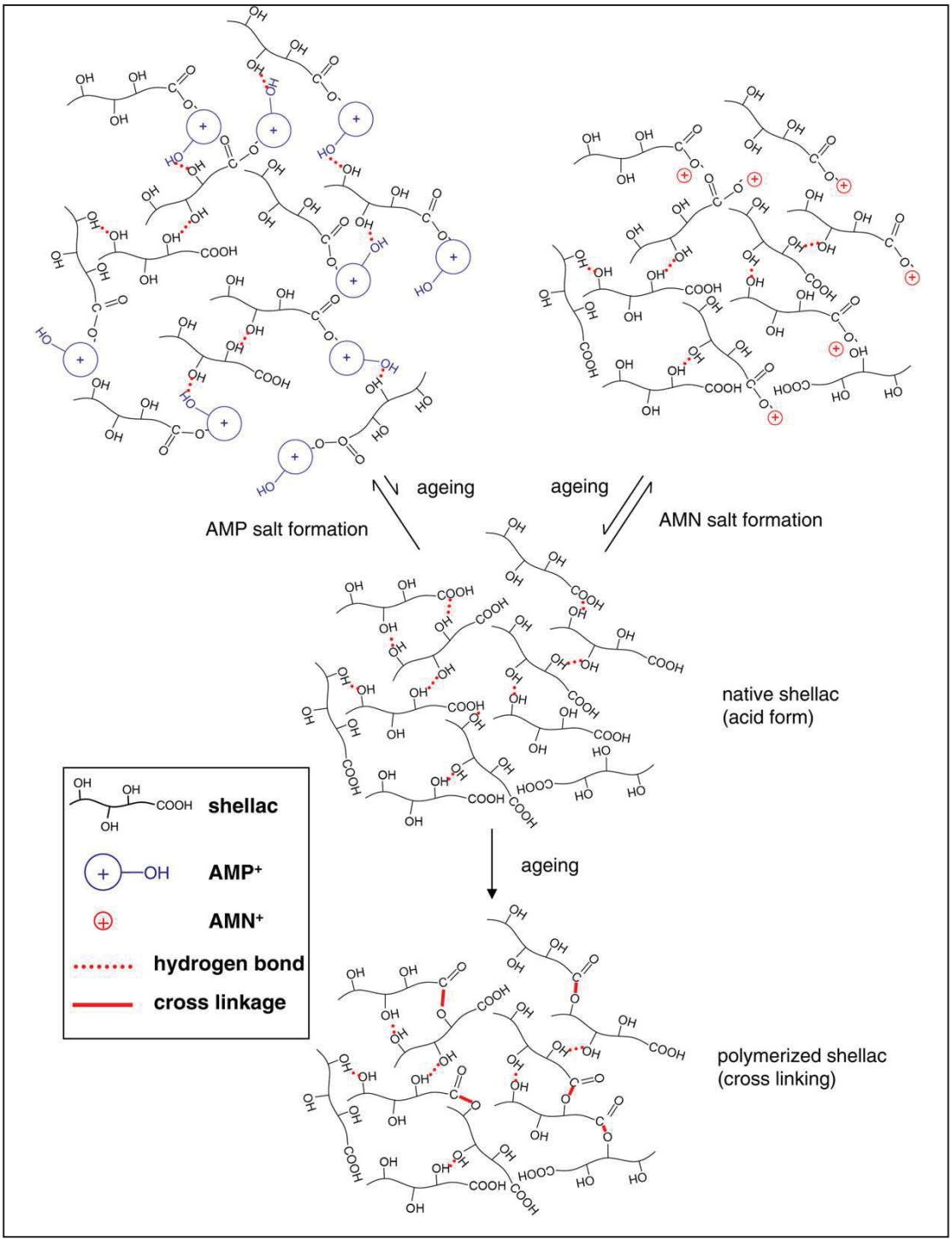


Figure 2 Proposed diagram of salt formation and aging of various forms of shellac

Source : Sontaya Limmatvapirat et al., "Enhanced enteric properties and stability of shellac films through composite salts formation." European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) : 690-698.

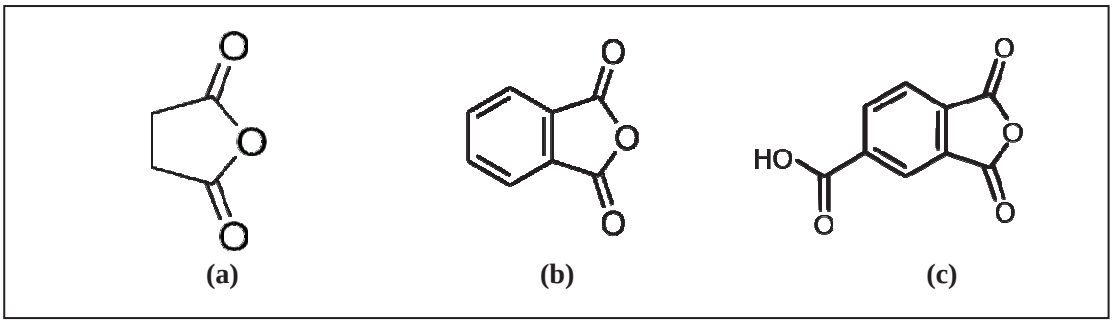


Figure 3 Chemical structure of cyclic anhydrides; succinic anhydride (a), phthalic anhydride (b), and trimellitic anhydride (c)

CHAPTER 2

LITERATURE REVIEWS

1. Enteric coating

1.1 Definition

An enteric coating is a barrier applied to oral medication that controls the location in the digestive system where it is absorbed. Enteric refers to the small intestine; therefore enteric coatings prevent release of medication before it reaches the small intestine.

1.2 Purpose of enteric coating

Enteric coating is the most established of delayed-release products, designed to pass through the stomach unaltered, later to release their medication within the intestinal tract. They are necessary for the following reasons:

1. To protect a substance from destruction by gastric fluids (e.g., antibiotics, proteins, and peptides)

2. To minimize gastric distress (eg., nonsteroidal anti-inflammatory agent) (Schoenwald 2001; Washington et al. 2001; Rathbone et al. 2002: 223-225; Allen et al. 2004: 260-261; Kendall and Basit 2006: 42-44)

3. To deliver substance to target site in the intestine for the treatment of local diseases.

4. To provide a delayed fraction for repeated action dosage forms

1.3 Physiological considerations

The most important gastrointestinal physiology factors affecting the function of enteric coatings are as follows.

1. pH of the stomach and intestinal contents

The pH of different parts of GI tract is the most important factors that affect on drug release from an enteric drug delivery system. The pH of the stomach is about 1.0-3.5, depending on the presence or absence of food and reflux of intestinal contents into the stomach. The pH of the intestine may range from about 3.8-6.9 in the small intestine to about 7.5-8.0 in the large intestine. Based on the pH of the stomach and small intestine, enteric coating must be designed to resist dissolution at pH values below 4 to avoid disintegration in the stomach, but to begin dissolving at pH 5 and above (Tarcha 1999: 39-66).

2. Ionic state

Most enteric coating polymers show anionic character, therefore, dissolution is depended on the ionic state of the gastrointestinal medium. Several studies have reported that drug released rate from anionic polymer coated dosage forms increased with increasing the ionic strength of the dissolution media (Kararli et al. 1995: 1813-6; Ibekwe et al. 2006: 52-60; Liu et al. 2009: 119-124). In addition, the dissolution rate of acid polymer was increased by the basic salt because of the easier proton transferring to water molecules (Liu et al. 2009: 119-124).

3. Gastric emptying

Gastric emptying of coated tablets is highly variable, and may vary from 30 min to 7 h, depending on the presence and type of food in stomach. Horton et al. (Horton et al. 1965: 1537-1539) reported that gastric emptying-time was variable

4-8 h after taken enteric coated granules of barium with a meal. Although most enteric coatings could resist gastric acid for 1 h, the coated tablets may not be able to remain intact if they reside in the stomach for longer period.

4. Enzyme activity of the gastrointestinal tract

A variety of enzymes in the intestine can break down various substances. The pancreatic juice, containing trypsin, chymotripsin, amylase, and lipase are enzymes which can hydrolyze various compounds. Fats have been used in the past as enteric coatings because they are not digested in stomach due to the absence of lipase.

5. Pathophysiological change

Gastrointestinal disorders greatly impair drug absorption. Depending on the condition, inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, may alter pH, ionic state, and gastric emptying time of gut. Changes in GI condition directly reduce dissolution of enteric coated drug at the precise point of action (Gubbins and Bertch 1991: 431-47; Schreier 2001).

1.4 Theory of enteric polymer performance

Based on the gastrointestinal physiology and the purpose of an enteric coating, an ideal enteric coating should possess the following properties: resist dissolution for as long as the dosage form remains in the stomach, impermeable to gastric fluids and drug while in the stomach, rapidly break down in the small intestine, stable during storage, nontoxic, easy to apply as a coating, and not be too expensive.

Almost enteric polymers contain ionizable carboxylic groups. In the gastric fluid, the carboxylic groups remain unionized, and the polymer coatings remain insoluble. In the intestine, the pH increase to 5 and above, allowing

carboxylate groups to ionize, and the polymer coatings dissolve and release their contents. pK_a of enteric polymer and the pH of medium are the main factors influencing the disintegration of coatings. The relationship between the pH of medium and pK_a of enteric polymer is given, in general, by the Henderson-Hasselbach Equation.

$$pK_a - pH = \log\left(\frac{\text{concentration of unionized form}}{\text{concentration of ionized form}}\right)$$

In general, the enteric polymer will be unionized and insoluble at pH values 2 units below its pK_a and completely ionized and soluble at pH values 2 units above its pK_a. Based on the range of pH of 1.0-3.5 in the stomach and 3.8-6.9 in the small intestine, the idea pK_a for enteric polymers would be in the range of 3.5-5.0 (Tarcha 1999: 39-66).

1.5 Enteric polymers

1.5.1 Synthetic polymer

1.5.1.1 Cellulose esters

Cellulose acetate phthalate (CAP) is the oldest of synthetic coating polymer used for enteric coating; it is prepared by reacting a partial acetate ester of cellulose with phthalic anhydride. Although CAP is the most wildly used for enteric coating, it has a number of disadvantages such as high pH dissolution (pH > 6), water vapor and gastric permeability, easily to hydrolysis during storage (Hogan 2002). CAP coating solution is prepared by dissolving in acetone, acetone/ethanol, acetone/methanol (1:3), or ethyl acetate/isopropanol and plasticizer is required.

Hydroxypropyl methylcellulose phthalate (HPMCP) is the newest of the phthalate-containing polymer used as enteric coating. It is prepared by hydroxypropyl methylcellulose with phthalic anhydride. HPMCP has various grades, including HP-50, HP55, HP55S, HP55F. The number that follows the HP letters indicates the pH of dissolution. Thus HP-50 and HP-55 dissolve at pH 5.0 and 5.5, respectively. HPMCP shows better solubility and stability as compared to CAP; however it is permeable to moisture and gastric fluid. For coating solution preparation, the best solvents for HPMCP are methylene chloride/ethanol, ethanol/water with or without plasticizer (Tarcha 1999: 39-66).

Hydroxypropyl methylcellulose acetate succinate (HPMCAS) is manufactured by Shin-Etsu, Japan. There are 3 types of HPMCAS that vary in their degree, type of substitution, and solubility at various pH values from 5 to 7. AS-LG (LF) and AS-MG (MF) dissolve at pH 5.0 and 5.5, respectively. While AS-HG (HF) dissolves at higher pHs and is used in sustained release coating. HPMCAS is more stable and flexible than CAP and HPMCP. This polymer is soluble in acetone, methanol, methylene chloride/ethanol, and ethanol/water (8:2). In addition, plasticizer is not essential.

1.5.1.2 Methacrylic acid copolymers (EUDRAGIT®)

The grades of methyl acid polymers used in enteric coatings are methacrylic acid methylmethacrylate copolymer (EUDRAGIT® L 100 and EUDRAGIT® S 100) and methacylic acid ethyl acrylate copolymer (EUDRAGIT® L 30D-55). EUDRAGIT® L 30D-55, EUDRAGIT® L 100 and EUDRAGIT® S 100 dissolve at pH 5.5, 6, and 7, respectively (Schreier 2001). The advantages of

EUDRAGITs are good storage stability against hydrolysis, not absorbed, not degraded, and excreted unchanged (Tarcha 1999: 39-66).

1.5.1.3 Polyvinylacetate phthalate (PVAP)

PVAP is prepared by the esterification of a partially hydrolysed polyvinyl acetate with phthalic anhydride. PVAP is not solvable in low pH media but dissolves immediately at pH 5. It demonstrates better water and gastric fluid protection, more resistant to hydrolysis, as compared with CAP. The good solvents for PVAP are methanol, ethanol, acetone/methanol, or methanol/methyl chloride, however, plasticizer is necessary.

1.5.2 Natural polymer

Shellac has been used as enteric polymer in pharmaceutical industry over the past several decades. It is applied from alcoholic and aqueous solutions. Alkaline shellac solution shows excellent film forming properties, easily preparation and good mechanical properties, however, the pharmaceutical application of shellac is greatly declined in present because of its characteristic (Karsa et al. 1996). Shellac and its details are elaborated on further topic.

2. Shellac

Shellac is the oldest natural polymer that has been used as enteric coating material. Lac is the name given to the resinous exudation of the tiny lac insect which is parasitic lived on tropical trees in Asia, particularly Thailand, India, and Myanmar.

The first scientific name of the lac insect, given by J.Kerr in 1782, was *Tachardia lacca* following the name of French Missionary Father “Tachardia”. It was

changed to *Laccifer lacca* (Kerr) and the other name was given as *Kerria lacca* (Singh 2006).

Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Hemiptera
Suborder	:	Homoptera
Super family	:	Coccoidea
Family	:	Lacciferidae
Genus	:	*Laccifer*
Species	:	*Lacca*

There are 85 species under 9 genera of lac insects found all over the world. However, the commonest occurring species of lac insect is *Laccifer lacca* (Kerr) which secretes commercial lac and *Kerria chinensis* is the other species which is found in Thailand and south-east Asia (Eastaugh et al. 2008; Bechtold and Mussak 2009).

2.1 Lac insect

The lac insect parasitically grows on the tropical trees, called “host plants”. Although lac insect is natural pest on host plant, it causes only impermanent and recoverable damage to the host plant. In Thailand, approximately 30 varieties of host plants are referred as host plant. For example, *Samanea saman*, *Butea monosperma*, *Cajanus cajan*, *Schleichera oleosa*, *Combretum quadrangulare*, *Uncaria gambir*, *Dalbergia cochinchinensis*, *Drypetes roxburghii*, *Zizyphus mauritiana*, *Flemingia lineate*, *Ficus racemosa*.

The life cycle of lac insect takes around half year. Every lac insect goes through four stages of development: egg, instars nymph, pupa and adult. A minute crawling scale lac insect finds a suitable branch of host plant. Female lays 200-500 eggs. Eggs hatch within a few hours, and then crawlers come out. The appearance of nymph may continue for 5 weeks. Nymphs, which live on the sap of the host plant, insert their suctorial beaks into plant tissue and suck the sap, juices and nourishment. Afterward, they grow in size and resinous material is secreted from the nymph body. A number of crawlers locate side by side. Their own bodies are covered with lac in the so called “Cell” and completely encase the twig. The resinous lac hardens on exposure to air which protects lac insect from predators. The lac insect larvae become mature after three molts in about 8 weeks. Only the male transforms to a complete another form; it loses beak and develops antennae, legs and a pair of wings. While the female retains her mouth parts but fails to develop any wing, eye and appendages (Figure 4). After fertilization, the increasing of female size is suitable for growing number of eggs and she assumes a sac like appearance. The female dies, the eggs hatch, the crawlers shift to a close uninfected area of the twig, and the process is repeated (Figure 5) (Jabde 2005; Singh 2006).

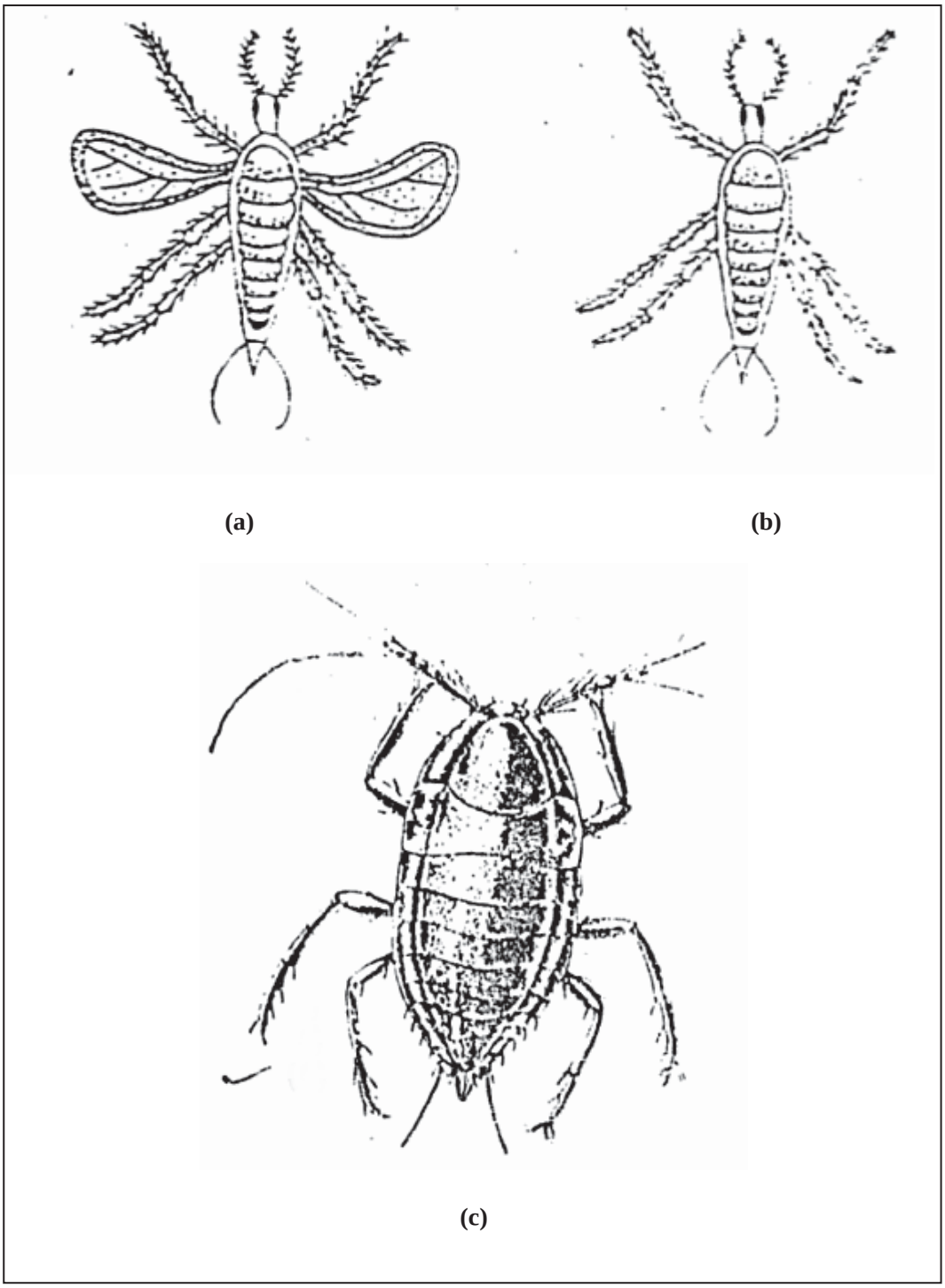


Figure 4 Lac insect; male with wings (a), male without wings (b), and female (c)

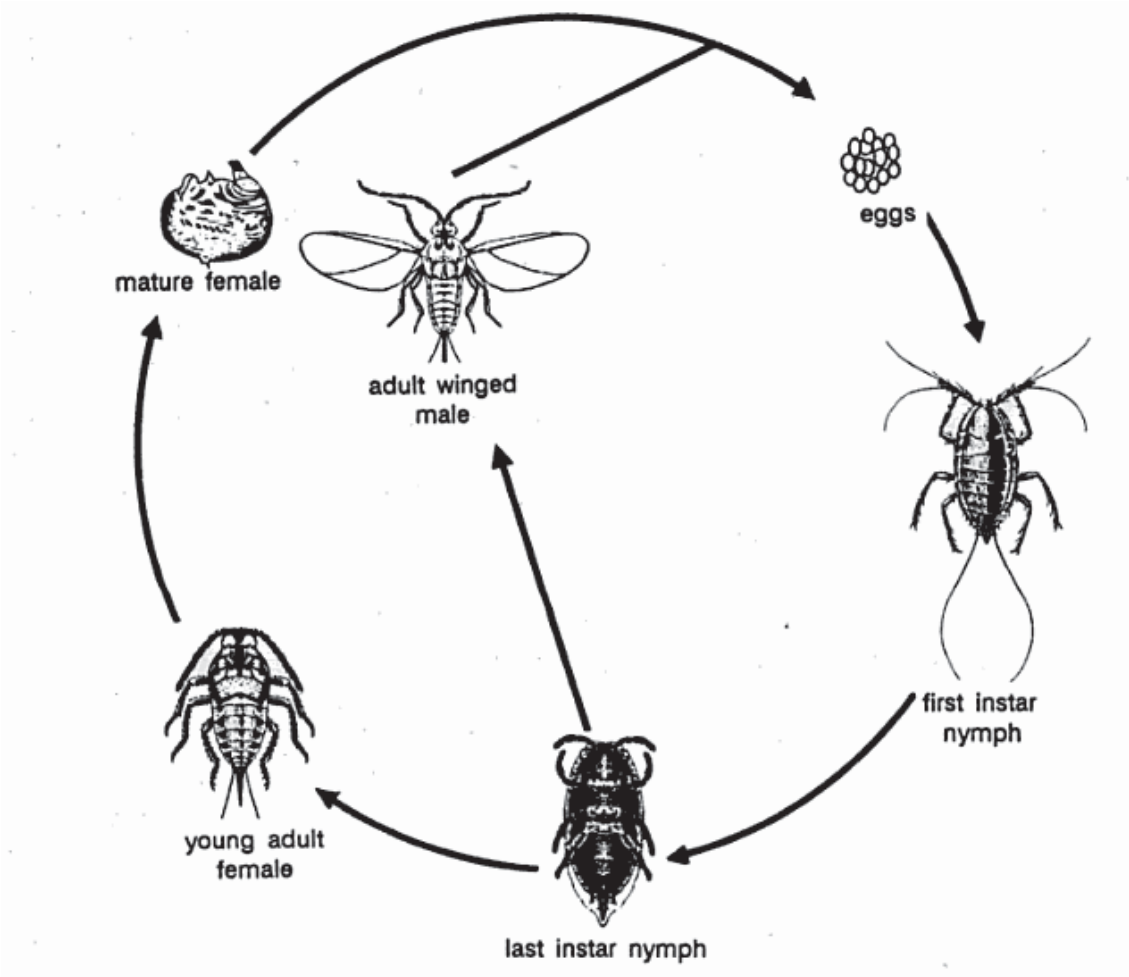


Figure 5 Life cycle of lac insect

2.2 Extraction of Lac

Lac cultivation is simple, no any large investment requirement. Harvesting is done by removing the lac encrusted twigs. The raw lac is known as “stick lac” (Figure 6a). The stick lac composes of resin, insect body, sand, coloring matter (lac dye) and twig debris. The stick lac become lump and the quality of lac is decrease by long term storage, so this problem can protect by keeping high moisture content of stick lac or converting into “seed lac”. Seed lac, which is granular lac, is prepared by grinding stick lac in crude mortar, sieving to remove sand and washing to

wash out the lac dye and twig debris. The general appearances of seed lac are yellow or reddish brown and small seed (about 10 mesh or smaller) (Figure 6b). In addition, the seed lac is washed, melted, spread out in a thin layer film and dried. The product obtained is called “shellac” (Figure 6c).

2.3 Shellac processing

Shellac is produced by three methods; hand made process, heat process or solvent process.

2.3.1 Hand made process

Seed lac is processed into shellac by hand that is traditional process. Seed lac is filled into long cloth bag and melts by charcoal-fired hearth. Molten lac is squeezed from twisting the bag and spread into thin sheets. Moreover, the molten lac is allowed to solidify in form of discs, and then it is known as “button Lac” (Figure 6d). Examples of hand made shellac are lemon one shellac, standard one shellac, superior shellac, etc.

2.3.2 Heat process

This process is utilized in manufacturing level. The seed lac is liquefied by hot stream and the molten lac is filtered by hydraulic pressure machine. Shellac sheet is prepared by drying the filtered molten lac and then broken it in the form of pieces called “flakes”. Many grades of machine made shellac, for example, orange shellac, orange fine shellac, lemon one shellac, lemon two shellac, etc.

2.3.3 Solvent process

Seed lac is dissolved in ethanol and then impurity and wax are removed by filtering through filter. Alcohol is recovered and the residue shellac is

stretched with a roller. The shellac products from this process are dewaxed platina, dewaxed blonde, dewax lemon, or dewax orange shellac.

Moreover, bleached shellac is produced by solvent process with base solution. The bleached shellac is prepared by dissolving seed lac in sodium carbonate solution at a high temperature, stirring with bleaching agent (such as sodium hypochlorite) and then filtering after cooling. The commercial bleached shellac are dewaxed bleached shellac and waxy bleached shellac.

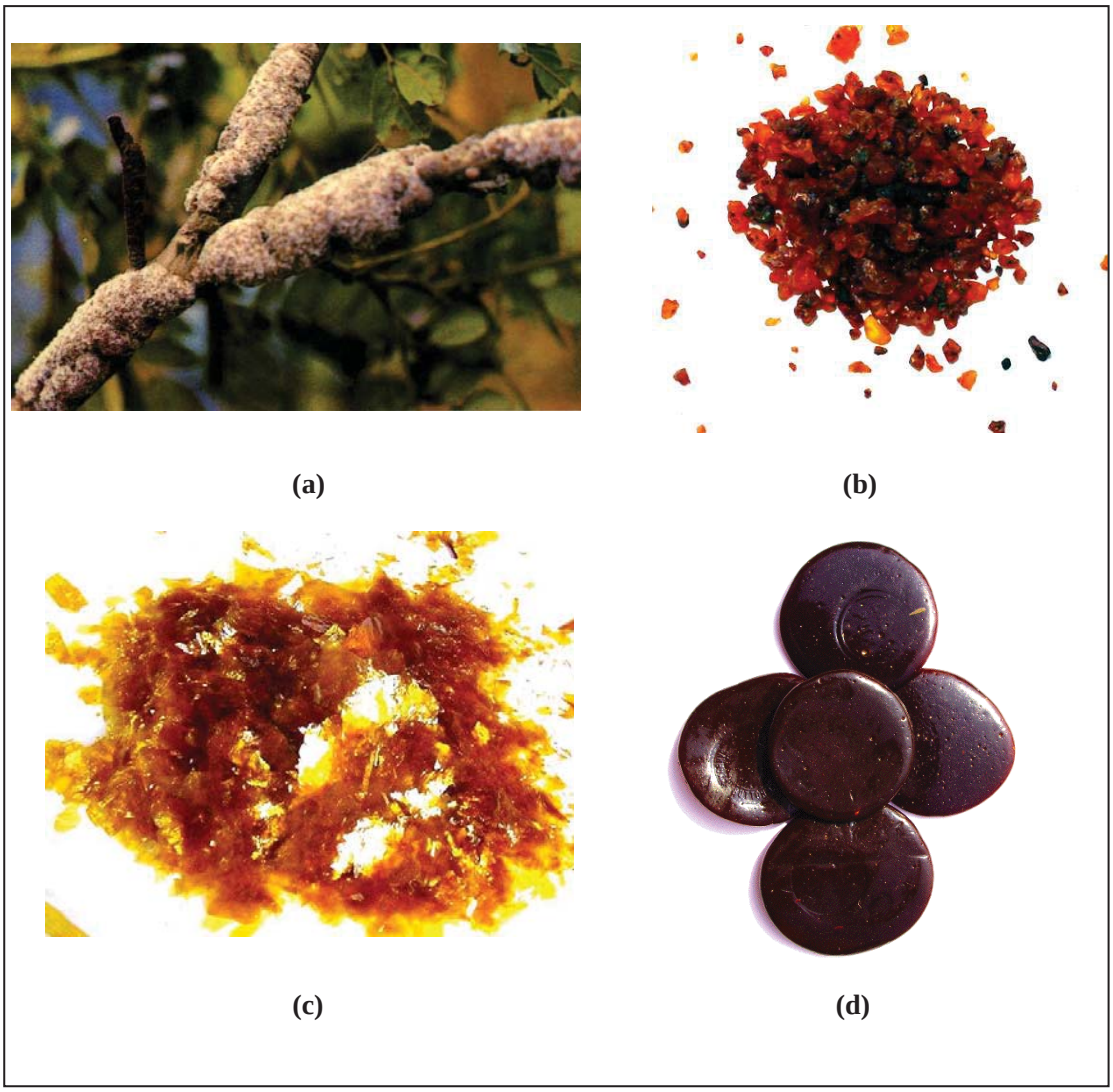


Figure 6 Stick lac (a), seed lac (b), shellac (c), and button lac (d)

According to the United State Pharmacopoeia-National Formulary (USP 30-NF 25), shellac is categorized into four grades: orange, dewaxed orange, regular bleached, and refined wax-free bleached shellac (United States Pharmacopeial Convention 2007: 3417). The grades differ in the manner in which the seed lac is treated. Orange shellac is obtained by the evaporation of filtered ethanolic solutions of seed lac. It may be dewaxed by further filtration to achieve dewaxed orange shellac. Regular bleached shellac is obtained by the method described above. The resin is removed by sulfuric acid precipitation after bleaching process. Refined wax-free bleached shellac required another filtration step to remove the waxes.

2.3.4 Chemical structure of shellac

The exact chemical composition of shellac is unknown. It appears to be composed of a network of hydroxy fatty acid esters and sesquiterpene acid esters with a molecular weight of about 1000. Shellac is always associated with odorous compound, wax and a mixture of dyes such as erythrolaccin and desoxyerythrolaccin (hydroxyanthraquinone derivatives). Three main components of shellac are hard resin, soft resine and wax. Both soft and hard resins contain hydroxyl groups. The composition of shellac depended on the source and harvest time of the stick lac. The variability may be a problem for commercial use of shellac. The physical properties of shellac are also varied. For example, the reported melting point is ranged from 77°C to 120°C (DerMarderosian and Beutler 2002), specific gravity is between 1.14 to 1.21, acid value is 65-75 and saponification value is 220-230 (Sharma et al. 1983: 261-271).

Table 1 Solubility of shellac

Solvent	**Solubility at 20ºC**
Alkalis	Soluble
Aqueous ethanolamine solution	Soluble
Benzene	1 in 10
Ethanol	1 in 2
Ethanol (95% v/v)	1 in 1.2 (very slowly soluble)
Ether	1 in 8
n-Hexane	Practically insoluble
Propylene glycol	1 in 10
Water	Practically insoluble

Source : Raymond C. Rowe et al., Handbook of pharmaceutical excipients (Michigan : Pharmaceutical Press, 2006), 650.

After alkaline treatment (Sharma et al. 1983: 261-271), shellac are hydrolysed to mixture of hydroxyl aliphatic and terpenic acids. The proportion between aliphatic and terpenic acids is about 50:50. The aliphatic acids are insoluble in water; the main constituent is aleuritic acid (35%). While the terpenic acids are easily soluble; the main constituent is jalaric acid (25%). Other acids are shellolic/epishellolic, laccijalaric and butolic acid (8%). The structure of shellac and its constituent acid are shown in Figure 1.

2.3.5. Properties of shellac

2.3.5.1 Low water vapor permeability

Moisture-protective polymer should prepare film which can prevent water vapor from entering the coated products. Several studies reported that shellac had the better effective barrier to water vapor, as compared to other polymers (Nantarat Pearnchob and Bodmeier 2003: 363-369; Bley et al. 2009: 59-65). Hence this characteristic of shellac was the important reason for utilizing shellac as excellent moisture barrier.

2.3.5.2 pH-dependent solubility

Because the structure of shellac consists of carboxyl groups, shellac is not dissolved at the acidic pH of the stomach and dissolved at a higher pH of the intestine (above pH 7.0) (Gad 2008). Therefore, shellac could be applied as enteric coating polymer.

2.3.5.3 Gloss

Gloss is an optical property, which is based on the interaction of light with physical characteristics of a surface. The factors that affect gloss are the refractive index, the surface topography and the angle of incident light. Shellac has high refractive index (1.5210-1.5272) and gloss (Rowe et al. 2006), so shellac is extensively applied for coating of woodwork and fruit because of this attractive property.

2.3.5.4 Low thermal conductivity

Shellac has a low thermal conductivity. It also has excellent dielectric properties, high dielectric strength, a low dielectric constant and a

good tracking resistance (Berger and Sicker 2009). Shellac was used as an insulator for several decades.

2.3.5.5 Good adhesive property

Alkaline solution and alcoholic solution of shellac provide spreadable films of high adhesive power. Shellac films exhibit good to excellent adhesion to a wide range of surface except Teflon and silicone coated glass (Ghosh 2008).

Based on these properties, shellac has been developed for various applications. Shellac is often used as a finish for fine furniture, an aqueous varnish for paper, wood and leather and an additive for some cosmetic products. Furthermore, shellac has been used for almost 100 years by the pharmaceutical industry. Examples of pharmaceutical applications include protective coating, enteric coating, microencapsulation and matrix forming agent for tablet (DerMarderosian and Beutler 2002). For enteric coating, shellac has been used since 1930.

Wruble reported that ammonia solution of shellac provide the best enteric coating, among a number of investigated solutions (Wruble 1930: 318).

Because of the disadvantages of shellac as a coating material is batch to batch variation. In addition, shellac undergoes an “aging effect” upon storage. The delayed disintegration and reduced solubility of shellac on storage is attributed to polymerization resulting from transesterification of the hydroxyl group of shellac molecule with the carboxyl group of other adjacent shellac molecule (Wruble 1930: 318).

The other disadvantage of shellac is poor solubility. The low amount of carboxylic acid group per shellac molecule and the high pK_a lead to the low intestinal

solubility. Since the pH in the small intestine, where most drug release is required, is between 3.8-6.9, failure of shellac-coated tablets to disintegrate at pH values below 7.0 is still a major disadvantage (Tarcha 1999: 39-66), and thus shellac has fallen into disfavor as the enteric coating material (DerMarderosian and Beutler 2002).

Pearnchob et al. developed the shellac-coated soft gelatin capsules that demonstrated ion faster disintegration in phosphate buffer pH 6.8 by the addition of pore-formers, such as organic acids and hydrophilic polymer. Ethanolic and aqueous shellac solutions were prepared and comparatively evaluated. Incorporation of additives effectively decreased the disintegration times in phosphate buffer pH 6.8, while the behavior in 0.1 N hydrochloric remained unchanged. The best disintegration was achieved with sorbic acid as pore-former. The disintegration time of ethanolic shellac-coated soft gelatin capsules decreased with increasing amount of pore-former. The longer disintegration time of aqueous shellac-coated soft gelatin capsules could be also decreased by the addition of hydroxypropyl methylcellulose (HPMC) (Nantarat Pearnchob et al. 2004: 313-321). Similarly, Qussi and Suess investigated aqueous shellac-coated pellets containing different amounts of polyvinyl alcohol, hydroxypropyl methylcellulose, and carbomer. The results showed that all coating systems could prevent the dissolution of drug in simulated gastric juice for 2 h and rapid release of drug within 45 min in simulated intestinal fluid. Moreover, polyvinyl alcohol and hydroxypropyl methylcellulose showed a positive effect on the stability of shellac-coating systems after storing at room temperature and 40-45 % RH for 3-12 months (Qussi and Suess 2005: 99-108).

Limmatvapirat et al. modified the structure of shellac by partial hydrolysis with 2% w/w sodium hydroxide solution at various times. The partially hydrolysed

shellac, having more carboxylic acid groups, demonstrated the solubility improvement nearby pH 7.0. The ammonium base film of hydrolysed shellac was more effective than ammonium base film of ethanol-based film, in term of better drug dissolution (Sontaya Limmatvapirat et al. 2004: 41-49). Furthermore, the enteric properties of shellac were improved by composite salts formation. The shellac samples were prepare by dissolving with 1-amino-2-methyl-1-propranol (AMP) and ammonium hydroxide (AMN) for different ratio of AMP:AMN. The results demonstrated that shellac was partially dissolved at pH 7.0 and completely dissolved at pH 7.3 and higher. The percent dissolved of shellac salts was significantly increased while the dissolving time was significantly decreased with increasing AMP content. The stability of shellac films was investigated by monitoring of insoluble solid and the change of percent dissolved at pH 7.0. After storage, the 60:40, 80:20, and 100:0 AMP:AMN salts did not show a clear increment of insoluble solid and the percentage of dissolved films was not changed with increasing storage time. The results indicated that the polymerization, which was the cause of instability, was inhibited after salt formation (Sontaya Limmatvapirat et al. 2007: 690-698). However, most research of shellac has just only concentrated on modification of carboxylic acid while modification of hydroxyl group has not been clearly investigated.

In addition, a number of researches reported the enteric properties improvement of polymers was achieved by reaction with cyclic anhydride (CAHs). Hydroxypropylmethylcellulose phthalate (HPMCP), prepared from esterification of hydroxypropylmethylcellulose (HPMC) with phthalic anhydride, was insoluble in simulated gastric fluid and provided protection against dissolution of the drug. The HPMCP coated products showed rapid drug release at upper intestinal pH (~ 5.5)

(Meehan 2006: 2-6; Rowe et al. 2006). Some natural polymers also demonstrated their enhanced solubility after reaction with CAHs. N-deacetylated chitin (DAC) was prepared via ring-opening reactions with various CAHs in lithium chloride/N, N-dimethylacetamide (LiCl/DMAc) system. The solubility of DAC derivatives was increased. Succinyl DAC-20 and phthaloyl DAC-20 were soluble at the pH range from 4.5 to 11.0 and 3.0 to 11, respectively (Shigemasa et al. 1999: 237-243). Moreover, the structure of starch was modified by esterification with succinic anhydride. The results demonstrated the thermal stability of starch succinate was changed. With an increasing in degree of substitution, thermal stability increased. The greater stability of the modified starch was explained by the lower amount of remaining hydroxyl groups after esterification. Since the decomposition mechanism of starch was the dehydration reaction among the hydroxyl groups of starch, the decrement of hydroxyl groups caused better thermal stability of the starch ester (Rudnik et al. 2005: 163-166). The esterification of corn starch by using other CAHs including dodecenyl succinic anhydride (DDSA) and octenyl succinic anhydride (OSA) was also reported. The mechanical properties; tensile strength, Young's modulus; of the film prepared from modified starch depended on thickness of surface esterification layer and the degree of substitution (DS). The starch films modified by using DDSA were more strong and rigid while the films modified with OSA were more flexible and ductile. OSA, which has shorter alkenyl group chain length, could give a higher DS, enhanced side chain mobility and reduced the number of free hydroxyl groups in starch molecule that could weaken interaction of starch molecule, resulting in high elasticity as compared to DDSA. Thus, the modification starch through esterification by using anhydride could affect starch structure and their

molecular mobility, resulting in change of mechanical properties (Ren et al. 2010: 1010-1013). In addition, the increasing carboxylic groups improved solubility and stability of starch. As shellac consists of a large number of hydroxyl groups, the esterification with CAHs may be an alternative way for improving enteric properties and stability of shellac.

3. Cyclic anhydrides (CAHs) (Kim et al. 2009)

An acid anhydride is a compound that formally arises by loss of H_2O between two oxoacid functions (Bunzli-Trepp 2007). The basic formula of CAHs is R-CO-O-CO-R´ (Morris 1992; Smith 2010). Succinic anhydride, phthalic anhydride and trimellitic anhydride is member of CAHs that utilized in this study by esterification with shellac.

3.1 Succinic anhydride (SUCA)

Formula: $C_4H_4O_3$

Molecular structure: 

Molecular Weight: 100.07 g/mol

Synonyms: Dihydro-2,5-furandione

Succinyl Peroxide

Succinyloxide

Dihydro-2,5-diketotetrahydrofuran

Succinic acid anhydride

Tetrahydro-2,5-dioxofuran

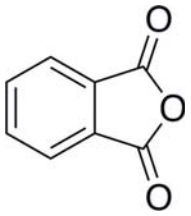
2,5-dioxotetrahydrofuran

	Butanedioic anhydride
Physical state:	White flakes
Melting point:	119.0-120.0 ºC
Boiling point:	261.0 ºC
Density:	1.572 g/cm^3
Vapor density:	3.5 (air = 1 at boiling point of compound)
Flash point:	157.0 ºC
Solubility:	2.40 g/100 mL water at 25 ºC
	2.56 g/100 mL ethanol at 25 ºC
	0.64 g/100 mL ether at 25 ºC
	0.87 g/100 mL chloroform at 25 ºC
Toxicity:	Oral rat LD50: 1510 mg/kg
	Eye rabbit: 0.75 mg (severe eye effect)

3.2 Phthalic anhydride (PHTA)

Formula: $C_8H_4O_3$

Molecular structure:



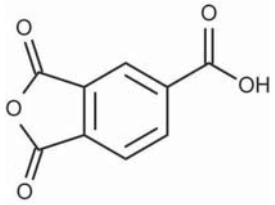
Molecular Weight:	148.12 g/mol
Synonyms:	Phthalic acid anhydride
	1,3-Isobenzofurandione
	Isobenzofuran-1,3-dione
	1,2-Benzenedicarboxylic acid anhydride

	1,2-Benzenedicarboxylic anhydride
	1,3-Dihydro-1,3-dioxoisobenzofurane
	1,3-Dioxophthalane
	1,3-Phthalandione
	Phthalandione
Physical state:	White flakes or needles
Melting point:	131.6 ºC
Boiling point:	284.5 ºC
Density:	1.527 g/cm^3
Vapor density:	5.1 (air = 1 at boiling point of compound)
Flash point:	152.0 ºC
Solubility:	0.62 g/100 mL water at 20 ºC
	Very slightly soluble in cold water
	Soluble in alcohol
	Soluble in ether
Toxicity:	Oral rat LD50: 4020 mg/kg
	Inhalation rat LC50: > 210 mg/m^3/h
	Skin rabbit LD50: > 10 g/kg

3.3 Trimellitic anhydride (TMTA)

Formula: $C_9H_4O_5$

Molecular structure:



Molecular Weight:	192.13 g/mol
Synonyms:	Trimellitic acid anhydride
	Benzene-1,2,4-tricarboxylic-1,2-anhydride
	Benzene-1,2,4-tricarboxylic acid 1,2-anhydride
	1,2,4-Benzenetricarboxylic acid 1,2-anhydride
	Anhydrotrimellitic acid
	1,2,4-Benzenetricarboxylic acid anhydride
	1,2,4-Benzenetricarboxylic anhydride
	4-Carboxyphthalic anhydride
	1,3-Dioxo-5-phthalancarboxylic acid
	Trimellitic acid 1,2-anhydride
	Trimellitic acid cyclic 1,2-anhydride
Physical state:	White flakes or needles
Melting point:	161.0-163.5 ºC
Boiling point:	240.0 ºC
Density:	1.54 g/cm^3 at 20 ºC
Vapor density:	6.6 (air = 1 at boiling point of compound)
Flash point:	227.0 ºC
Solubility:	49.6 g/ 100 mL acetone
	38.4 g/ 100 mL cyclohexanone
	36.5 g/ 100 mL 2-butane
	21.6 g/ 100 mL ethyl acetate
	15.5 g/ 100 mL dimethylformamide

	0.4 g/ 100 mL xylenes
	0.1036 g/ 100 mL water
	0.002 g/ 100 mL carbon tetrachloride
Toxicity:	Oral rat LD50: 2730 mg/kg
	Inhalation rat LC50: > 2330 mg/m^3/h
	Skin rabbit LD50: > 2 g/kg

4. Green Chemistry

If two or more elements or chemical compound come into contact with the enough energy, a chemical change may take place. The chemical change can be called a chemical reaction (Lew 2008; Wolny 2011). Most chemical reactions have utilized organic solvents as media for synthesis. For examples, a modification of polymer with cyclic anhydrides, the organic solvent, e.g. acetone, ethanol and toluene was famously used in a synthesis process such as chitosan succinate (AlKhatib et al. 2008: 804-812), polyvinyl acetate phthalate (Nesbitt et al. 1985: 215-226), poly(styrene-alt-maleic anhydride) (Lai et al. 2008: 66-73), respectively. However, organic solvents showed several disadvantages, including toxicity of solvent to worker and environment, destruction of ozone, miscarriages and birth defects from exposure to solvents, fires, explosion and high cost (Matlack 2010). Therefore, many countries have faced with environmental problems, thus a number of scientists have attempted to process under the concept of development the novel synthesis “green chemistry”.

Green chemistry, also called sustainable chemistry, is environmentally friendly chemistry. It covers such concept as: the design of process to maximize the amount of raw material that ends up in the product , the use of safe, environment

benign substances, including solvents, whenever possible, the design of energy efficient processes and the best form of waste disposal (do not create it in the first place).

The twelve principles of green chemistry are listed below (Doble and Kruthiventi 2007; Lancaster 2010).

1. Prevention; it's better to prevent wastes than to treat or clean them up after it has been formed during process.

2. Atom economy; synthesis reaction should be designed to maximize the incorporation of all materials used in the process into the final product.

3. Less hazardous chemical synthesis; synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to people, public and the environment.

4. Designing safer chemicals; chemical products should be designed to perform their function while minimizing their toxicity.

5. Safer solvents and auxiliaries; the use of auxiliary substances (such as solvents or separation agents) should be made unnecessary. If their use cannot be avoided, they should be used as innocuously as possible.

6. Design for energy efficacy; energy requirements should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. Use of renewable feedstock; a raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. Reduction of derivatives; utilization of blocking group, protection/de-protection, and temporary modification of physical/chemical processes should be minimized if possible.

9. Catalysis; catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

10. Design for degradation; chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. Real-time analysis for pollution prevention; analytical methods need to be improved to allow for real-time, in-process monitoring and control prior to the formation of hazardous materials.

12. Inherently safer chemistry for accident prevention; substances and the form of a substance used in a chemical process should be chosen to reduce the potential for chemical accidents, including releases, explosions, and fires.

“Solid state reaction”, is also a reaction that obeys the principles of green chemistry. The detail of the solid state reaction, including definition, step and types, would be discussed as follows.

5. Solid state reaction

A solid state reaction (a dry media reaction, a solventless reaction or a solvent-free reaction) is a chemical reaction in the absence of a solvent. In a normal reaction, the reactants are placed in a solvent before the reaction occurs. These reactants react and a new substance can appear. After the completion of reaction, the

new product is removed from the solvent. However, a solid-state reaction allows the reactants to chemically react without the solvent.

Solid state reaction provides a number of advantages. The elimination of solvents could cut off the budgets of starting material. This will make the products cheaper. Usually, the residual solvent must remove from the product after a solvent reaction has finished. For solid state reaction, this step is eliminated and therefore the purification time is decreased. Additionally, eliminating of solvent from the reaction means that reactants could more intimately contact each other and the yield of products is improved. The solid state reaction is also environmentally friendly. Since the lack of organic solvent, there is no harmful waste to eliminate at the end of the reaction. However, the limitation of solid state reaction is a high viscosity in reactant system that is sometimes hard to be handling. The reactants should be mixed to a homogeneous system. Additionally, the reaction is not suitable for solvent assisted chemical reaction.

The solid state reaction generally involved with these four steps (Gad 2008):

1. Loosening of molecules at the reaction site. It is reasonable to assume that crystal imperfection achieves the mobility required to accomplish the next step.

2. Molecular change. This step is the actual reaction of interest and is similar to the corresponding solution reaction where the bonds of the reactants are broken and the bonds of the product are formed.

3. Solid solution formation. During the early stages of the reaction, a small amount of the product in the starting crystal is formed at the site of reaction and solid

solution is randomly mixed. However, after enough product forms, the products will separate.

4. Separation of product. This step gives a new phase that can be crystalline or amorphous. The new compound is either randomly oriented or with an orientation governed by the crystals of the starting material.

There are several conditions, affecting solid state reaction, as follows (West 1991; Byrn et al. 2001: 115-136).

1. Reagents

The starting materials are selected, depending on the reaction types and the expected nature of product. The reactants should be dried thoroughly prior to weighing. As increase in surface area enhance the reaction rate, fine powder reactants are used if possible.

2. Mixing

After the required amounts of reactants have been weighed out, they must be throughly mixed. For manual mixing of small amounts (≤ 20 g total), a pestle and mortar are usually employed. In order to mix large quantities of the reactants, a mechanical mixing is adopted using a planetary ball mill. The effect of grinding is to maintain a high surface area, as well as increase new contacting surfaces. This process may take several hours.

3. Container material

For the subsequent reaction at high temperature, it is necessary to choose a suitable container material which is chemically inert to the reactants under the heating conditions used. The noble metals, platinum and gold, are usually suitable. Platinum has higher melting point about 1700 ºC and is considerably harder, as

compared with gold (1063 ºC). Gold-based alloys such as Au-Pd are sometimes used as they are harder than pure gold. For low temperature reactions, other metals like nickel (below 600-700 ºC) can be used.

4. Heat treatment

The heating program is used, depending on the form and reactivity of the reactants. The reaction rate may increase by grinding the samples prior to heating, as a result of enhancing contact area between the materials. In order to speed up reaction rates, the higher temperature is used as possible. However, the problems may occur at high temperature such as volatilization and instability of one or more components of the reacting mixture.

A number of studies have reported that solid state reaction was accelerated by irradiation (radiation-induced reaction) and annealing with oven (thermally-induced reaction) (Thomas 1979: 1065-1082).

5.1 Radiation-induced reaction

Most photochemical and other radiation induced reactions have been studied at room temperature or above. The atom or molecule of materials must absorb light then the photons of light activate the substance molecule. When a photon is absorbed, it transfers energy to the absorbing molecule, the molecule is excited to a higher energy state. The advantages of radiation-induced reaction are clean reaction (no photon residues), reduced reaction time (rapid energy transfer) and high selectivity and yields. However, the limitations of this method are high equipment cost and not applicable for production scale (Kappe et al. 2009; Lancaster 2010).

Several studies reported the radiation-induced reaction through solid state reaction. Byrn et al. reviewed the solid state reaction of drug substances. The

oxidation of three model peptides (DL-Ala-DL-Met (a zwitterionic dipeptide), *N*-formyl-Met-Leu-Phe methyl ester (a neutral tripeptide), and Met-enkaphalin acetate salt (a weakly acidic pentapeptide)) in crystalline and amorphous forms were investigated by using UV radiation (254 nm). The results demonstrated that the oxidation of both forms of each peptide could take place. However, the amorphous material showed faster oxidation and degradation as compared to the crystalline material because it had more mobility (Byrn et al. 2001: 115-136). The other radiation-induced reactions were the syntheses of caprolactam and vitamin D_3. The production of caprolactam could be successful by inducing at visible region light (535 nm), using a low-pressure mercury lamp doped with thallium iodide. The vitamin D_3 had also been produced using a photochemical electrocyclic ring opening of 7-dehydrocholesterol at the wavelength of 300 nm (Lancaster 2010). In recent years, microwave irradiation has been applied for solid state synthesis. Kuan et al. synthesized methionine complex of iron by using of microwave oven (2.45 GHz frequency, 700 W powers). A high yield of complex was successfully achieved by employing microwave irradiation under the optimum reaction condition (microwave heat time, 260 s; quantity of reactant, 15 g and the airflow temperature, 100 ºC) (Wang et al. 2008: 315-323). To use microwaves as a source of heating in a solid state reaction, at least one of reactants needs to be microwave susceptible (Lalena and Cleary 2008). This was demonstrated by Safari et al., who prepared 3-arylidenephthalides from quinoline, phthalic anhydride and acetic anhydride. The advantage of microwave was the fast reaction time. The 3-arylidenephthalides could be successfully synthesized after 20-150 s (Safari et al. 2007: 236-240). Li et al. also reported the rapid synthesis of oligosaccharide from phosphoric acid and glucose. The

yield ratio of product was 76.56% after irradiation for only 10 min (Li et al. 2006: 274-281). The mechanism is dielectric materials absorbed microwave radiation and converted it to heat. The microwave effect is microwave-induced ionic transport, as responsible for increased ionic diffusion in dielectric solids irradiated with microwave.

The microwave effect is a non-thermal effect that results in enhanced reaction rates as well as properties in the final product which differ from those obtained by thermal processing such as ceramic processed with microwave heating demonstrated larger grain size, higher density and lower sintering temperature, as compared with thermal processing (Kararli et al. 1995: 1813-1816).

5.2 Thermally-induced reaction

Solid state reactions are carried out by keeping a mixture of reactant at room temperature or elevated temperature. A common technique of thermal solid state reaction is oven technique, which use high temperatures to encourage reactions without solvents. Thermal activation induced solid solution formation, as described above. The advantages of thermally-induced reaction are simplicity of sample preparation, product synthesis and the low cost equipments. On the other hand, the decomposition of thermal-sensitive compound and volatility of component are the disadvantages of this method.

The thermal solid state reaction has been investigated by a number of studies. Zhao et al. studied the esterification of poly (vinyl alcohol) and maleic anhydride through solvent-free reaction. The chemical reaction was achieved by milling and annealing. The presence of ester linkages was evaluated by FTIR spectroscopy and DSC was used for characterizing thermal properties of product

(Zhao et al. 2007: 1353-1356). The formation of poly(isothianaphathene) from phthalic anhydride or phthalide with phosphorus pentasulfide in one step was reported by Asselt et al (Asselt et al. 1995: 65-70). The product was also easily obtained by mixing and heating at elevated temperature ($T \geq 120$ ^{o}C). In addition, Montazerozohori et al. investigated the fast solid state oxidation of thiols by grinding at room temperature. The reaction rapidly occurred within 5 min after grinding (Montazerozohori et al. 2007: 694-702). A variety of reactions could occur through solid state reaction. For examples, benzoylation reactions of phenol and naphthols, bromination reactions of olefins (Toda 2002) and the oxidation reaction of vitamin A (Byrn et al. 2001: 115-136). The other reaction types were inclusion reaction, condensation of carbonyls, electron transfer, cycloadditions, proton transfers, nucleophillic substitutions, oxygenation, aromatic substitutions, hydrogenations, cyclizations, halogen addition reactions, nitrations, elimination, diazotizations, Sandmeyer reactions, carboxylation, cascade reactions and catalyzed reactions (Kaupp et al. 2001: 55-61).

In some cases, the reactants were melted together. The melted reactants interacted in the liquid state and become a paste which consequently changed into hardened solid. As mentioned above, this technique was known as a melt technique. Some reactants were highly reactive in the presence of a gas. Therefore investigators expose the substance to a stream of reactive gas at high temperature. This process is called a gas reaction (Kidwai 2001: 147-151; Toda 2002).

Under the concept of green chemistry, avoiding organic solvents during the solid state reaction lead to a safe, clean, efficient, and economical technology (Kidwai 2001: 147-151). This reaction with the safeguard of the

environment is one of the challenges of the new millennium and interesting innovative approach for present and future time (Tundo et al. 2000: 1207-1228).

Since various chemical reactions are achieved through solid state reaction, so the chemical reactions through this method are interesting. However, the synthesis of shellac derivatives by, solid state reaction was not yet reported. Therefore, the purpose of this study were to modify shellac structure and improve enteric and stability properties by solid state reaction, The shellac and shellac esters was then comparatively evaluated for formation and their film and tableting properties.

CHAPTER 3
MATERIALS AND METHODS

1. Materials

1. Shellac (Thananchai Part., Ltd., Thailand)

2. Succinic anhydride (Lot No. S4949283 747, Merck Darmstadt, Germany)

3. Succinic acid (Lot No. K 38387882 917, Merck Darmstadt, Germany)

4. Phthalic anhydride (Lot No. S4848092 744, Merck Darmstadt, Germany)

5. Phthalic acid (Lot No. S4535398 738, Merck Darmstadt, Germany)

6. Trimellitic anhydride (Lot No. S4764738 706, Merck Darmstadt, Germany)

7. Trimellitic acid (Lot No. S05583 529, Merck Darmstadt, Germany)

8. Ammonia solution (Lot No. K39606032 910, Merck Darmstadt, Germany)

9. Sodium hydroxide (Lot No. B0274298 827, Merck Darmstadt, Germany)

10. Potassium dihydrogen phosphate (Lot No. A837073 707, Merck Darmstadt, Germany)

11. Hydrochloric acid (Lot No. 05 07 0162, Lab scan, Germany)

12. Ethyl alcohol absolute (Lot No. J32T04, Mallinckrodt, Malaysia)

13. Calcium chloride anhydrous (Lot No. 7D027277D, Carlo Erba, France)

14. Sodium chloride (Lot No. 0811292, Ajax Pty Ltd, New Zealand)

15. Tri-Sodium phosphate (Lot No. A0121178 008, Merck Darmstadt, Germany)

16. Methanol (Lot No. K36193607 627, Merck Darmstadt, Germany)

17. Acetonitrile (Lot No. I050291 233, Merck Darmstadt, Germany)

18. Methanol-d4 (Lot No. S5033528 939, Merck Darmstadt, Germany)

19. Orthophophoric acid (Lot No. A3B017, Asia Pacific Special Chemical Limited, Australia)

20. Potassium bromide (Lot No. B0351607 905, Merck Darmstadt, Germany)

21. Paracetamol (Lot No. 0135816, Rhodia organique, France)

22. Microcrytalline cellulose (Complecel® M102 D^{+}) (Lot No. 71268, Mingtai chemical, Taiwan)

23. Sodium starch glycolate (Explotab®) (Lot No. E9963, JRS pharma LP, Germany)

24. Lactose monohydrate (Supertab®) (Lot No. FQ010012 T1120, Fonterra, New Zealand)

25. Silicon dioxide (Aerosil® 200) (Lot No. 5042 0110/069, Degussa AG, Germany)

26. Magnesium stearate (Lot No. YW01M0301, Glaxo Wellcome Vidhyasom, Thailand)

27. PEG 400 (Lot No. 1403539, Sigma-Aldrich, Germany)

28. Talcum (Lot No. 000949, Vidhyasom, Thailand)

29. HPMC (Pharmacoat 606) (Lot No. YW0540102, Shin-Etsu, Japan)

2. Equipments

1. Planetary ball mill (Retsch® PM 100, Germany)

2. pH meter (Mettler Toledo seveneasy®, Switzerland)

3. Hot air oven (Heraeus UT 6200, Germany)

4. Stability environmental chamber (Hotpack, USA)

5. Fourier transform infrared spectrometer (Nicolet 4700, USA)

6. Hydraulic press (Atlas GS15011, USA)

7. Differential scanning calorimetry (Perkin-Elmer Pyris Sapphire DSC, Japan)

8. Nuclear magnetic resonance spectroscopy (Bruker AVANCE 300, Germany)

9. Powder X-ray diffractometer (Rigaku Miniflex II, Japan)

10. High-performance liquid chromatography (Jasco AS-2050 plus, Japan)

11. HPLC column 15cm x 4.6mm, 5μm (Ascentis™ C18, USA)

12. Texture analyzer (Stable Micro Systems TA.XT. plus Texture Analyzer, UK)

13. Disintegration testing apparatus (Sotax DT3, Switzerland)

14. Dissolution testing apparatus (Erweka DT70, Germany)

15. UV/VIS spectrophotometer (Perkin-Elmer Lamda 2, Germany)

16. Friabilator (Yeo Heng Factory, Thailand)

17. Magnetic stirrer and magnetic bar

18. Analytical balance (Sartorius CP 224s, Germany)
19. Moisture balance (Sartorius YTX01L, Germany)
20. Hardness tester (Pharmatest PTB311, Germany)
21. Drop shape analysis (FTA 100, USA)
22. Hot stage microscopy (Mettler Toledo FP82HT, Switzerland)
23. Vortex mixer (Gibthai VX-100, Thailand)
24. Desiccator
25. Coating thickness gauge (Minitest 600B, Germany)
26. Tablet press machine (Yeo Heng Factory, Thailand)
27. Film coating machine (Rama Cota, Narong Karnchang, Thailand)
28. Cube mixter (Yeo Heng Factory, Thailand)

3. Methods

3.1. Preparation of shellac esters

3.1.1 Preparation of shellac-cyclic anhydrides physical mixture

Shellac (SHL)-cyclic anhydrides (CAHs) physical mixture was prepared by simple mixing 144.79 g of SHL with 105.21 g of succinic anhydride (SUCA), 120.97 g of SHL with 129.03 g of phthalic anhydride (PHTA), or 104.00 g of SHL with 146.00 g of trimellitic anhydride (TMTA) in a 1:4 molar ratio using plastic bag.

3.1.2 Preparation of shellac-cyclic anhydrides ground mixture

The ground mixtures of SHL and CAHs were prepared by grinding of corresponding physical mixtures (Figure 7). The physical mixture was filled in a 500 mL stainless steel grinding bowl together with 5 stainless steel grinding

balls having a radius of 30 mm and then ground by planetary ball mill (Retsch® PM 100, Germany). The grinding speed was set to 400 rpm and the reverse rotation interval was 2 min. To avoid excessive heat, the mill was stopped every 10 min and the grinding bowl was dipped into ice bucket until the ground mixture cooled. The total grinding time was fixed to 90 min (excluded cooling time). The ground mixture was then stored in a freezer prior use for next step.

3.1.3 Preparation of shellac: cyclic anhydrides annealing mixture

Esterification of SHL-CAHs was activated by annealing the ground mixtures for various temperatures and times. After annealing at a predetermined period, a minor portion of annealed mixtures was also collected for characterization. The annealed mixtures were washed with purified water to remove excess CAHs. The shellac esters were collected and storage in a freezer before further characterization.

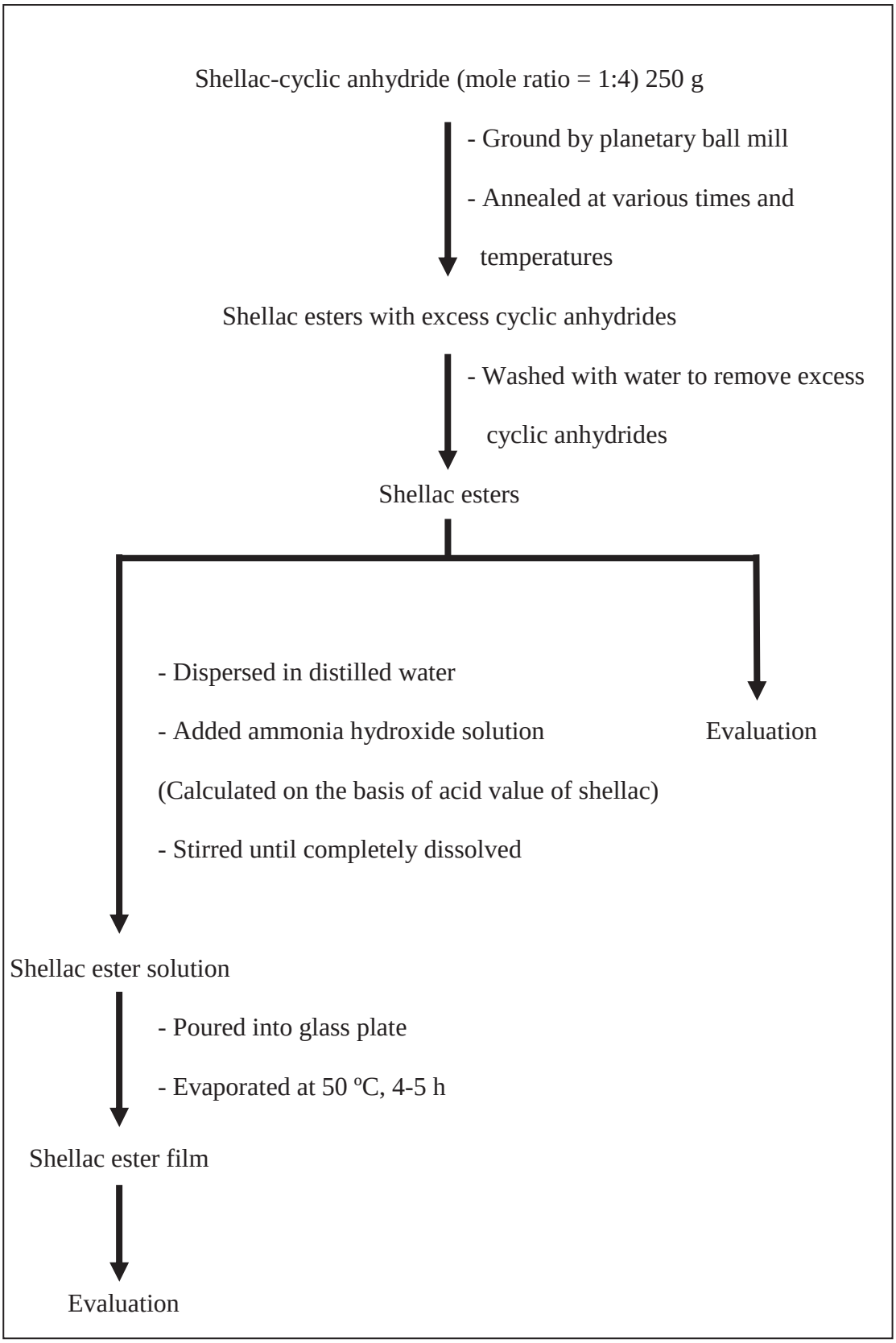


Figure 7 Preparation of shellac esters and their films

3.2 Characterization of shellac esters

3.2.1 Acid value

Acid value (AV) was measured by the method adapted form USP 30-NF 25. An accurately weight of 3 g of shellac sample was dissolved in 95% w/w ethanol overnight and adjusted to the final weight of 39 g with 95% ethanol. The solution was centrifuged at 4500 rpm for 10 min and filtered through Whatman® filter paper No.1. The 26 g of filtrate was titrated with 0.1 N sodium hydroxide VS. The equivalent point was determined by potentiometric titration (pH meter) instead of color indicator due to the dark color of shellac. The acid value was expressed as mg of potassium hydroxide per 1 g of shellac sample. The average of two measurements was used for evaluation.

3.2.2 Alcohol insoluble solid

The insoluble solid on filter paper from 3.2.1 was washed with excess 95% w/w ethanol and dried at 70 ºC for 12 h, and then the percentage of insoluble solid was calculated.

3.2.3 Differential scanning calorimetry (DSC)

The thermogram of shellac sample was determined by DSC using indium as a standard. About 4-5 mg of powder sample was accurately weighted and placed in a closed aluminium solid pan. The aluminium pan was then transferred into the furnace. The thermal behavior of sample was determined at heating rate of 10 ^{O}C per min from 10-250 ^{O}C using an empty closed aluminium solid pan as a reference. The measurement was done under nitrogen gas at a flow rate of 10 mL per min. DSC measurements were performed in duplicate.

3.2.4 Fourier transformed infrared (FTIR) spectroscopy

FTIR spectra of shellac samples were recorded with FTIR spectrophotometer (Nicolet 4700, Thermo Electron Corporation, USA) using the KBr disc method. Each sample was dried over silica gel and pulverized. The ground sample was blended with KBr powder and then compressed with pressure of 5 tons for 30 seconds. The KBr discs were placed in the sample holder and scanned from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}.

3.2.5 Nuclear magnetic resonance (NMR) spectroscopy

Each shellac sample was dissolved in methanol-d_4 to obtain solution. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 Spectrometer operating at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR, using residual solvent peaks at δ_H 4.84 and at δ_C 49.05 ppm as chemical shift reference signals. The chemical shifts were reported in δ(ppm).

Percent substituted at hydroxyl group of shellac ester was estimated by ^{1}H NMR, as compared to that of SHL. % substituted was calculated from the equation:

$$\% \text{ substituted at OH} = \left(1 - \frac{I_{\text{-CH-OH group of shellac ester}} / I_{\text{-CH=C- in cyclohexene of shellac ester}}}{I_{\text{-CH-OH group of SHL}} / I_{\text{-CH=C- in cyclohexene of SHL}}} \right) \times 100$$

where

$I_{\text{-CH-OH group of shellac ester}}$ was the number resulting from division of the integrals of hydrogen of -CH-OH group at 3.0-3.6 ppm from shellac ester structure by amount of proton (=7),

$I_{\text{-CH=C- in cyclohexene of shellac ester}}$ was the number resulting from division of the integrals of hydrogen of –CH=C- group at 6.3-6.7 ppm from shellac ester structure by amount of proton (=1),

$I_{\text{- CH - OH group of SHL}}$ was the number resulting from division of the integrals of hydrogen of -CH-OH group at 3.0-3.6 ppm from SHL structure by amount of proton (=7), and

$I_{\text{-CH=C- in cyclohexene of SHL}}$ was the number resulting from division of the integrals of hydrogen of –CH=C- group at 6.3-6.7 ppm from SHL structure by amount of proton (=1).

3.2.6 Powder X-ray diffractometry (PXRD)

Each shellac sample powder was loaded into PXRD plate and scanned by powder X-ray diffractometer (Rigaku, Miniflex II, Japan). The PXRD data were recorded on a Rigaku Miniflex System using Ni-filtered, Cu-K (alpha) radiation, 30 kV, 15 mA with a scanning speed of 4°/min.

3.3 Preparation of shellac films

Shellac films were prepared using a casting/solvent evaporation technique. Each shellac sample, including shellac and its esters, was dispersed in water and then ammonium hydroxide was added. The amount of added ammonium hydroxide was calculated in accordance with the acid value of each shellac sample. The mixture was stirred until the shellac sample was completely dissolved and kept stirring overnight. The final concentration was adjusted to 6% w/w with water. The solution was poured onto a glass plate, whose surface was treated with Aquasil®, and

allowed to evaporate at 50 °C for 4–5 h. The film was peeled off and stabilized at 25 °C, 75% RH prior to testing.

3.4. Characterizations of films

3.4.1 Water vapor permeability of film

Water vapor permeability (WVP) was measured according to the method adapted from the Annual Book of ASTM Standards (ASTM 1989). The cell consisted of a glass bottle, filled with dried granular calcium chloride, and a cap with an opened circular hole of 3–4 cm in diameter was used for WVP measurement. The prepared film with an exposed area of 800–1000 mm^2 was placed inside the cap and then sealed tightly. The cell was then kept in a cabinet at 40 °C, 75% RH. The weight change was recorded periodically. The WVP coefficient of at least six cells for each film was then calculated using the following formula.

$$\text{WVP coefficient} = \frac{(W \times t)}{(A \times \Delta P)}$$

where

WVP coefficient is water vapor permeability coefficient ($g.h^{-1}.m^{-1}.Pa^{-1}$),

W is the amount of water permeated through the film (g/h),

t is the thickness of film (m),

A is exposed area of film (m^2), and

ΔP is the vapor pressure difference ($\Delta P = 5.39 \times 10^4$ Pa)

3.4.2 Mechanical properties (puncture test)

The mechanical properties of the shellac films were measured by a puncture test. A texture analyzer (Stable Micro Systems TA.XT. plus Texture Analyzer, UK), equipped with a spherical puncturing probe (diameter 5 mm) was

employed. The film was placed in a holder with a cylindrical hole (radius = 1.0 cm). The probe was driven through the film with a speed of 0.1 mm/s and force–displacement curve was recorded through a 50 N load cell. The maximum load and the maximum displacement of film at the break point was measured, and then converted to puncture strength, elongation at puncture and modulus at puncture. The parameters, from at 5 determinations, were then calculated using the following equations (Sontaya Limmatvapirat et al. 2004: 41-49; Sontaya Limmatvapirat et al. 2007: 690-698):

$$\text{Puncture strength} = \frac{F_{\max}}{A_{CS}}$$

Where F_{max} is the maximum applied force at the break point (N), A_{CS} is the cross-sectional area of the edge of the film located in the path of the cylindrical hole of the film holder, with A_{CS} = 2rδ, where r is the radius of the hole and δ is the thickness of the film (mm). The unit of puncture strength was MPa.

$$\text{Elongation} = \frac{\sqrt{\left(r^2 + d^2\right)} - r}{r} \times 100$$

Where *r* is the radius of the film exposed in the cylindrical hole of the film holder (mm) and *d* represents the displacement of the probe from the point of contact to point of puncture (mm).

$$\text{Modulus at puncture} = \frac{\text{puncture strength}}{\text{elongation}(\%)}$$

The unit of modulus at puncture is MPa.

3.4.3 pH solubility profiles

The film was cut in a square of 1 cm × 1 cm and weighed. The film was then placed in each of the six tubes of the basket of USP disintegration

apparatus, which maintained the temperature at 37 ± 2 °C. The simulated gastric fluid (SGF) was used as immersion fluid for the first 2 h. After immersion in SGF, the film was transferred to the buffer solutions at various pH values for the next 3 h. The resulting film was dried at 70 °C for 12 h, and reweighed. The percentage of dissolved film was calculated from the percent weight loss of film. In case the film was completely dissolved within 3 h in buffer solutions, the dissolving time was recorded.

3.5 Fabrication of shellac esters coated tablets

3.5.1 Preparation of compressed core tablets

Formulation of core tablets

Paracetamol	10.0	%w/w
Complecel® M102 D^+ 30.0	%w/w	
Explotab®	4.0	%w/w
Supertab®	55.0	%w/w
Aerosil® 200	0.5	%w/w
Magnesium stearate	0.5	%w/w

In order to study the enteric properties of shellac coated tablets, paracetamol core tablets were prepared. Prior to compression, each excipient was dried and passed through an 80 mesh sieve. The tablet ingredients (paracetamol, Complecel® M102 D^+, Explotab® and Supertab®) were mixed for 10 min in cube mixter (Yeo Heng Factory, Thailand), then magnesium stearate and Aerosil® 200 were added. The powder mixture was further mixed for 5 min. Tablets were produced in a single-punch tablet press (Yeo Heng Factory, Thailand). The concave tablets were compressed using punch with a 103 mm diameter. The weight of each tablet was maintained at 1000 mg.

3.5.2 Coating solution preparation and coating condition

3.5.2.1 Shellac succinate coated tablets

The core tablets were coated by aqueous solution of shellac or shellac succinate. The formulation of coating solution was as followed.

Shellac or shellac succinate	8.0	%w/w
Ammonium solution	equivalent to acid value	
PEG 400	0.4	%w/w
Talcum	1.6	%w/w
Water q.s. to	100.0	%w/w

3.5.2.2 Shellac phthalate coated tablets

The core tablets were coated with shellac or shellac phthalate. The coating formulation contained the following components.

Shellac or shellac phthalate	7.2	%w/w
HPMC	0.8	%w/w
Ammonium solution	equivalent to acid value	
PEG 400	1.2	%w/w
Talcum	1.6	%w/w
Water q.s. to	100.0	%w/w

Coating conditions are as follows:

Conditions	Shellac coated tablets
Tablet weight (kg)	1.5
Inlet temperature (ºC)	50
Feed rate (mL/min)	6-9
Atomization pressure (bar)	1.5
Pan speed (rpm)	12

The film coating machine (Narong Kanchang, Rama Cota 18, Thailand) was used

3.6 Evaluation of coated tablets

3.6.1 Weight variation

To study weight variation, 20 tablets of uncoated tablets and coated tablets at different coating level were weighed individually using four decimals digital analytical balance (Sartorius CP 224s, Germany). The mean and standard deviation were determined.

3.6.2 Thickness and diameter

The thickness and diameter of the tablets were determined using a digital thickness gauge (Minitest 600B, Germany). Ten tablets from each coating level were used and the average and standard deviation values were calculated.

3.6.3 Hardness

Ten tablets were sampled and individually subjected to test for hardness using a hardness tester (Pharmatest PTB311, Germany). The mean and standard deviation of the tablet hardness were calculated.

3.6.4 Disintegration

The disintegration test was carried out as described in USP 30-NF 25. A total of 6 coated tablets were weighed and placed in each tube of a disintegration basket, using a disintegration testing apparatus (Sotax, DT3, Switzerland) and immersed in simulated gastric fluid (SGF, pH 1.2) for 60 min. At the end of operation, the basket was removed from the SGF and the coated tablets were checked for the absent of swelling or broken tablets. Then, the coated tablets were subsequently immersed in simulated intestinal fluid (SIF, pH 6.8). The temperature of both SGF and SIF was maintained at 37 $\pm$ 2 ºC. The time to achieve disintegration of all 6 coated tablets was recorded.

3.6.5 Dissolution

Dissolution studies were performed according to the paddle method defined in USP 30 (Erweka DT70, Germany).The rate of paddles was set as 50 rpm and the temperature was 37.0 ± 0.5 °C. Experiments were conducted in triplicate. In acidic step, 750 mL of 0.1 N HCl was used for 2 h. Aliquots of 5 mL were withdrawn of each vessel at 15, 30, 60, 90, 120 min and equal volume of fresh medium was replaced to maintain a constant total volume. In basic step, after 2 h in the acidic medium, 250 mL of 0.2 M tribasic sodium phosphate was then added to a pH of 6.80 ± 0.05 and continued testing for 6 h. At the sampling time, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min, 5 mL was removed with a syringe connected with a sampling device. The sample was withdrawn through a cylindrical polyethylene filter stick connected to the end of the sampling device. The concentration of dissolved drug was monitored by UV/VIS spectrophotometer (Perkin-Elmer Lamda 2, Germany) at 243 nm. The dissolution profiles were

constructed from the average of the percentage of cumulative drug dissolved from three vessels at each sampling time point.

3.7 Cytotoxicity test

Cytotoxicity of shellac samples was studied by the MTT cytotoxicity assay. Caco-2 cells were harvested and seeded in 96-well plates at a seeding density of 2 x 10^4 cells per well and preincubated for 24 h. Then, the cells were treated with shellac at various concentrations ranging from 0.001-2 mg/mL in medium (pH 7.4) and incubated for 24 h. After treatment, shellac solution was removed, and fresh medium was added and incubated for 4 h to stabilize the cells. Finally, the cells were incubated with MTT containing medium (0.1 mg/ml) for 4 h. The medium was removed and the formazan crystal that formed in the living cells was dissolved in 100 µl DMSO/well. The relative viability (%) was calculated based on absorbance at 550 nm using a microplate reader (Packard BioScience AOPUS01, USA). Viability was determined by comparing absorbance in wells containing treated cells with that of untreated cells. Eight replicates were measured for each concentration. The relative cell viability was calculated according to the following equation, and the IC_{50} was calculated as a shellac concentration that inhibited the growth of 50% of the cells relative to non-treated control cells

$$\text{Relative cell viability} = \frac{[OD_{550,\text{sample}} - OD_{550,\text{blank}}]}{[OD_{550,\text{control}} - OD_{550,\text{blank}}]} \times 100$$

Where $OD_{550,\text{ sample}}$, $OD_{550,\text{ control}}$ and $OD_{550,\text{ blank}}$ are optical density at 550 mM of shellac sample, control and blank solution, respectively.

3.8. Stability study

The stability of films and coated tablets were studied under the acceleration conditions in 40 ºC and at 75 % in Hotpack® stability chamber for 6 months. During the stability test, Samples were periodically withdrawn at 15, 30, 60, 90, 120, 150 and 180 days and analyzed according to the method previously described in section 3.2 and 3.6.

3.9. Statistical analysis

The statistical data was expressed as means ± standard deviation (SD). The statistical analysis was carried out using analysis of variance (ANOVA). A multi-comparison among pairs of means was performed using a Scheffé test. A *p*-value < 0.05 was considered significantly different. All analyses were performed with SPSS 11.5 for Windows.

CHAPTER 4
RESULTS AND DISCUSSION

1. Preparation of shellac esters

Native shellac consists of a large number of hydroxyl and carboxyl groups as shown by chemical structure (Figure 1). These functional groups have an effect on solubility and aging effect (polymerization) of shellac. Several works attempted to improve its solubility and stability. The hydrolysis treatment was utilized for solving solubility problem of shellac. The partial hydrolyzed shellac showed better solubility as compared to native shellac. Furthermore, the improved enteric property of shellac by salt formation was investigated. The shellac salts demonstrated improved solubility and stability (Sontaya Limmatvapirat et al. 2004: 41-49; Sontaya Limmatvapirat et al. 2005: 41-46; Sontaya Limmatvapirat et al. 2007: 690-698). However these studies only focused on modifying carbonyl groups of shellac while shellac composes of a large amount of hydroxyl group that were available for reaction. Therefore, the modification of hydroxyl groups may be an alternative way for resolving both solubility and stability problems.

The objectives of this study were to enhance the solubility and stability of shellac by esterification with cyclic anhydride through solid state reaction. The increasing carboxylic groups, possessing lower pK_a, of shellac esters was proposed to increase polarity and improve solubility of shellac especially below pH 7.0. As shellac dissolved at pH 7.0 or higher, drug was not released from shellac coated pharmaceutical product in small intestine (pH 3.8-6.6). So the increment of carboxylic

groups from cyclic anhydrides may be a way to solve this problem. In addition, the aging effect was proposed to be inhibited by the decreasing hydroxyl groups of shellac. The polymerization or trans-esterification of the hydroxyl group of shelloic or aleuritic acid molecule with the carboxyl group of another molecule was assumed to be reduced.

From a preliminary investigation, a variety of cyclic anhydrides such as succinic anhydride (SUCA), phthalic anhydride (PHTA), trimellitic anhydride (TMTA), were used to react with shellac (Figure 3). The solid state reaction was employed for studying of formation of shellac esters. The advantages of this technique were no organic solvent utilization, no organic waste, environmental friendly, easy, and high reactivity (Kidwai 2001: 147-151; Kappe et al. 2009). Mechanochemistry with thermal activation, one of solid state reaction, was applied in this study. Shellac and each cyclic anhydrides in the mole ratio 1:4 (based on the amount of hydroxyl groups of jalaric acid) were mixed and ground by planetary ball mill for 90 min. The ground mixture (GM) was then annealed at 60 ºC for various times (Figure 7). A part of annealed mixture (AM) was further processed to remove the excess acid by washing with water. Then all samples were comparatively evaluated by FTIR spectroscopy.

1.1 Shellac-succinic anhydride system

Succinic anhydride was the smallest molecule of the cyclic anhydrides, used for the study. The process described above and FTIR spectroscopy were applied for preliminary of study the formation of succinate ester.

Figure 8 shows FTIR spectra of SHL-SUC systems. In SHL spectrum (Figure 8a), the IR peak at 3400-3000 cm^{-1} was attributed to O-H stretching. The IR

peak at 1716 cm^{-1} and 1255 cm^{-1} were due to the carbonyl stretching and C-O stretching, respectively (Ortega-Aviles et al. 2005: 164-172). SUCA (Figure 8b) showed two characteristic stretching bands in the carbonyl region. The carbonyl peaks were located at 1863 cm^{-1} and 1783 cm^{-1} which resulted from asymmetrical and symmetrical carbonyl stretching modes, respectively. The C-O stretching peak of SUCA was observed at 919 cm^{-1} (Silverstein et al. 2005). The FTIR spectrum of physical mixture of SHL-SUC demonstrated a complete superimposition of the spectra of SHL and SUCA (Figure 8c). On the spectrum of SHL-SUC GM, similar results were observed as SHL-SUC PM and no new peak was found, indicating the esterification between SHL and SUCA could not occur in the grinding process (Figure 8d). After annealing at 60 ºC 12 h, the intensity of SUCA peak decreased obviously and the spectrum of SHL-SUC AM (before washing) changed as compared to SHL and SHL-SUC PM spectrum (Figure 8e). As illustrated by stars in Figure 8f, the new peaks of SHL-SUC AM were clearly observed after removing of excess SUCA. The O-H stretching band in the region of 3400-3000 was different, suggesting the hydroxyl groups of the ester-linked succinate moieties. The esterification was confirmed by the new peaks at 1251 cm^{-1} and 1163 cm^{-1} that were assigned to C-C(=O)-O and O-C-C stretching of the ester linkage. Furthermore, the increasing of the relative peak intensity of carbonyl stretching at 1736 cm^{-1} (yellow arrow) was noticed, supporting the succinate formation after grinding and thermal activation.

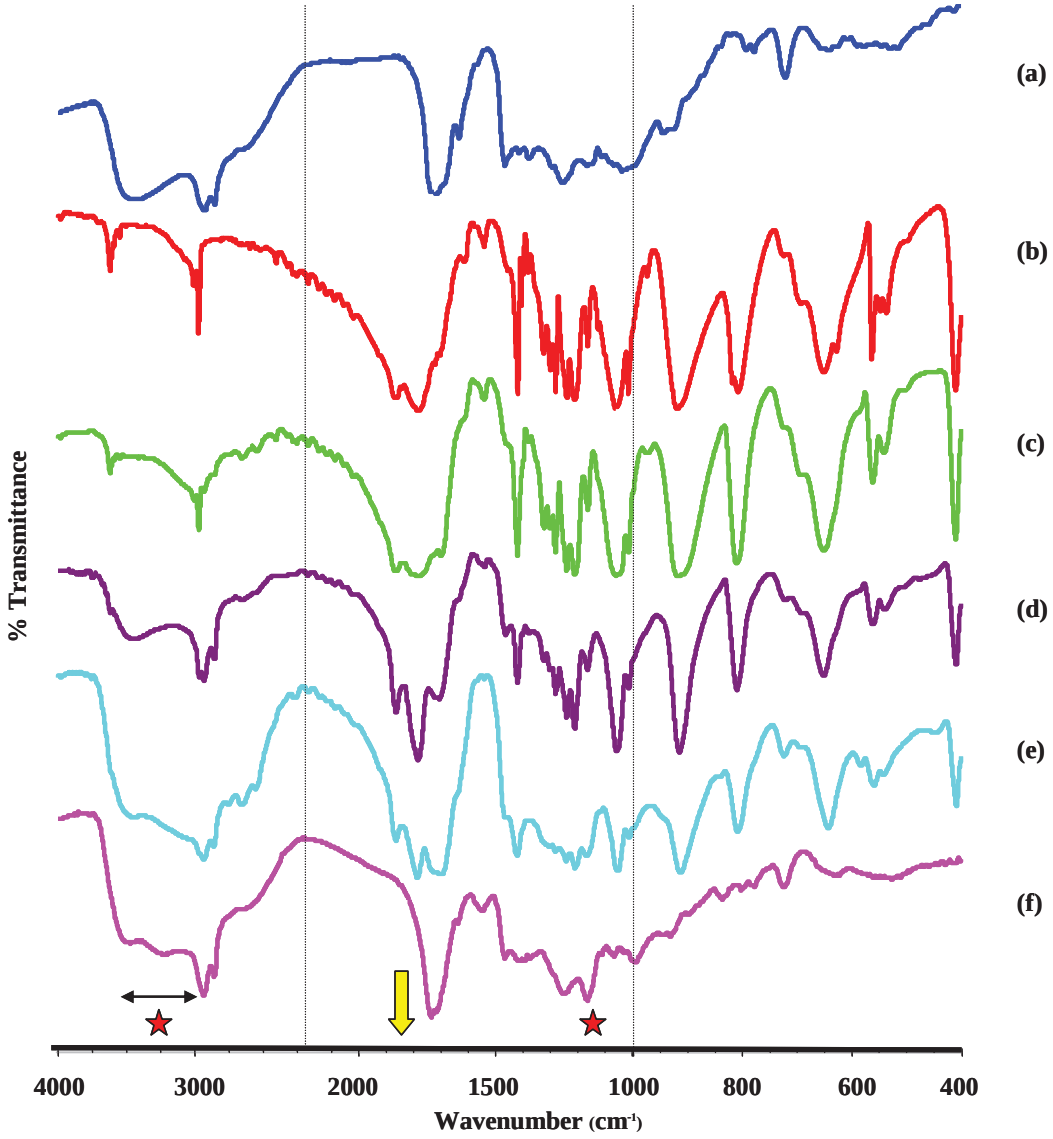


Figure 8 FTIR spectra of SHL-SUC systems; SHL (a), SUCA (b), SHL-SUC PM (c), SHL-SUC GM (d), SHL-SUC AM (before washing) (e), and SHL-SUC AM (after washing) (f)

1.2 Shellac-phthalic anhydride system

To study the effect of molecular size of CAHs, phthalic anhydride (PHTA), which is slightly larger than succinic anhydride (SUCA), containing one benzene ring was used for the study. The esterification of SHL by PHTA was prepared by the same method as described in SHL-SUC system.

Figure 9 illustrates FTIR spectra of SHL-PHT system. PHTA displayed two strong C=O stretching peaks at 1850 cm^{-1} and 1762 cm^{-1}, resulting from asymmetrical and symmetrical carbonyl stretching, respectively. Other strong peak appeared at 1258 cm^{-1} as a result of C-O stretching. In addition, the peaks at 1598 and 713 cm^{-1} arised from aromatic C-C stretching and C-H out-of-plane bending, respectively, were described (Figure 9b). SHL-PHT PM and SHL-PHT GM were the superimposition of spectral pattern of SHL and PHTA, suggesting that the reaction did not occur during grinding process (Figure 9c and 9d). The FTIR spectrum of SHL-PHT was changed after thermal activation and the relative peak intensity of PHTA decreased (as illustrated by the carbonyl stretching peaks at 1850 cm^{-1} and 1762 cm^{-1}) (Figure 9e). Furthermore, the new peaks (as shown by stars) were observed and more clearly seen after washing. As compared as SHL and SHL:PHT 1:4 GM, the peaks at 1600 cm^{-1} and 1580 cm^{-1} , were due to aromatic C=C from PHTA, were appeared (Velayutham et al. 2009: 367-371). The peaks at 1125 cm^{-1} and 742 cm^{-1} that were assigned to O-C-C stretching of ester linkage and aromatic C-H out-of-plan bending of phthalate moieties, respectively, were observed. The results confirmed the substitution of PHTA in the reaction. In addition, the increased relative intensity of C=O and C-O stretching bands at 1724 cm^{-1} and 1290 cm^{-1}, respectively,

and the different O-H stretching bands at 3400-3000 cm^{-1} were supported the esterification of SHL and PHTA.

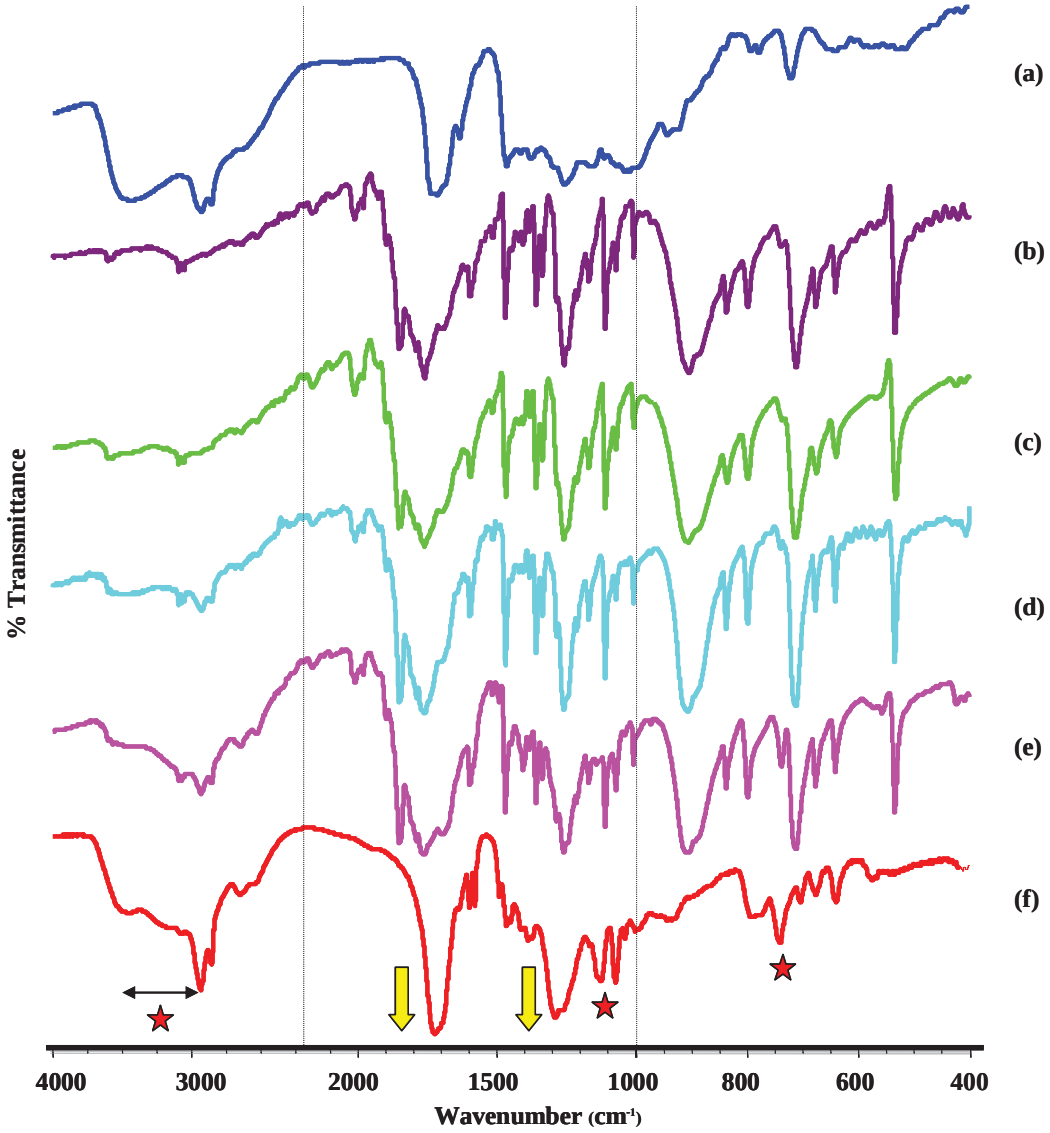


Figure 9 FTIR spectra of SHL-PHT systems; SHL (a), PHTA (b), SHL-PHT PM (c), SHL-PHT GM (d), SHL-PHT AM (before washing) (e), and SHL-PHT AM (after washing) (f)

1.3 Shellac-trimellitic anhydride system

Trimellitic anhydride (TMTA) has similar structure to PHTA with the exception of the third carboxylic group on aromatic ring, and was the biggest molecule of the selected cyclic anhydrides in this study. The formation of trimellitate ester was study by the same method described in SHL-SUC and SHL-PHT systems.

As illustrated in Figure 10, TMTA demonstrated the characteristic peaks of carbonyl stretching at 1864, 1778 and 1720 cm^{-1} (Figure 10b). SHL-TMT PM and SHL-TMT GM preserved the characteristic peak of SHL and TMTA and did not show any new peaks, confirming no reaction after grinding (Figure 10c and 10d). SHL-TMT AM (before washing) did not clearly show the new peak (Figure 10e). After removing excess TMTA (Figure 10f), the small peaks at 1067 and 760 cm^{-1}, assigned as the aromatic C-H in-plane bending and C-H out-of-plane bending from trimellitate moiety, were indicated. In addition, the tiny peak of C-O stretching at 1250 cm^{-1} and the slightly increased of relative intensity of carbonyl stretching at 1717 cm^{-1} were observed, indicating the reaction of SHL-TMT system might occur. However, the intensity of new peaks was very low; the data demonstrated that the less esterification of SHL-TMT systems might be occurred as compared to SHL-SUC and SHL-PHT systems under the same conditions.

The degree of esterification might relate with the molecular size of CAHs used. The detail of formation and properties of each SHL esters would be described later.

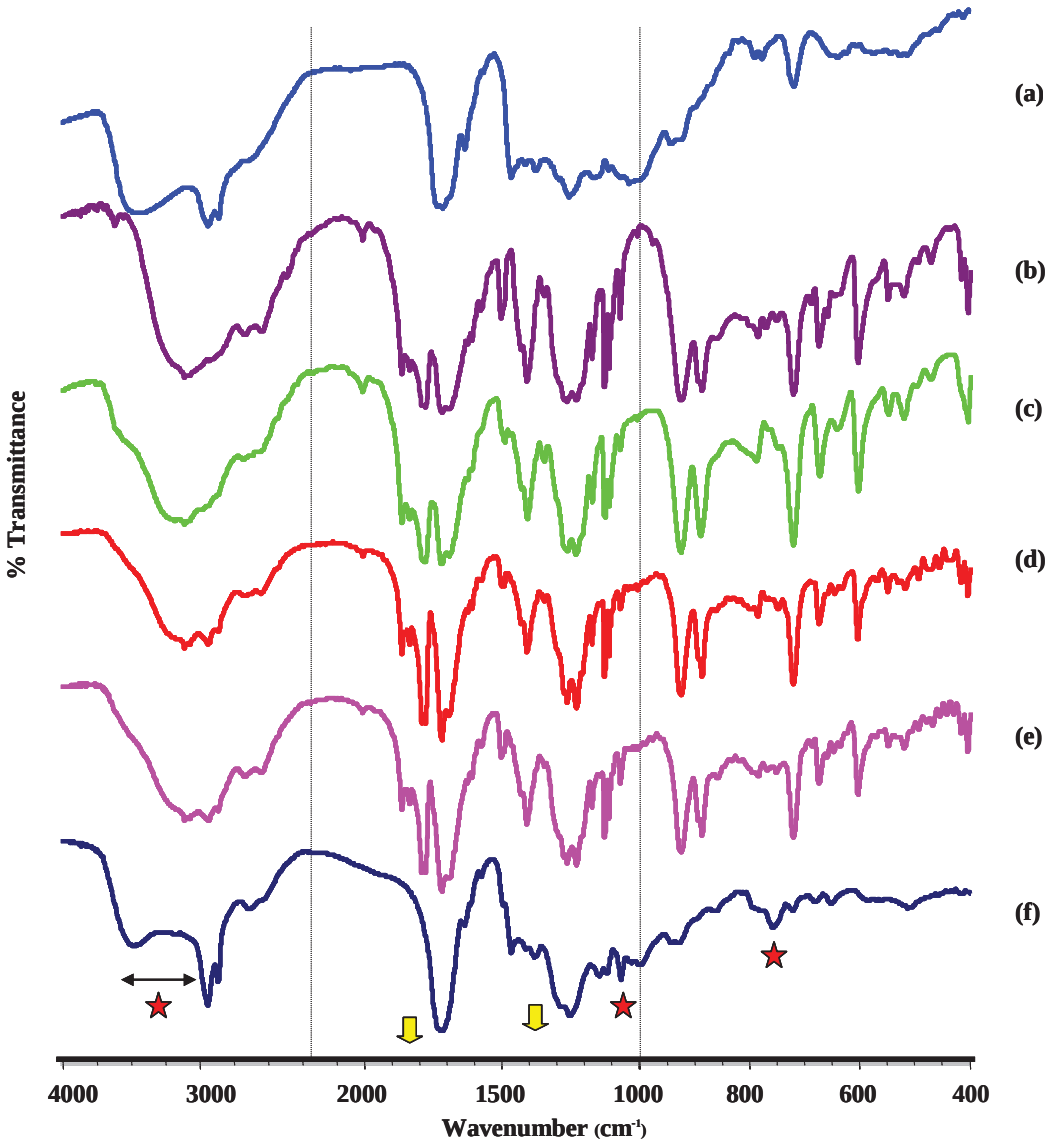


Figure 10 FTIR spectra of SHL-TMT systems; SHL (a), TMTA (b), SHL-TMT PM (c), SHL-TMT GM (d), SHL-TMT AM (before washing) (e), and SHL-TMT AM (after washing) (f)

2. Characterization of shellac esters

2.1 Characterization of shellac succinate and shellac succinate films

2.1.1 Effect of annealing time on shellac succinate formation and characterization of shellac succinate ester

The preliminary study indicated the esterification of SHL and SUCA by grinding with annealing at 60 ºC for 12 h. The formation of shellac succinate was suggested by FTIR spectroscopy (Figure 8). In order to confirm the formation of shellac succinate, other physicochemical and thermal properties of shellac ester were further investigated by measurement of acid value, percentage of insoluble solid, FTIR spectroscopy, DSC, PXRD and NMR spectroscopy. In addition, the effect of annealing time on shellac succinate formation was also studied.

2.1.1.1 Acid value and percentage of insoluble solid

Acid value represents amount of free acid in a given amount of sample, and is defined as milligrams of potassium hydroxide (KOH) required to neutralize the free acid presenting in one gram of sample. The acidity of shellac was determined by the potentiometric titration (Gardner and Sward 1972: 599; Nielsen 2010: 602; Ghatak 2011). Figure 11 demonstrates the effect of annealing time on acid value (bar chart) and percent insoluble solid (line chart) of SHL-SUC AM after washing. The result showed that the acid value of SHL-SUC was rapidly raised as increased annealing time and the value reached plateau after annealing for 9 h. The acid value of SHL-SUC 24 h AM was two-fold increased as compared with that of SHL. Since the acid value indicates the number of carboxylic acid in molecule, the augmentation of acid value suggested the increment of substituted carboxyl group of succinate moiety at the shellac molecule. Several studies also reported the succinate

formation of some natural materials such as chitosan (Aiedeh and Taha 2001: 159-168; AlKhatib et al. 2008: 804-812), cellulose (Fukui et al. 2001: 97-107; Schreier 2001) and starch (Bhandari and Singhal 2002: 277-283). However, the harmful organic solvent was utilized in the synthesis process of those studies.

Since shellac consists of a number of hydroxyl and carboxyl groups as shown in Figure 1. Instability may occur by the polymerization among the functional group. As a result of the polymerization, the acid value was decreased while the polymerized products (insoluble solid) were increased. Since thermal treatment was used to activate the formation of succinate ester, the insoluble solid was measured during annealing process to monitor stability of SHL-SUC AM (Wruble 1930: 318). The percent insoluble solid of SHL-SUC AM during annealing process is illustrated in Figure 11 (line chart), the result indicated that insoluble solid of SHL-SUC after annealing at 60 C for various times were not increased (< 1%w/w), confirming that SHL-SUC was stable during the heat treatment.

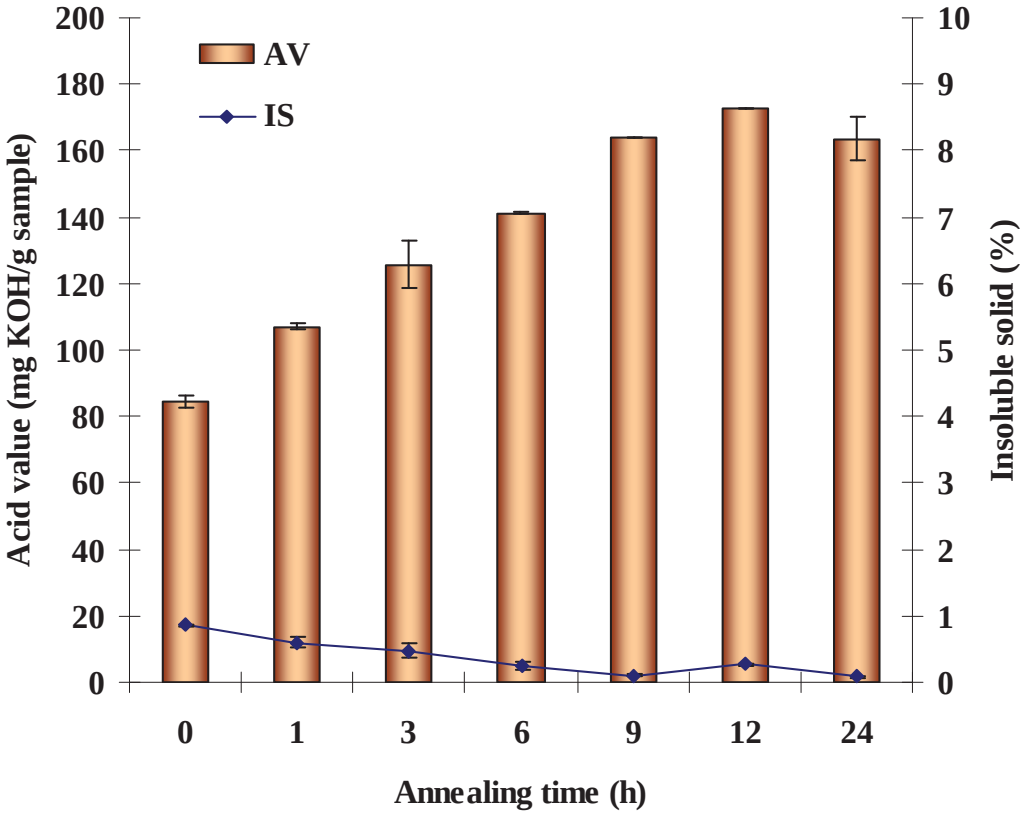


Figure 11 Acid value and insoluble solid of SHL-SUC AM after annealing at 60 °C for various times

2.1.1.2 Fourier transformed infrared (FTIR) spectroscopy

Figure 12 shows FTIR spectra of SHL-SUC AM after annealing at 60 ºC for various times (before washing). The peak at 3400-3000 cm^{-1} was obviously changed as increased annealing time because of the changed O-H stretching. Asymmetric and symmetric carbonyl stretching of SUCA at 1863 and 1783 cm^{-1}, respectively, were decreased and the intensity of carbonyl stretching from the attached succinate moiety at 1736 cm^{-1} was increased as prolonged annealing time.

Figure 13 demonstrates FTIR spectra of SHL-SUC AM after annealing at 60 ºC for various times (after washing). The new peaks of the SHL-SUC AM were more clearly seen as compared to unwashed samples. The washed SHL-SUC GM (no thermal activation) showed the similar spectrum as SHL, suggesting that the SUCA was completely removed and the esterification was not occurred during grinding. The intensities of new peaks of succinate moiety part were gradually increased in FTIR spectra of SHL-SUC AM by increasing annealing time, supporting the succinate formation. The proposed structure of shellac succinate and the peak assignment were displayed in Figure 14.

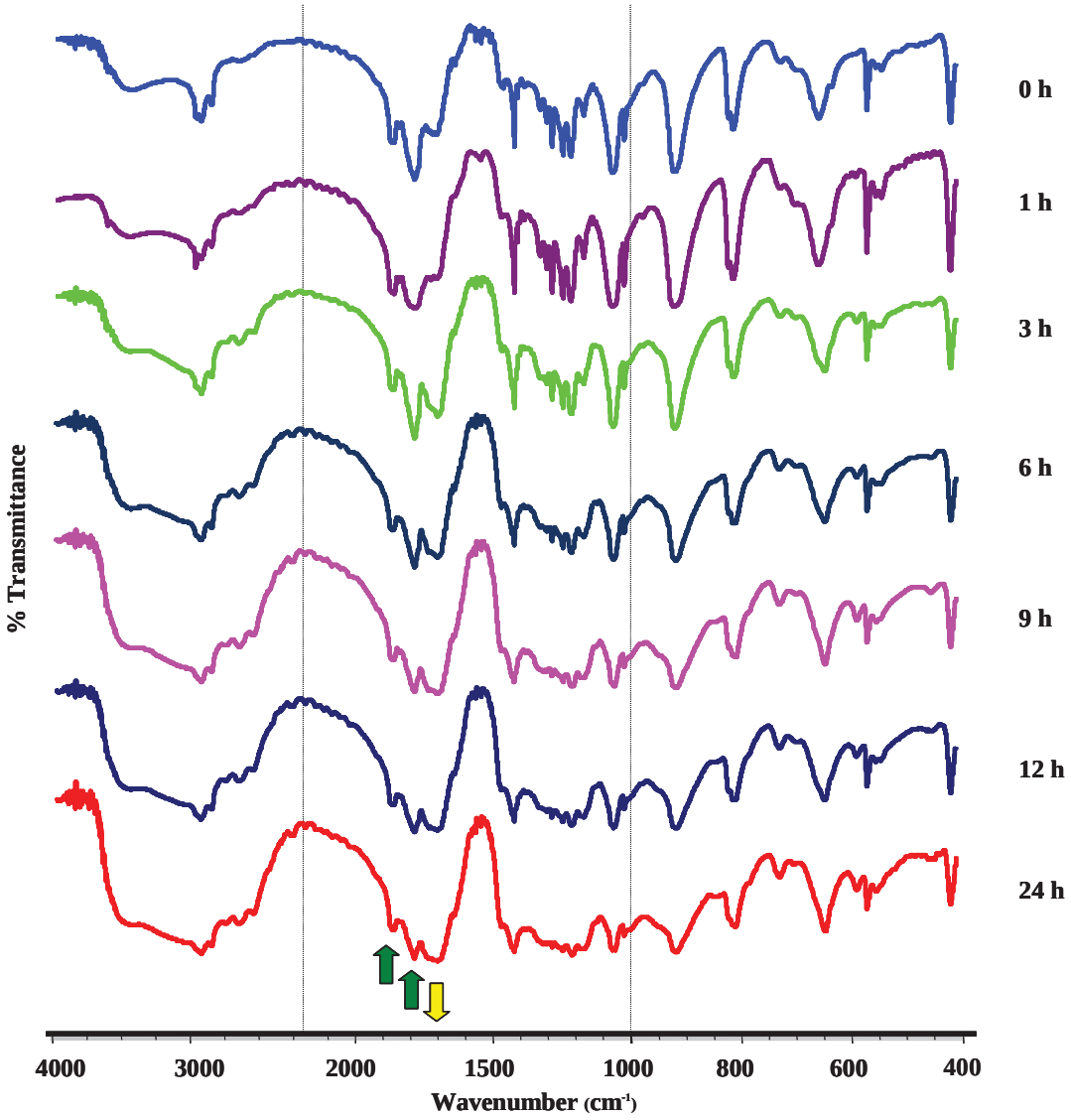


Figure 12 FTIR spectra of SHL-SUC AM after annealing at 60 ºC for various times (before washing)

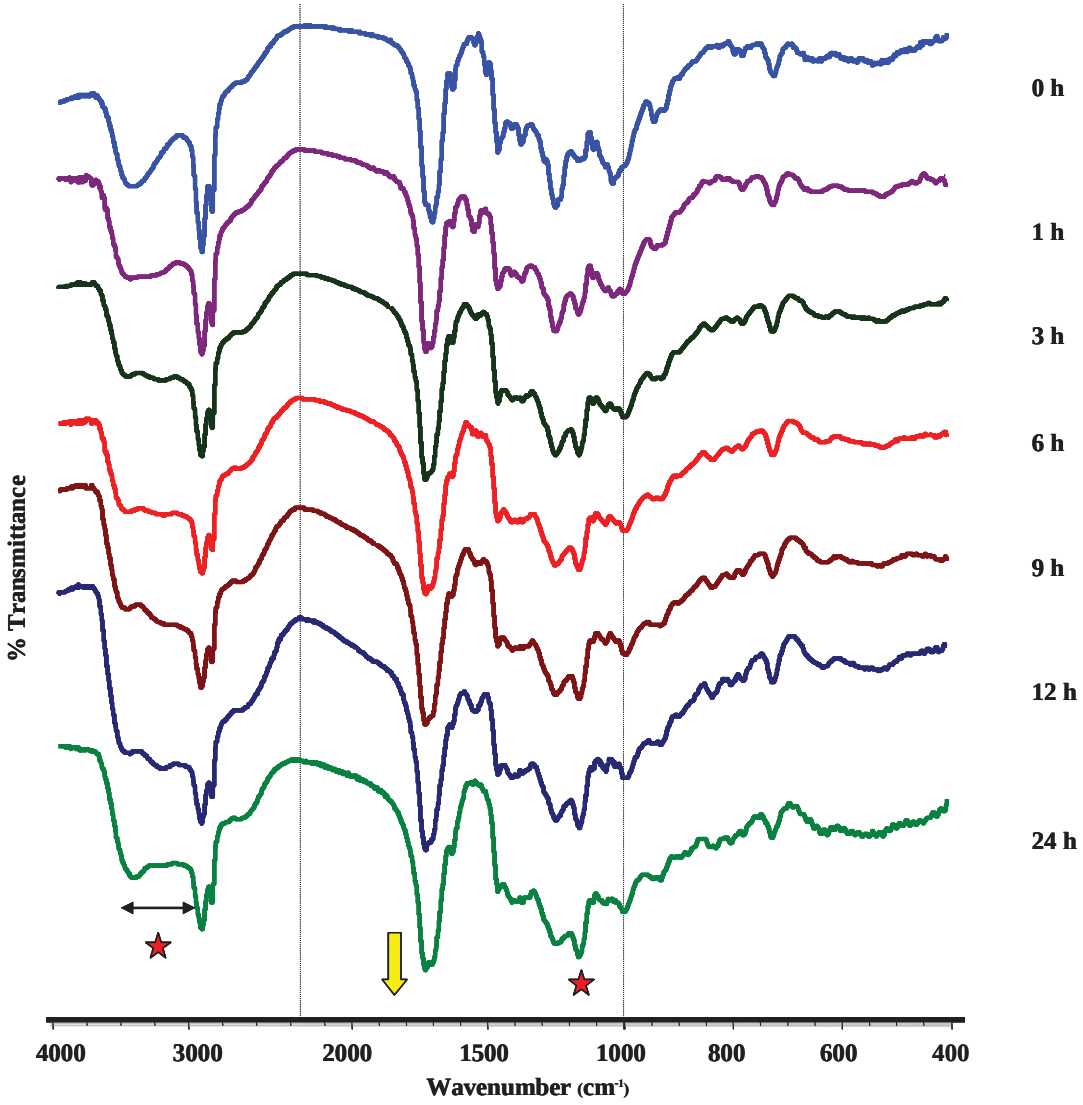


Figure 13 FTIR spectra of SHL-SUC AM after annealing at 60 ºC for various times (after washing)

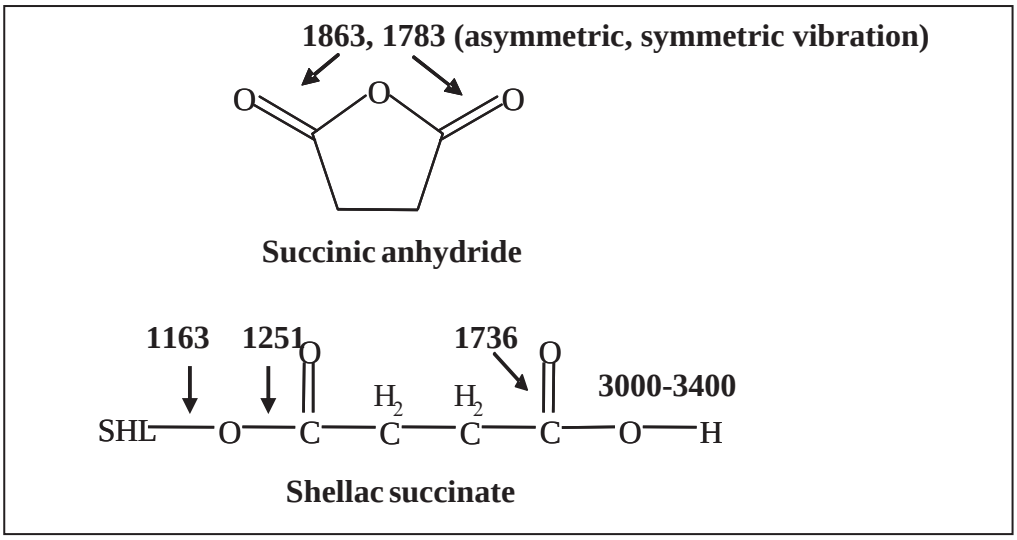


Figure 14 FTIR peak assignment of succinic anhydride and shellac succinate

2.1.1.3 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was employed to study the thermal behavior of samples and to confirm the succinate formation. Figure 15 shows DSC curves of SHL-SUC AM after annealing at 60 °C for various times (before washing). SHL-SUC GM showed the endothermic peak at 119 °C due to melting of SUCA. With prolong of annealing time, the enthalpy of endothermic peak of SUCA continuously decreased. The result indicated that SUCA was used for the reaction during annealing process. The results were agreed with the decreased intensity of peaks due to SUCA observed in FTIR spectra of SHL-SUC AM (Figure 12).

The DSC curves of SHL and the washed SHL-SUC AM samples are shown in Figure 16. SHL indicated a clear endothermic peak at 51 °C, while SHL-SUC 1 h AM and SHL-SUC 6 h AM showed a broad endothermic peak region in the range of 20-100 °C and SHL-SUC 24 h AM did not demonstrate

the endothermic peak on the DSC curve, which indicated the structural change of SHL after succination.

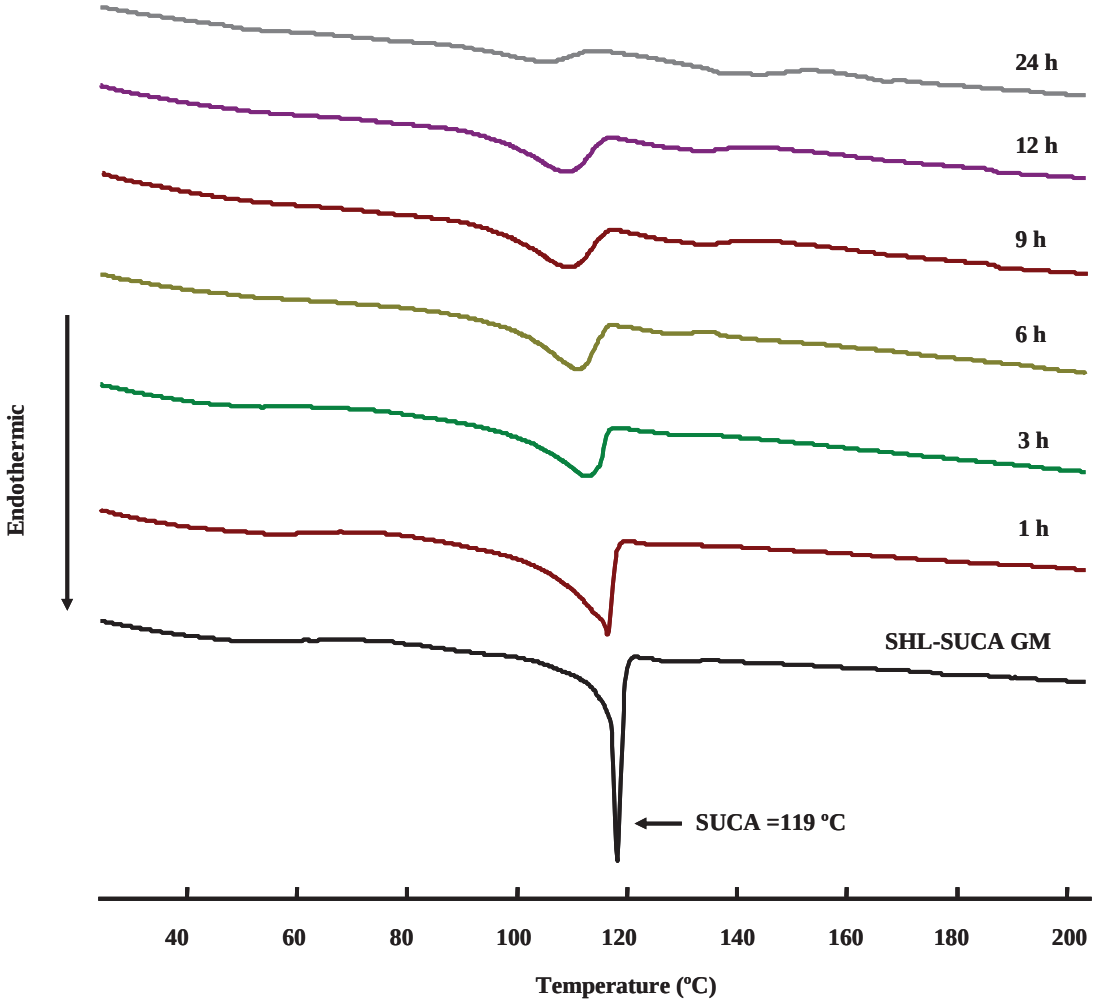


Figure 15 DSC curves of SHL-SUC AM after annealing at 60 °C for various times (before washing)

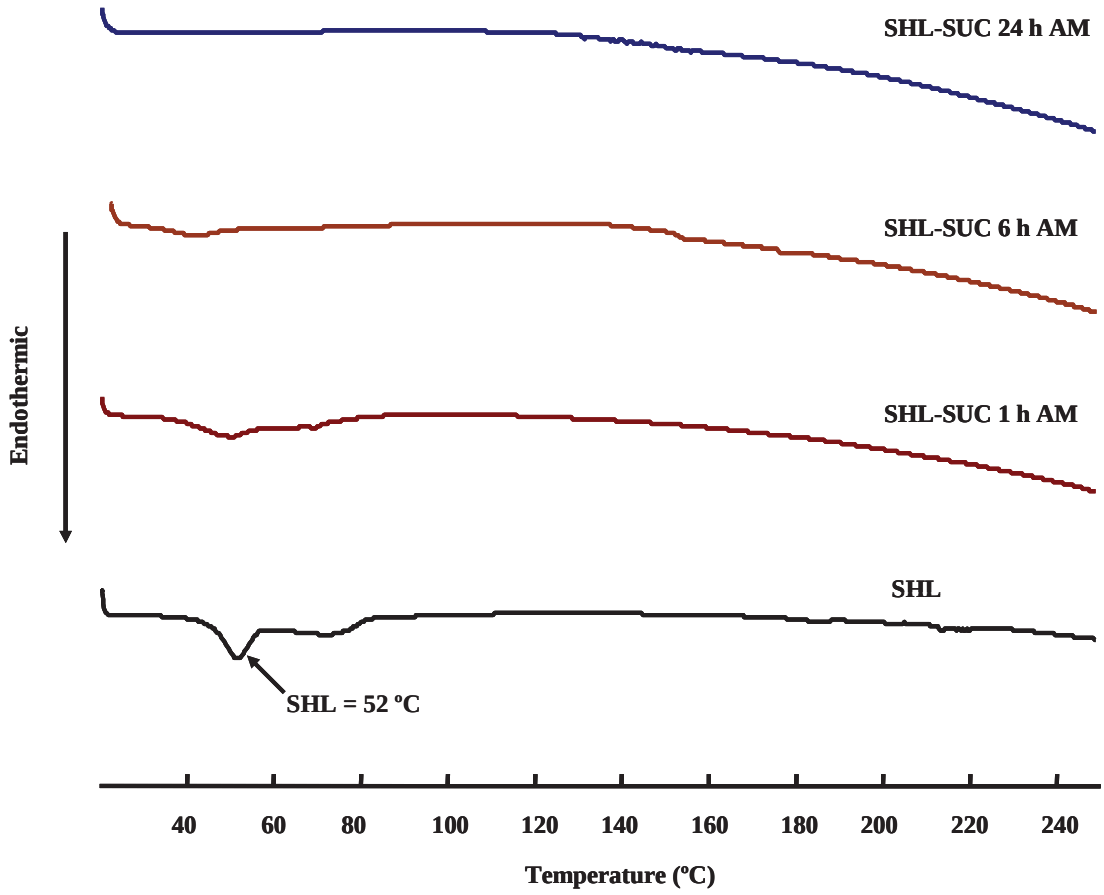


Figure 16 DSC curves of SHL, SHL-SUC AM (after washing)

2.1.1.4 Powder X-ray diffraction

Powder X-ray diffraction (PXRD) has been used for the identification of crystalline compounds by their diffraction pattern. Figure 16 shows the PXRD patterns of SHL and SHL-SUC systems (before washing). A halo pattern of SHL was clearly observed because of its amorphous phase (Figure 17a). The PXRD patterns of SHL-SUC PM (Figure 17c) and SHL-SUC GM (Figure 17d) were the superimposition of PXRD patterns of SHL and SUCA. With increase of annealing time, the intensity of diffraction peaks of SUCA gradually decreased (Figure 17e-17g), supporting that SUCA was utilized for the reaction during thermal activation process. After washing, the PXRD patterns of SHL-SUC AM changed to halo pattern as shown in Figure 18. These results suggested that the physical state of SHL and shellac succinate was amorphous. The succinate formation did not change the crystal properties of SHL.

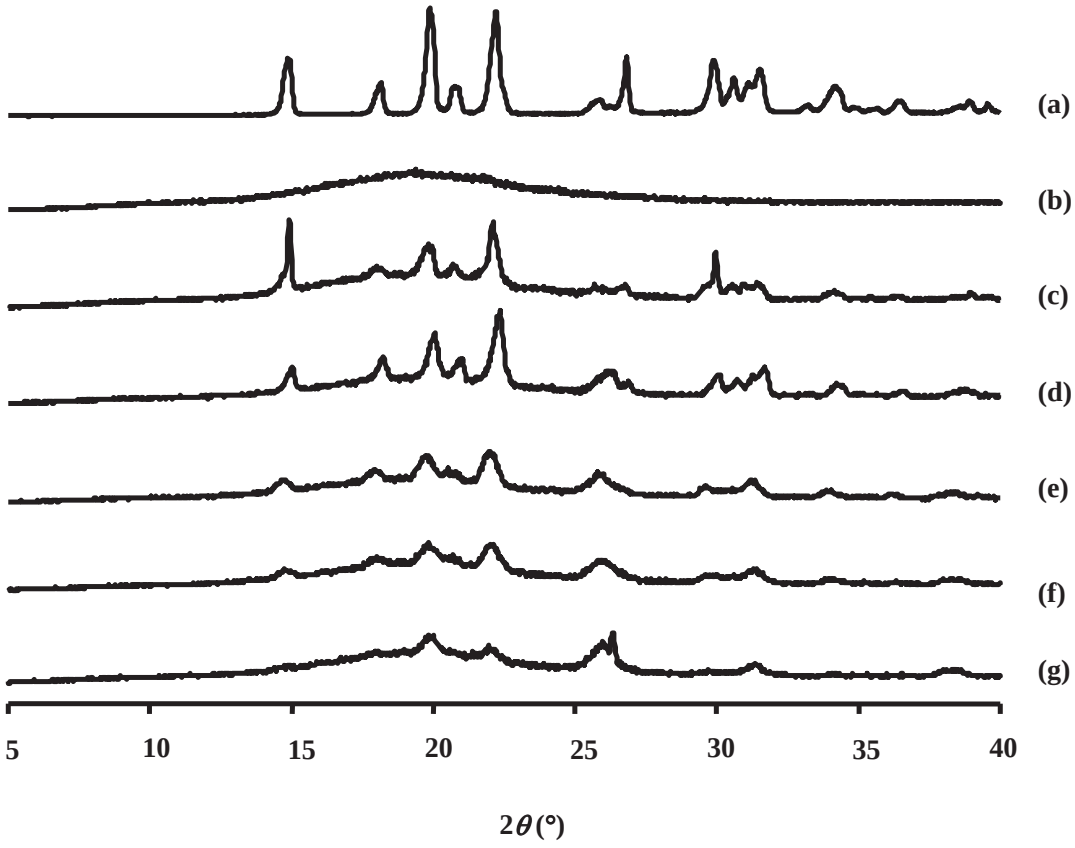


Figure 17 Powder X-ray diffraction patterns of SHL-SUC systems before washing: SUCA (a), SHL (b), SHL-SUC PM (c), SHL-SUC GM (d), SHL-SUC 1 h AM (e), SHL-SUC 6 h AM (f) and SHL-SUC 24 h AM (g)

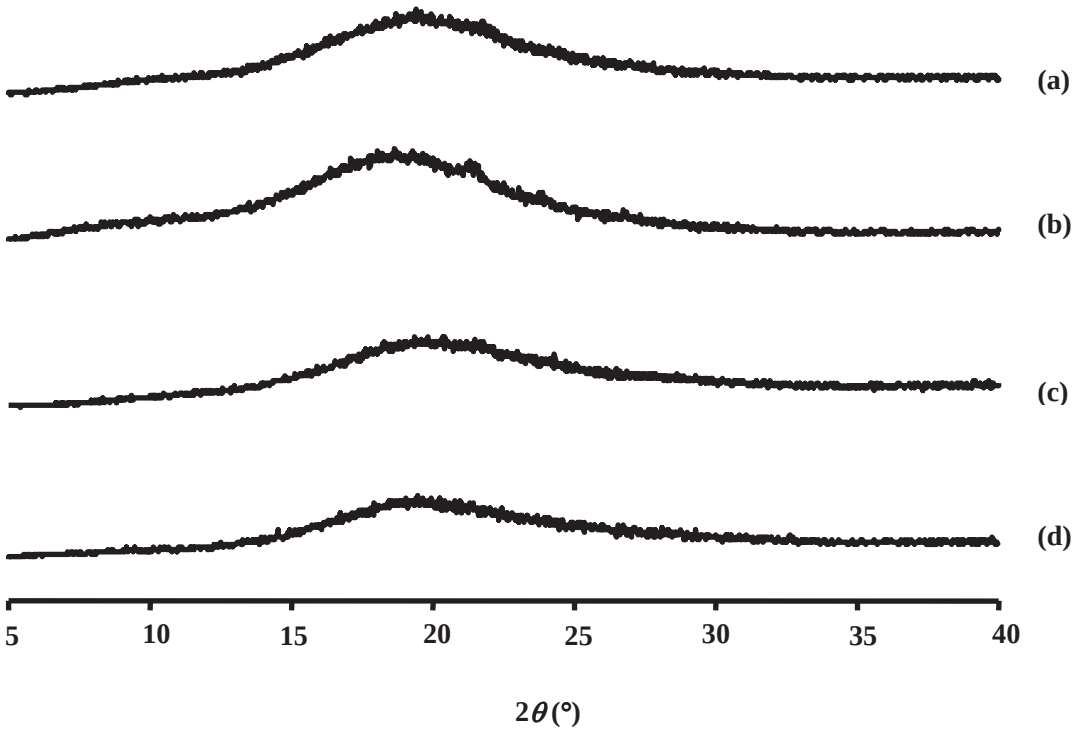


Figure 18 Powder X-ray diffraction patterns of SHL-SUC systems after washing: SHL (a), SHL-SUC 1 h AM (b), SHL-SUC 6 h AM (c) and SHL-SUC 24 h AM (d)

2.1.1.5 Nuclear magnetic resonance (NMR) spectroscopy

Since the development of NMR spectrometer in the 1950s, NMR spectroscopy has been an instrument of choice for the elucidation and confirmation of both newly synthesized and natural product structures by placing the sample solution in a strong magnetic field. The NMR signal give the information about the molecular structure in which the atom resides (Holzgrabe et al. 1999; Jacobsen 2007).

To confirm the esterification between SHL and SUCA, the ^{1}H NMR spectrum of SHL-SUC 24 h AM (after washing) (Figure 19c) was recorded and compared with SHL (Figure 19a). The loss of the relative resonance at

3.49 and 3.25 ppm, corresponding to methylene group (-CH_2-OH) and methine group (-CH-OH) of SHL, respectively, and the addition of resonance at 3.96 ppm attributable to an oxygenated methylene of SHL-SUC, were apparently observed. In addition, the attached succinate moiety was confirmed as seen by an addition of singlet resonance at 2.46 ppm from methylene group (-CH_2-CH_2-) of succinate (Graaf 2007). The finding was also supported by ^{13}C NMR (Figure 20), the increasing of the number of signals at 174.32 and 175.97 ppm, referred to carboxyl groups of succinyl, was obviously seen on the spectrum of SHL-SUC 24 h AM. Furthermore, the reducing of the relative resonance at 75.32 and 63.02 ppm, corresponding to methine group (-CH-OH) and methylene group (-CH_2-OH) of SHL, respectively, and the resonances of addition succinyl group at 65.86 and 72.68 ppm, were indicated the present of succinate chain.

Regarding with the percent substituted at hydroxyl group, the calculation was based on the reduction of resonance of proton attached at the methylene (-CH_2-OH) and methine group (-CH-OH) of SHL. The initial value of substituted OH (non-esterified SHL) was 0 %. After succination, % substituted at hydroxyl group of SHL-SUC was increased to 70 %, suggesting that the number of hydroxyl groups was esterified with SUCA.

From the results above, shellac succinate could easily achieve through toxic organic solvent free reaction by grinding and heat treatment. So shellac succinate films and their enteric properties are interesting to prepare and to characterize for proving the better alternative coating polymer.

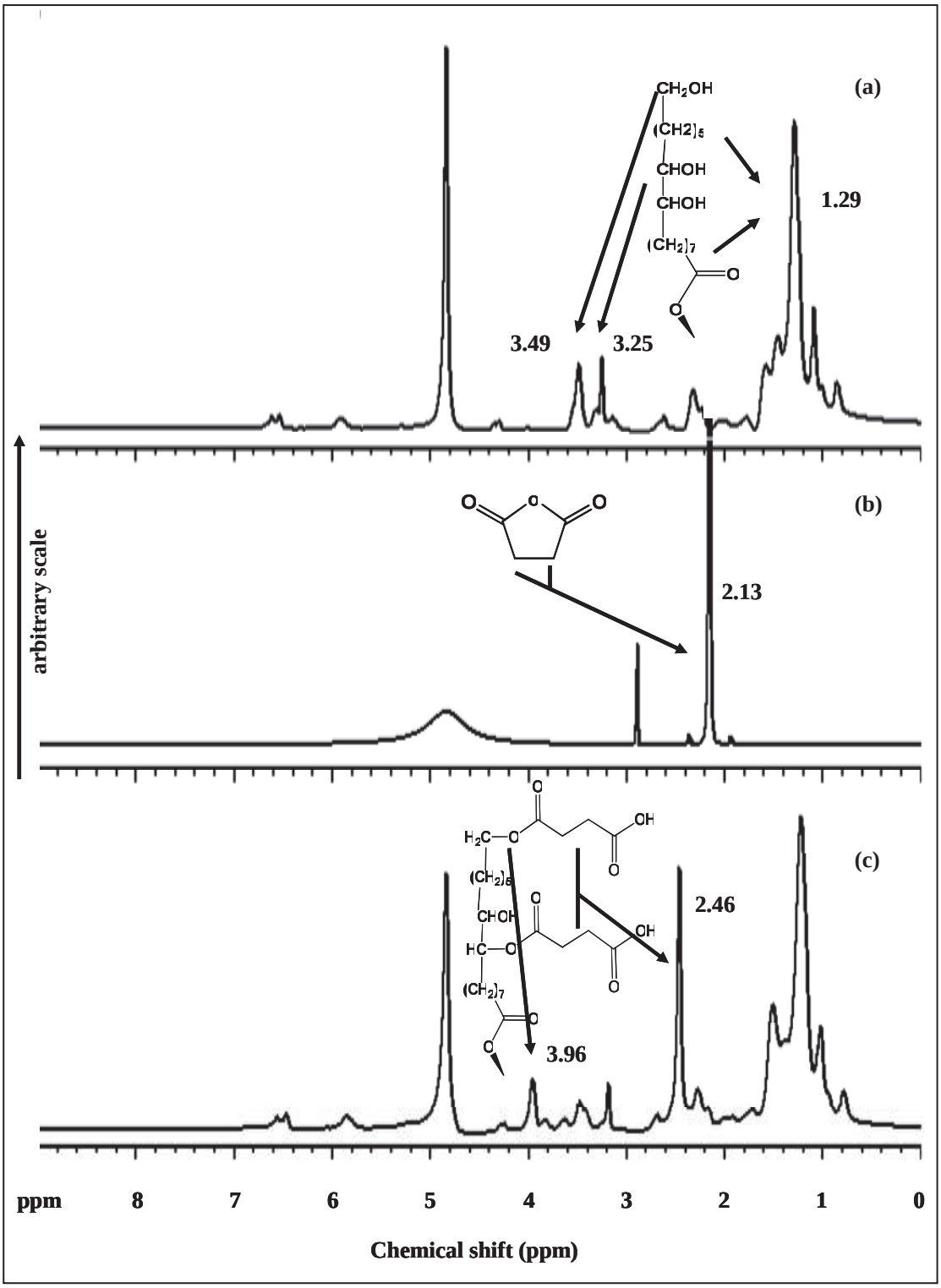


Figure 19 ^{1}H NMR spectra of SHL (a), SUCA (b) and SHL-SUC 24 h AM (c)

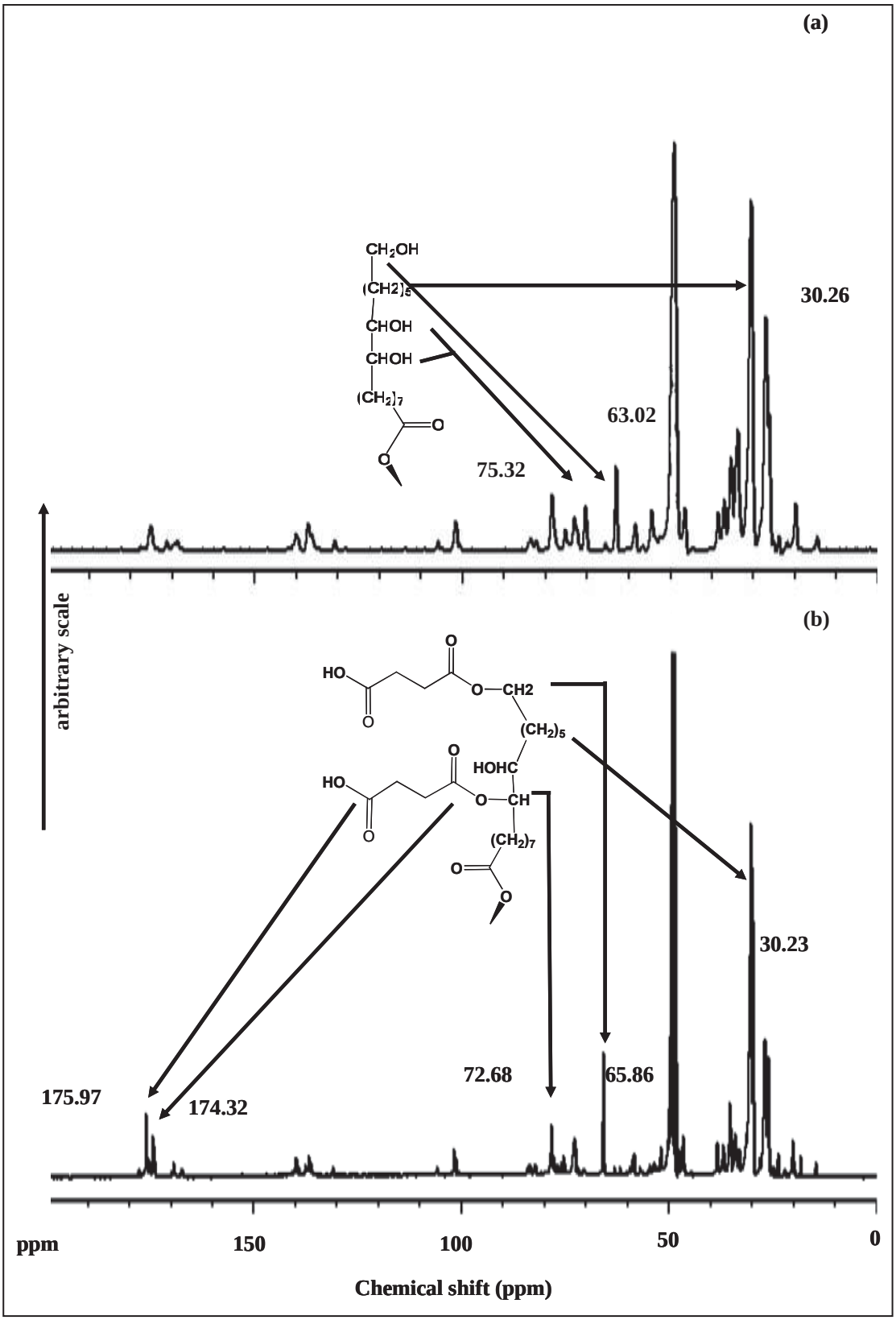


Figure 20 ^{13}C NMR spectra of SHL (a) and SHL-SUC 24 h AM (b)

2.1.2 Characterization of shellac succinate film

Films of SHL-SUC, varied in acid value, were prepared and investigated their film properties. The films were comparatively evaluated for physicochemical properties (such as solubility, water vapor permeability) and mechanical properties.

2.1.2.1 Physical appearance

All films of SHL-SUC were clear transparent, dark yellow or brown in color and were not clearly different as compared to that prepared from SHL. However, the SHL-SUC films demonstrated more hydroscopic and soft characteristic.

2.1.2.2 Mechanical properties (puncture test)

As shellac film was brittle and easily cracked (Pharmaceutical Society of Great Britain 1901; Manee Luangtana-anan et al. 2007: 687-692), several approaches including adding organic acid (Nantarat Pearnchob et al. 2004: 313-321), plasticizer (Qussi and Suess 2006: 403-412; Manee Luangtana-anan et al. 2007: 687-692) and hydrophilic polymer (Qussi and Suess 2005: 99-108), has been used to improve the mechanical properties of shellac. In the present study, the structure modification of shellac by esterification was prepared. Since the succinate formation affected molecular structure of shellac, the change of mechanical properties of SHL-SUC film might be observed. The mechanical properties of shellac, including puncture strength, elongation and modulus at puncture, are presented in Figure 21. The modulus at puncture of SHL showed the highest value about 243 kPa. The modulus was gradually decreased by prolonging annealing time (Figure 21a). As the modulus indicated the rigidity of film, the succinate moiety might increase the

flexibility of film. The result was supported by the lowest percentage of elongation of SHL (Figure 21b). For SHL-SUC films, the percentage of elongation was significantly increased as compared with SHL (p<0.01), suggesting the succinate formation led to the increment of elasticity of film. The puncture strength of SHL-SUC films was significant higher than SHL film. The brittle characteristic of SHL might be the reason of the lower puncture strength even though the modulus was high as compared to SHL-SUC films. With the increasing of the succinate moieties, the puncture strength was declined. The results confirmed the higher flexibility of SHL-SUC films.

A number of studies reported that the mechanical properties of polymer were improved by adding of plasticizer. The effect of plasticizer on mechanical properties of β-lactoglobulin film was determined. Various plasticizers (such as glycerol, sorbitol, PEG 200, PEG 400 and sucrose) were used and the results showed that the increasing of elongation and elasticity of film was dependent on type of plasticizer and the incremental amount of plasticizer (Rungsinee Sothornvit and Krochta 2001: 149-155). In addition, several natural polymers were investigated e.g. chitosan (Srinivasa et al. 2007: 1113-1122), oat starch (Galdeano et al. 2009: 532-538), gelatin (Cao et al. 2009: 729-735). The increment of film elongation from adding plasticizer was due to reduced intermolecular chain interaction. Furthermore, while polymer reacted with plasticizer, the hydrogen bonding between the outer hydrophilic part of plasticizer and water might easily occur, resulting in a higher flexibility (Rungsinee Sothornvit and Krochta 2001: 149-155; Belgacem and Gandini 2008). Shellac succinate had a higher polarity because of the increasing carboxylic group of the attached succinate moieties. Therefore, the

succinate moieties might act as plasticizer which could form the hydrogen bond with water. In addition, the polymer chain of shellac was separated by the steric effect of succinate part, leading to the change of mechanical properties of shellac.

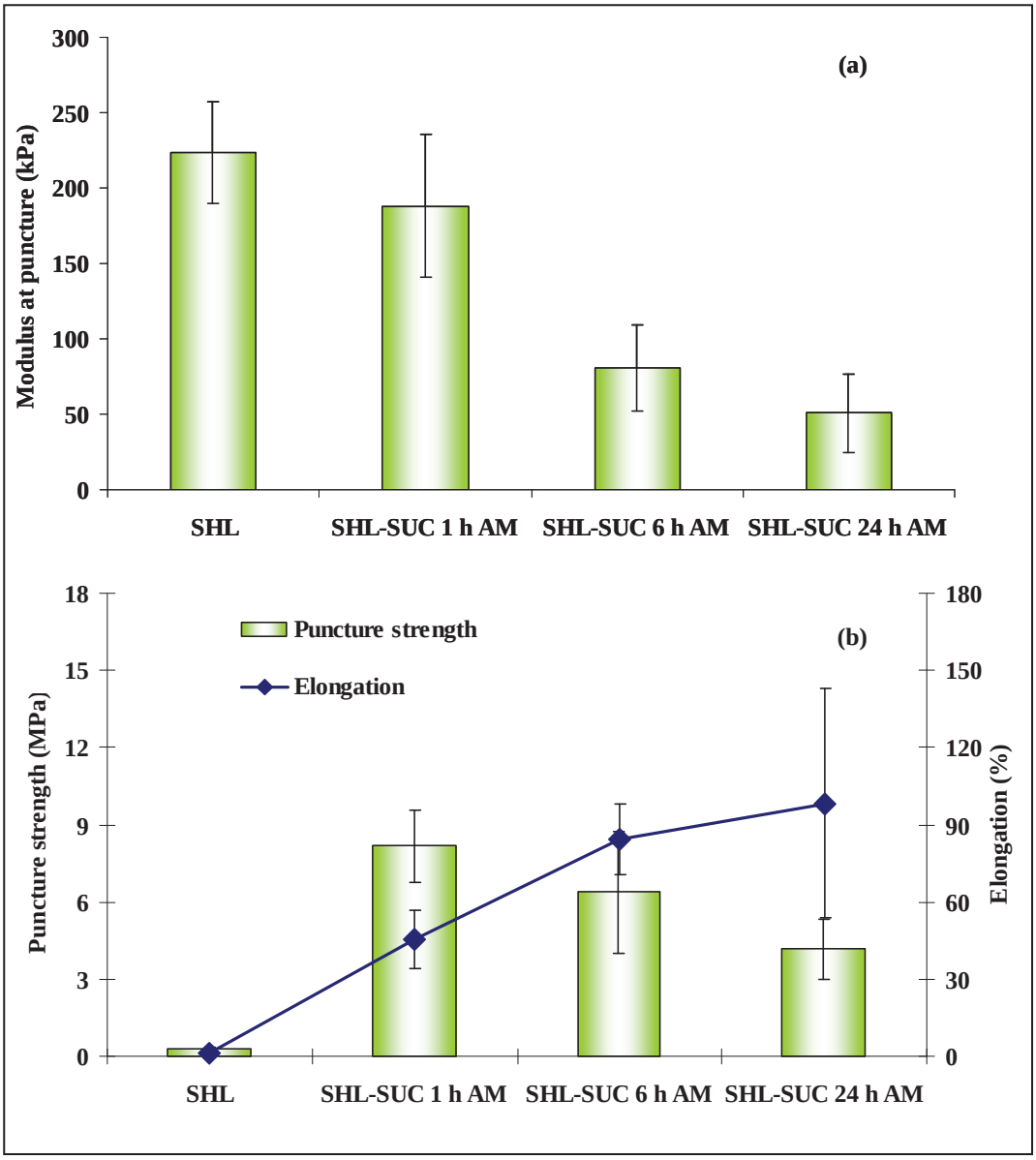


Figure 21 Mechanical properties of film prepared from SHL-SUC: modulus at puncture (a), puncture strength and percentage of elongation (b)

2.1.2.3 Water vapor permeability

Shellac is one of the effective coating barriers against the passage of water vapor (Francis 2000) and it has been used for moisture protection in a variety of fields. Since the succinate formation might affect the molecular structure of shellac, the water vapor permeability (WVP) of film prepared from SHL and SHL-SUC were comparatively determined. Figure 22 illustrates the WVP coefficient of SHL and SHL-SUC. The SHL showed lower WVP coefficient (2.78 x 10^{-8} $g.h^{-1}.m^{-1}.Pa^{-1}$) as compared with SHL-SUC. However, the WVP coefficients of SHL-SUC were not significantly increased as increasing annealing time. Additionally, the WVP coefficients of all SHL-SUC films were still in a low value as compared to several other enteric polymers such as Eudragit® (Sauer et al. 2009: 20-28), cellulose acetate phthalate (Rao and Diwan 1997: 47-51).

The more hydrophilicity of carboxylic groups from succinate moieties might promote the diffusion of water vapor through the film. The results were well agreed with previous studies. Eudragit® RL has more polarity of quaternary ammonium groups demonstrated higher WVP coefficient, as compared to Eudragit® RS. In some study, the polarity of Eudragit® was increased by reacting with lactic acid, resulting in higher porosity and water permeation (Omari et al. 2004: 85-96). The adding of hydrophilic plasticizer could increase water penetration of Eudragit® (Wu and McGinity 2000: 277-284), ethyl cellulose (Frohoff-Hulsmann et al. 1999: 67-75) and some protein (Rungsinee Sothornvit and Krochta 2001: 149-155).

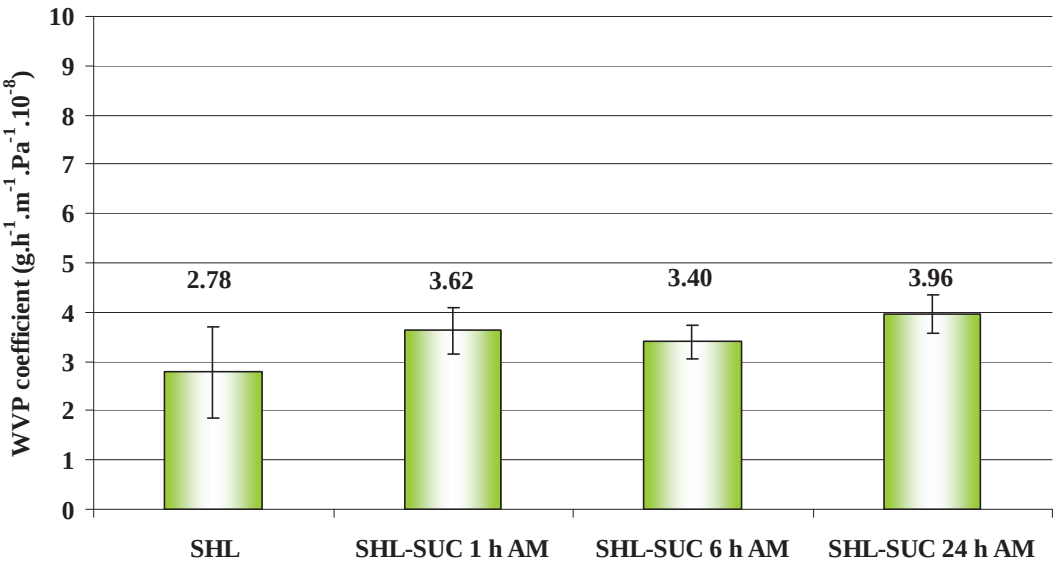


Figure 22 WVP coefficient of SHL and SHL-SUC AM systems

2.1.2.4 pH solubility profiles

The importance characteristics of enteric polymer are to prevent drug releases in the gastric environment and to rapidly dissolve intestinal pH to allow drug release. Shellac shows very good gastric resistance; however a problem of shellac is low solubility at the pH of small intestine. As indicated in previous chapter, SHL-SUC demonstrated more carboxyl groups which might solve the solubility problems of SHL. Hence the pH solubility profiles of SHL and SHL-SUC were comparatively evaluated. SHL and SHL-SUC films were soaked in simulated gastric fluid (SGF) at pH 1.2 for the first 2 h prior to test in buffer solution at various pH values for the next 3 h. The result showed that percent dissolved of films prepared from SHL-SUC in SGF was slightly higher as compared to SHL. The percent dissolved exhibited low value (less than 5%), suggesting that the gastric resistance of SHL-SUC was still in an acceptable range (Figure 23).

Figure 24 shows the percent dissolved of all shellac film after soaking in buffer solution at various pH values. The results demonstrated that the percent dissolved of all films was increased as increasing of pH of buffer solution. SHL and SHL-SUC 1 h AM were completely dissolved at pH 7.0 while SHL-SUC 6 h AM, SHL-SUC 24 h AM began to completely dissolved at pH 6.4 and 6.1, respectively. The result suggested the more solubility of SHL-SUC at intestinal pH. Furthermore, the dissolving time at which all shellac films were completely dissolved (pH 7) was comparatively determined (Figure 25). The declined dissolving time of films were clearly observed with extended annealing time ($p<0.01$), supporting the solubility improvement of shellac succinate film at intestinal condition. As previous described, the increasing carboxylic acid of succinate moieties, having lower pK_a, might provide higher ionization at lower pH, resulting in the solubility enhancement. The effect of the increment of carboxylic acid on solubility of enteric polymers was supported by several studies (Nesbitt et al. 1985: 215-226; Bruce et al. 2003: 85-96; Marťnez-Gonzlez and Villafuerte-Robles 2003: 183-193; AlKhatib et al. 2008: 804-812).

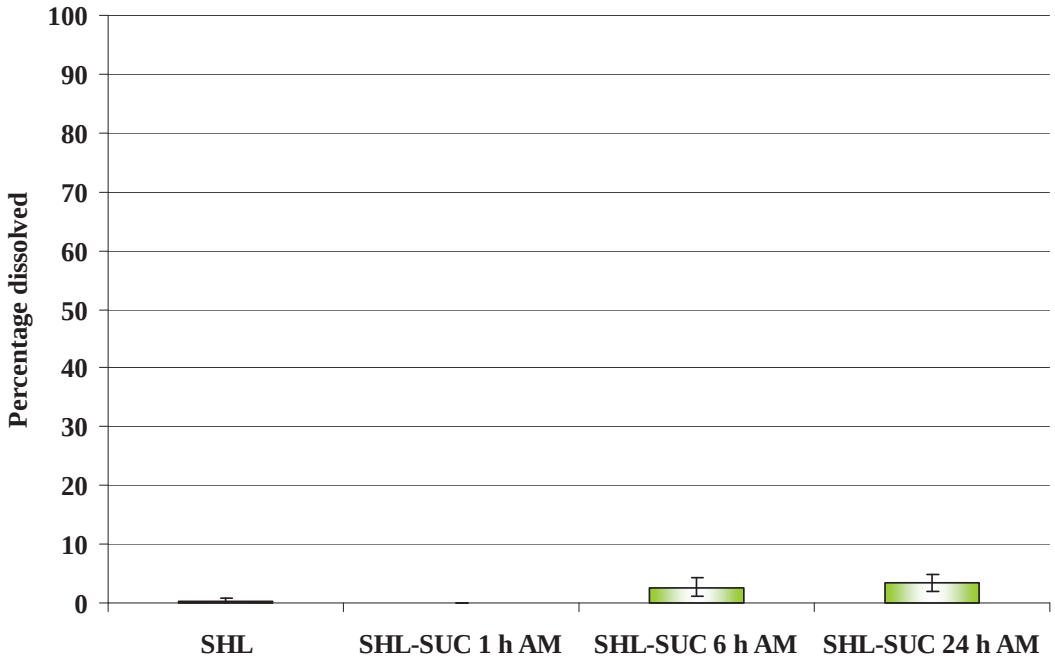


Figure 23 Percent dissolved of SHL and SHL-SUC in SGF

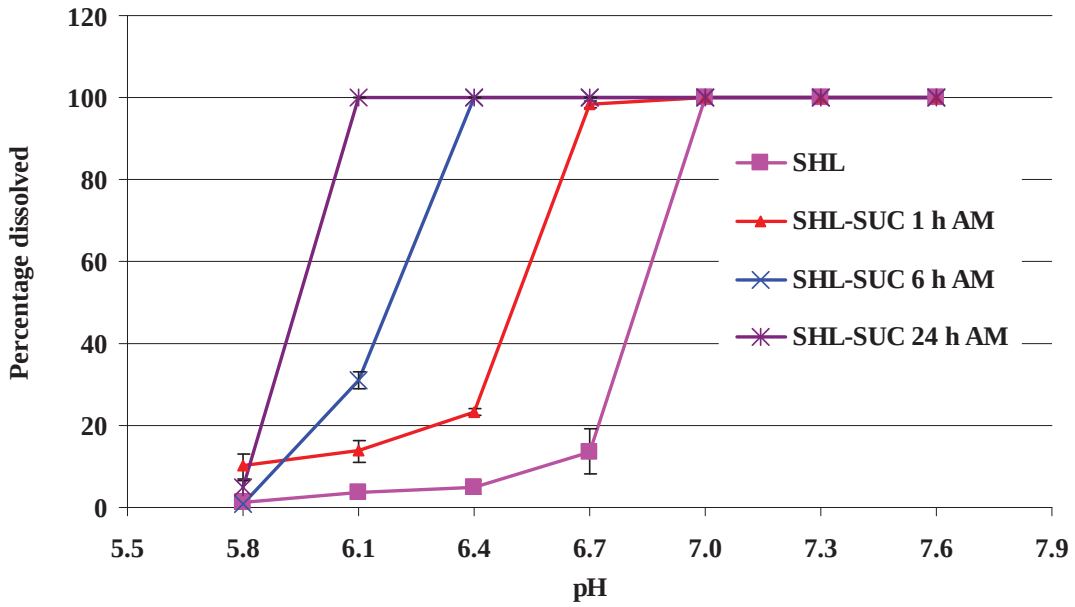


Figure 24 pH solubility profiles of SHL and SHL-SUC

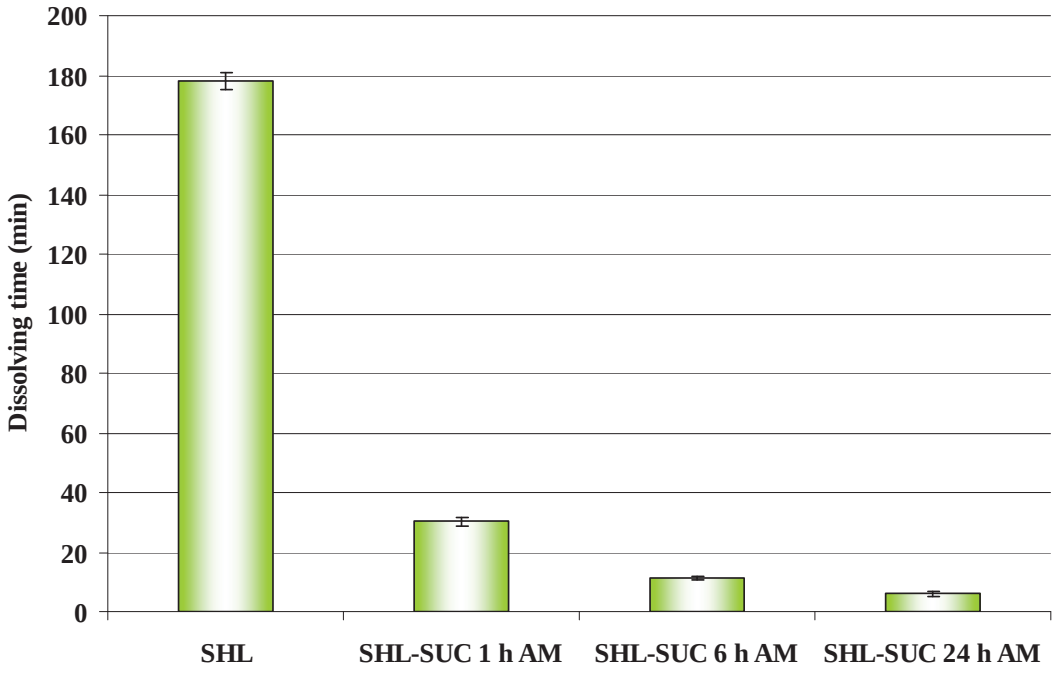


Figure 25 Dissolving time of SHL and SHL-SUC in pH 7.0 buffer

2.2 Characterization of shellac phthalate and shellac phthalate films

From preliminary results, the phthalate formation could occur after grinding with annealing at 60 ºC for 12 h. The structure of SHL-PHT was evaluated by FTIR spectroscopy, as shown in Figure 9. Then acid value of SHL-PHT AM was determined. The acid value of SHL-PHT AM was slightly increased (acid value= 92.7 ± 1.9 mg KOH/g sample), while SHL-SUC AM, which was prepared at same condition, showed two-fold increase as compared with SHL (acid value= 80.9 ± 0.6 mg KOH/g sample). The result indicated the esterification of SHL-PHT GM at 60 ºC for 12 h could occur at low level, so the higher annealing temperatures of SHL-PHT GM were further investigated.

The melting point of SUCA and PHTA was 119-120 ºC and 131.6 ºC, respectively, so it might be possible that should be easily changed to more liquid state

of SUCA, as compared to PHTA at the same annealing temperature. The high degree of ester formation of SHL-SUC (higher acid value) was well correlated with the small molecular size and lower melting point of SUCA (Papaspyrides and Vouyiouka 2009).

In order to examine the esterification SHL by PHTA, the optimum annealing condition was evaluated. Since the acid value of SHL-PHT AM at 60 ºC 12 h was still low value, so we investigated the SHL-PHT formation at higher annealing temperatures for 12 h.

Figure 26 illustrates the effect of heating temperature on acid value and insoluble solid of annealing mixture. The acid value was dramatically increased and the value reached a constant after thermal activation at 80 ºC. The acid values of SHL-PHT AM after annealing at 60 ºC, 80 ºC and 100 ºC for 12 h were 92.7 ± 1.9, 151.6 ± 10.2 and 149.1 ± 18.2 mg KOH/g sample, respectively. As the acid value of the 80 ºC annealed mixture showed about 2 times as compared to that of SHL and the value was similar to that of the 100 ºC annealed mixture. In addition, the percentage of insoluble solid of SHL-PHT AM after annealing at 80 ºC and 100 ºC was still in a low value (< 3%). The annealing temperature was set at 80 ºC for further study of shellac phthalate formation.

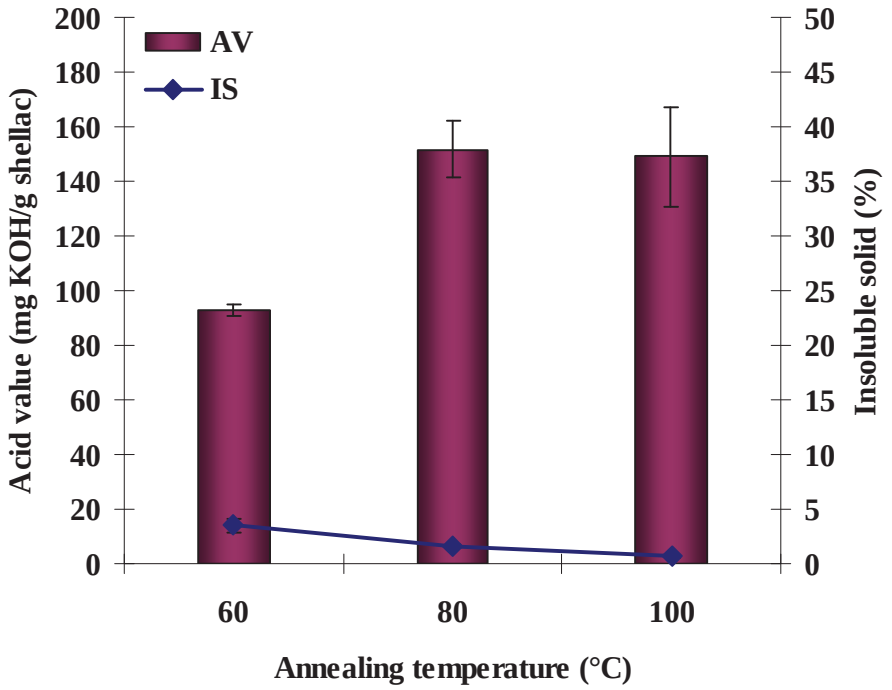


Figure 26 Effect of annealing temperature on acid value and insoluble solid of SHL-PHT AM (after washing)

In order to investigate the effect of annealing time on shellac phthalate formation, SHL-PHT was prepared by annealing at 80 ºC for various times. Figure 27 illustrates the effect of annealing time on acid value (bar chart) and percent alcohol insoluble solid (line chart) of SHL-PHT. The acid value was dramatically increased as prolonged annealing time. After the 12 h annealing time, the acid value reached plateau and the increment of value was approximately 2 times as compared to that of SHL and SHL-PHT 1 h AM.

The percent insoluble solid of SHL-PHT AM was not increased after heat treatment (< 3% w/w). The result indicated that the aging effect of SHL-PHT AM should not occur even heat treatment up to 24 h. As the acid value of the 12 h annealed mixture similar to that of the 24 h annealed mixture and the lower amount of

insoluble solid were observed in SHL-PHT 12 h AM. So SHL-PHT 12 h AM was further investigated for confirming the formation of shellac phthalate.

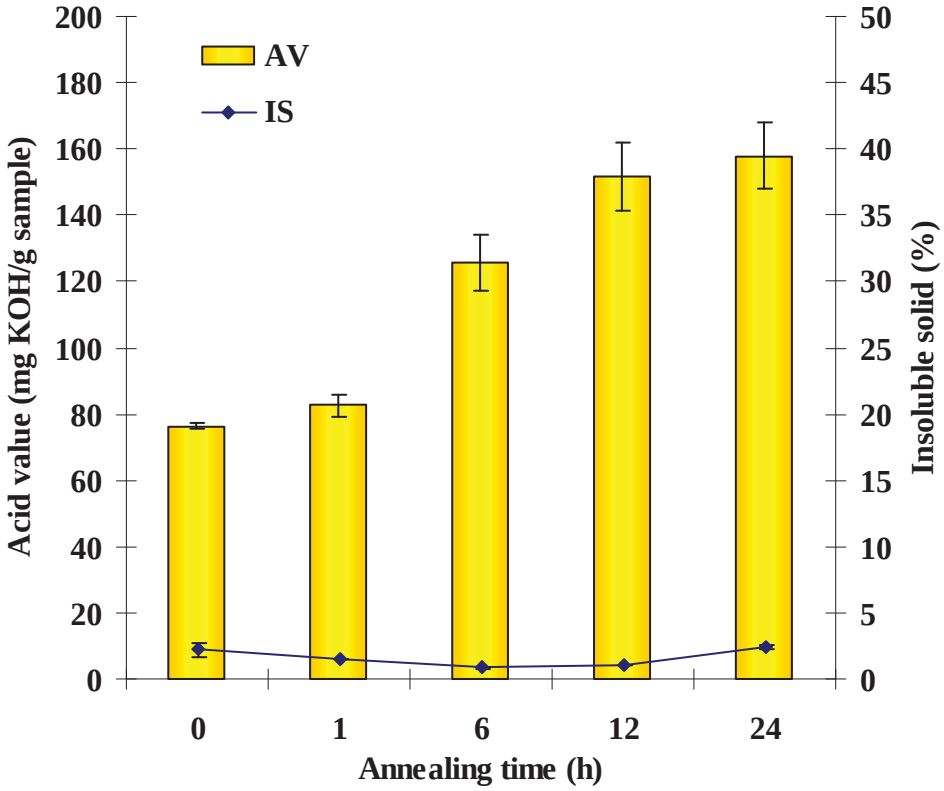


Figure 27 Acid value and insoluble solid of SHL-PHT AM after annealing at 80 ºC for various times

2.2.1 Characterization of shellac phthalate

The preliminary study indicated the esterification of SHL and PHTA by grinding with annealing at 80 ºC for various times. The formation of shellac phthalate was indicated by acid value (Figure 27). In order to confirm the formation of shellac phthalate, other physicochemical and thermal properties of SHL-PHT were

further evaluated by measurement of FTIR spectroscopy, DSC, PXRD and NMR spectroscopy.

2.2.1.1 FTIR spectroscopy

FTIR spectra of SHL and SHL-PHT after annealing at 80 ºC for various times are demonstrated in Figure 28. SHL-PHT (after annealing and removal of excess PHTA) illustrated the new peaks assigned as O-C-C stretching of the ester linkage at 1125 cm^{-1} and the out of plane C-H bending at 742 cm^{-1} (as demonstrated by star). The intensity of new peaks was significantly increased as prolongation of annealing time (peak assignment was shown in Figure 29). In addition, the different O-H stretching band in the range of 3400-3000 cm^{-1} and the increment of relative peak intensity at 1724 cm^{-1} and 1290 cm^{-1} which was due to C=O and C-O stretching were observed, supporting the phthalate formation.

2.2.1.2 Differential scanning calorimetry

Figure 30 demonstrates the DSC curves of SHL, PHTA and SHL-PHT which was annealed at 80 ºC for various times (before washing). SHL and PHTA demonstrated endothermic peak, due to melting at approximately 52 ºC and 131 ºC, respectively. The physical mixture of SHL and PHTA showed two endothermic peaks at 52 and 125ºC which were assumed as the melting point of SHL and PHTA, respectively. After annealing, the enthalpy of endothermic peak of PHTA was clearly declined as increased of annealed time. As supported by the results of FTIR spectra (Figure 28), PHTA should be utilized for esterification during thermal activation process.

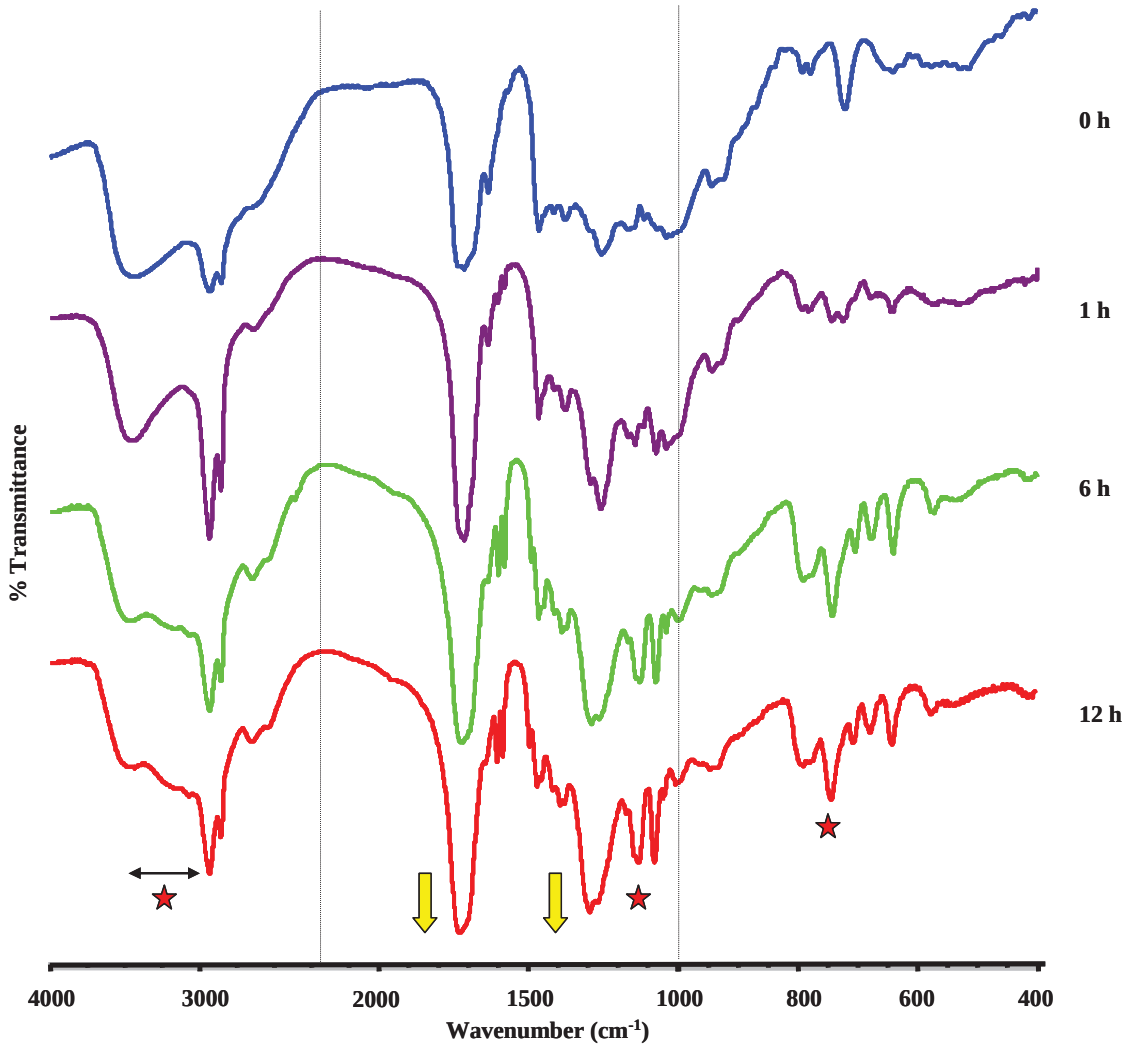


Figure 28 FTIR spectra of SHL-PHT AM after annealing at 80 ºC for various times (after washing)

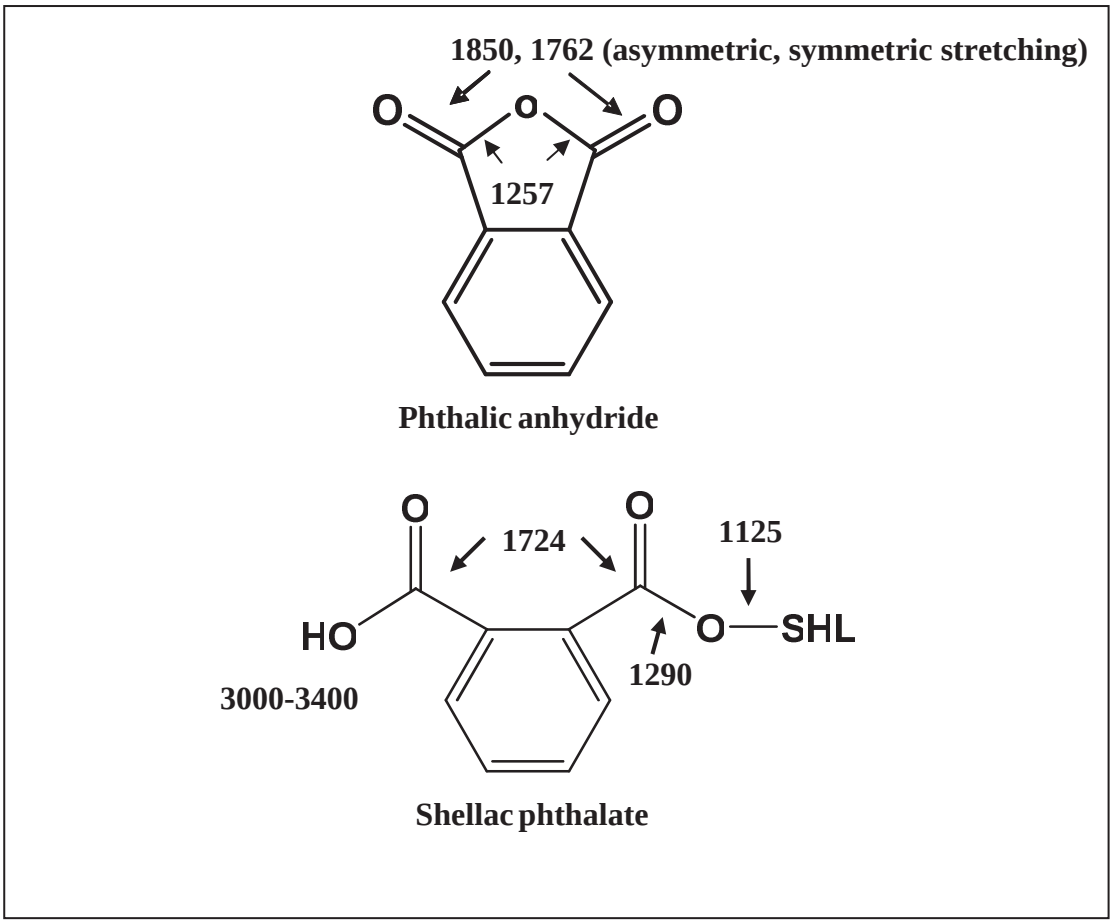


Figure 29 FTIR peak assignment of phthalic anhydride and shellac phthalate

The DSC curves of SHL-PHT AM washed samples are displayed in Figure 31. SHL showed an endothermic peak at 52 ºC, while SHL-PHT AM did not show the clear endothermic peak on the DSC curve, suggesting that the structure of SHL was changed after phthalation.

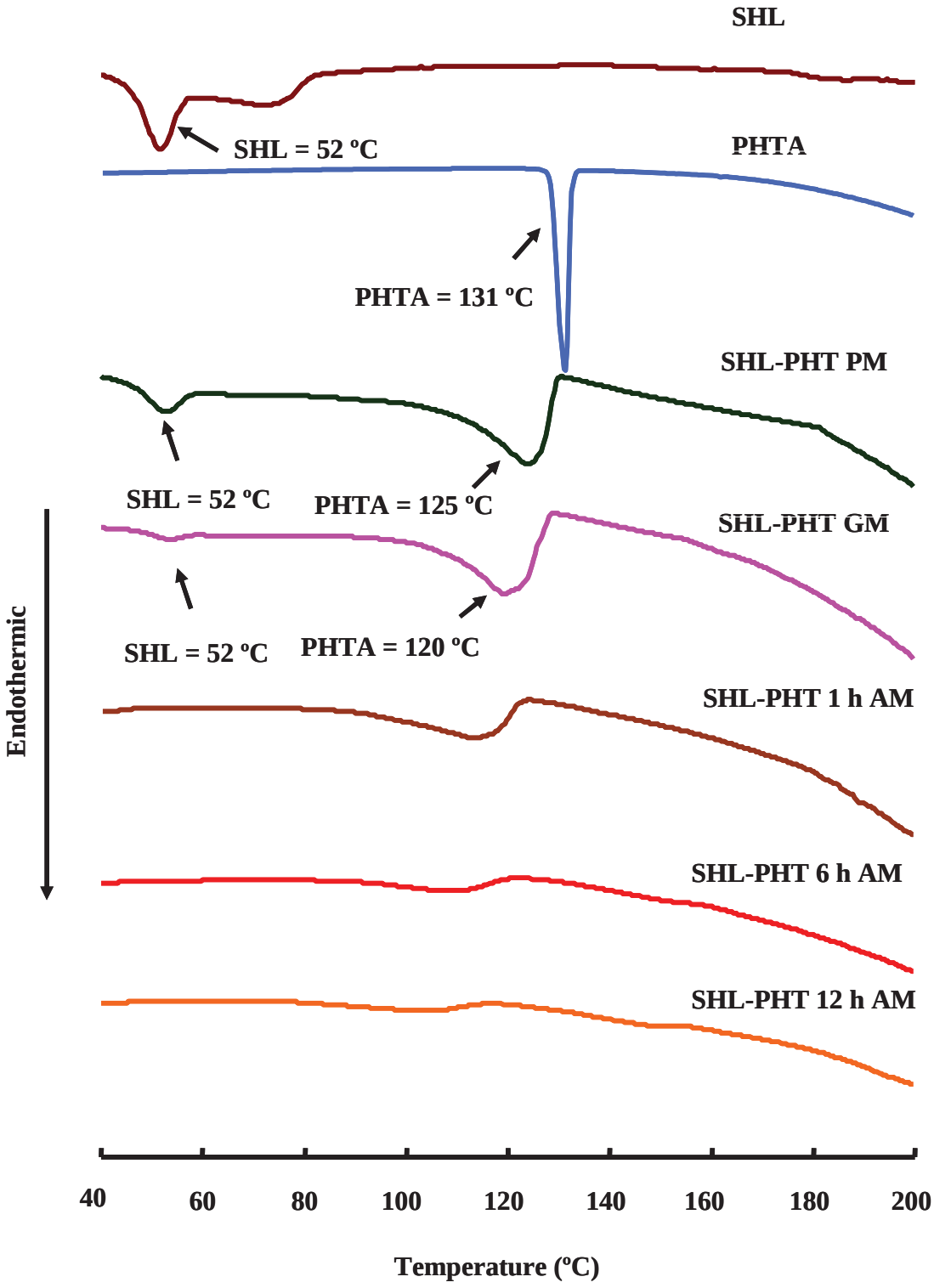


Figure 30 DSC curves of SHL-PHT AM after annealing at 80 °C for various times (before washing)

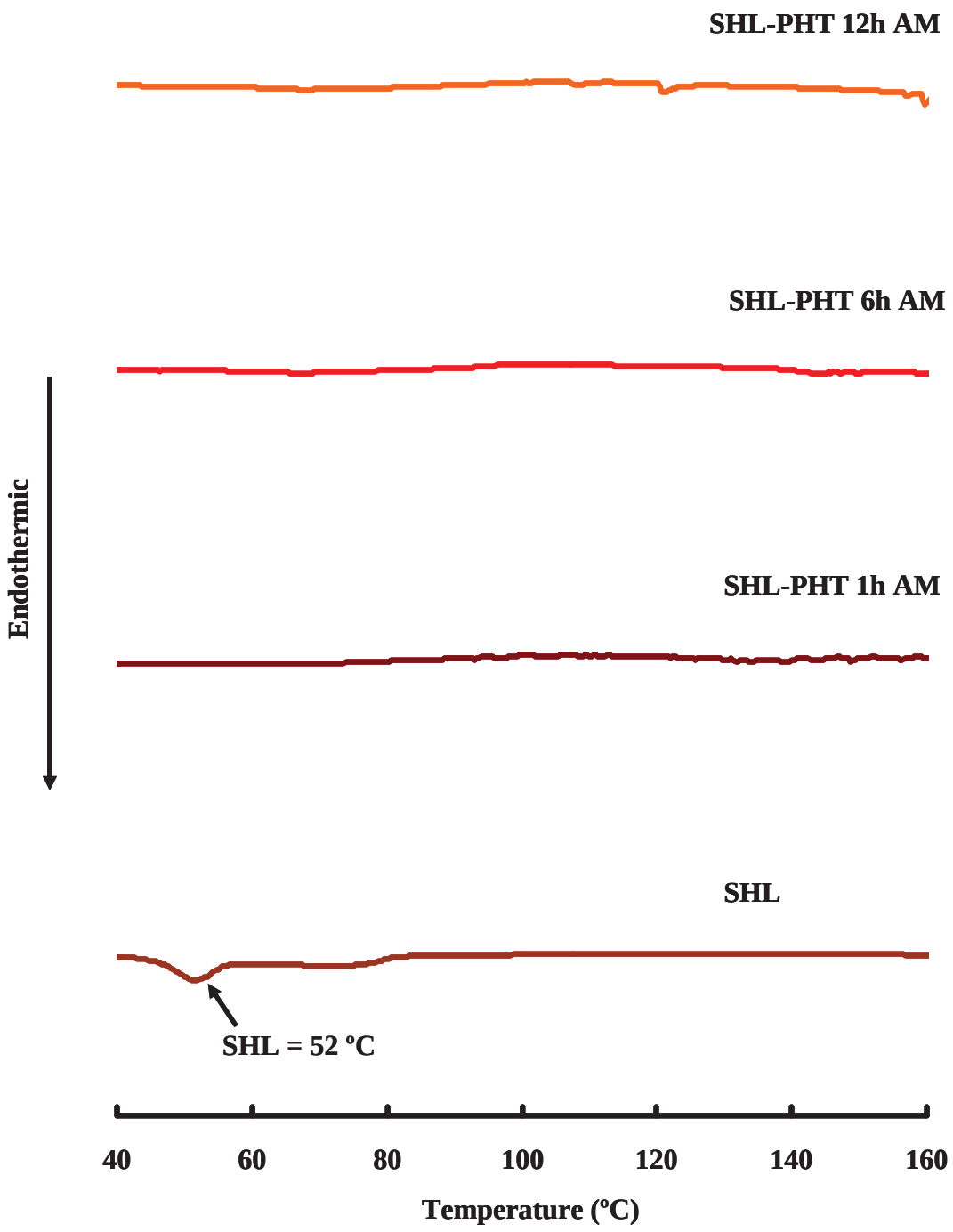


Figure 31 DSC curves of SHL and SHL-PHT AM after washing

2.2.1.3 Powder X-ray diffraction

PXRD patterns of SHL and SHL-SUC systems (before washing) are shown in Figure 32. SHL demonstrated a very broad peak (halo pattern) (Figure 32a). The PXRD patterns of SHL-PHT PM (Figure 32c) and SHL-PHT GM (Figure 32d) were the combination patterns of SHL and PHTA. The PXRD pattern of PHTA was decreased as increased annealing time (Figure 32e-32g), indicating that PHTA was consumed during phthalate formation. After removing of excess PHTA by washing, the PXRD patterns of all SHL-PHT AM demonstrated the halo pattern. The data indicated the amorphous state of SHL-PHT (Figure 33).

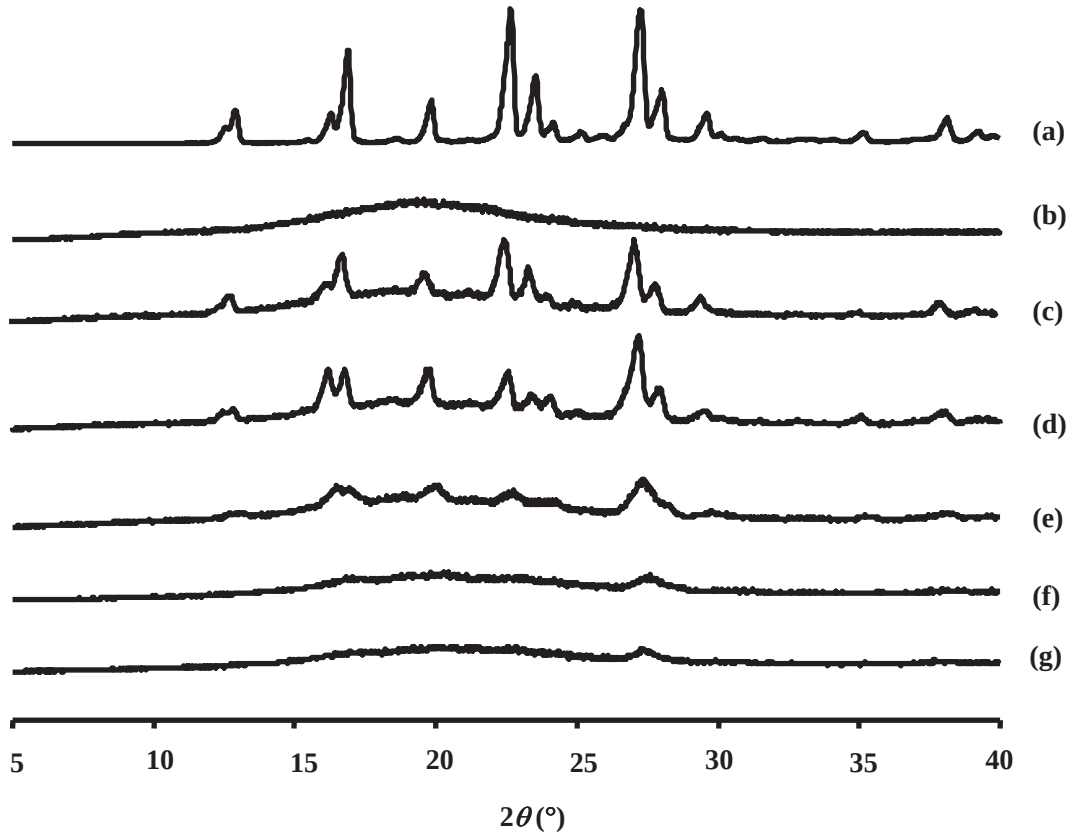


Figure 32 Powder X-ray diffraction patterns of SHL-PHT systems before washing: PHTA (a), SHL (b), SHL-PHT PM (c), SHL-PHT GM (d), SHL-PHT 1 h AM (e), SHL-PHT 6 h AM (f) and SHL-PHT 12 h AM (g)

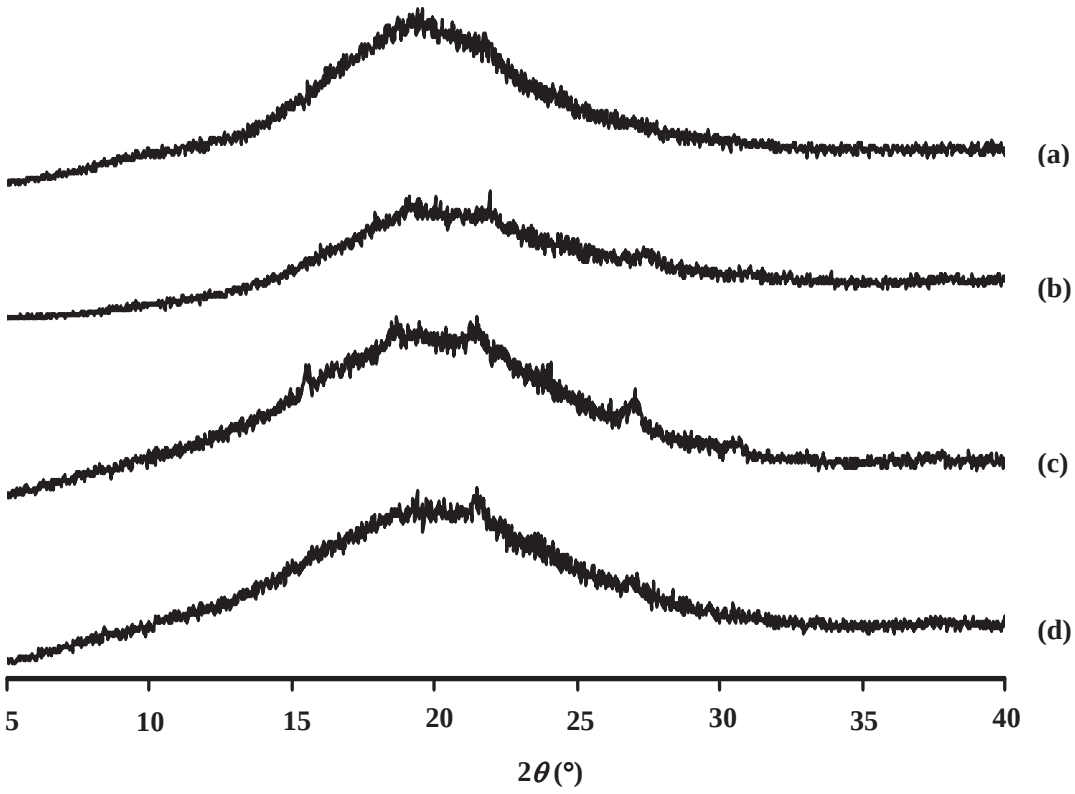


Figure 33 Powder X-ray diffraction patterns of SHL-PHT systems after washing: SHL (a), SHL-PHT 1 h AM (b), SHL-PHT 6 h AM (c) and SHL-PHT 12 h AM (d)

2.2.1.4 NMR spectroscopy

In order to support the esterification between SHL and PHTA, NMR spectroscopy was also employed to characterize the structure of shellac ester. The NMR spectra of SHL-PHT AM (after washing) were comparatively evaluated with SHL as shown in Figure 34 (^{1}H NMR spectra) and Figure 35 (^{13}C NMR spectra). The ^{1}H NMR spectrum of SHL demonstrated the methylene group (-CH_2-OH) and methine group (-CH-OH) at 3.49 and 3.25 ppm, respectively (Figure 34a). After esterification, the significant loss of the relative resonance of the peaks of

SHL-PHT 12 h AM observed for the methylene group and methine group and the new resonance of oxygenated methylene group of the attached phthalate moiety at 4.06 ppm. In addition, the addition of resonance attributable to an aromatic ring of ester part was observed in the range of 7.38-7.57 (Figure 34c). The result was supported by ^{13}C NMR spectra. The carboxyl group and benzene ring of phthalate part was clearly seen on the spectrum of SHL-PHT 12 h AM at 170.46 ppm and in the range of 132.03-129.98 ppm, respectively (Figure 35b). The data confirmed shellac phthalate formation.

Since SHL was esterified with PHTA, the increment of percent substituted at hydroxyl group of SHL-PHT was observed. Percent substituted at hydroxyl group of SHL-PHT was 62 % as compared with SHL, indicating the reduction of free hydroxyl group. As compared to SHL-SUC, SHL-PHT showed the lower percent substituted at hydroxyl group which correlated with the lower acid value, suggesting the less esterification of SHL-PHT.

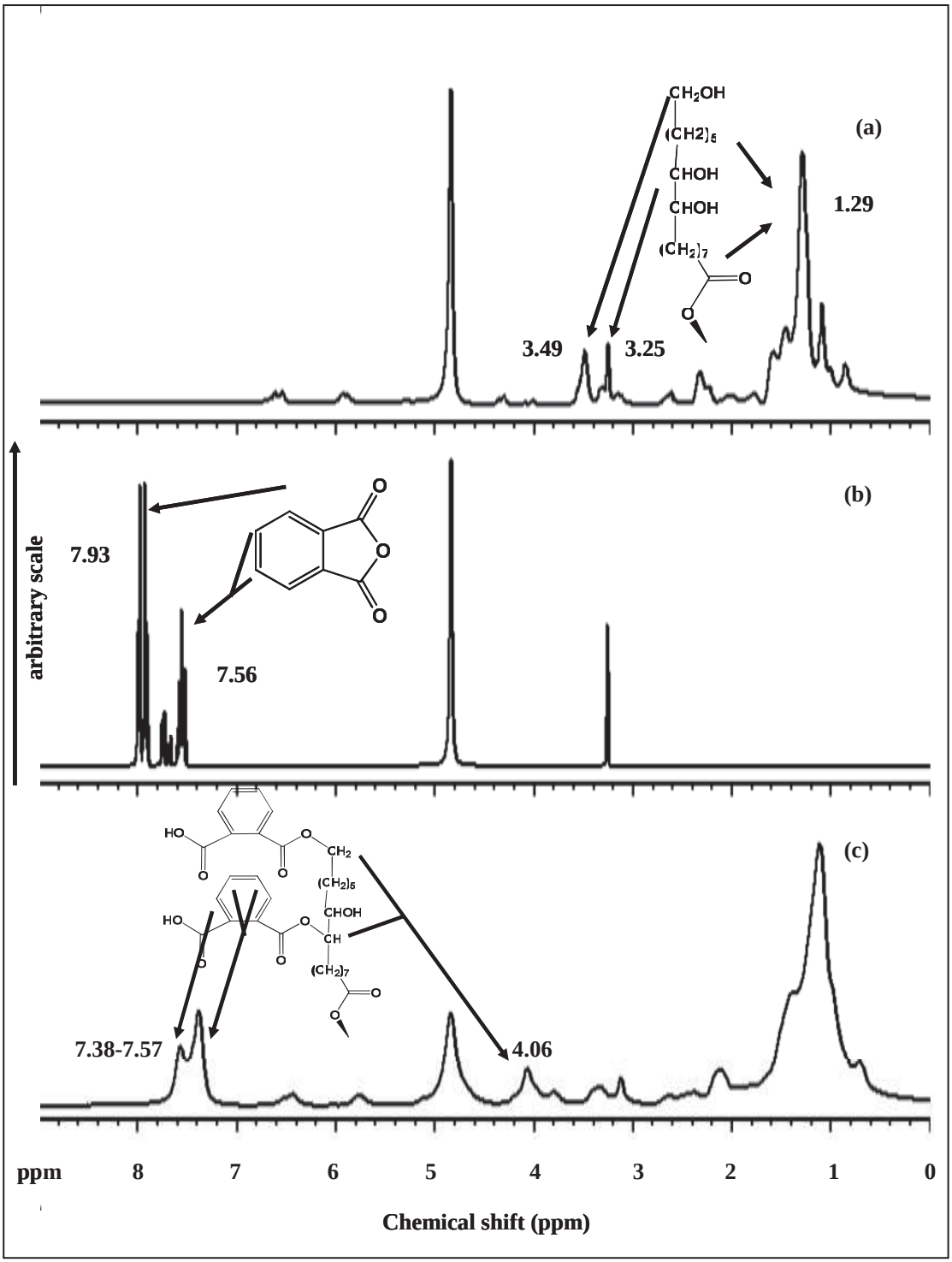


Figure 34 ^{1}H NMR spectra of SHL (a), PHTA (b) and SHL-PHT 12 h AM (c)

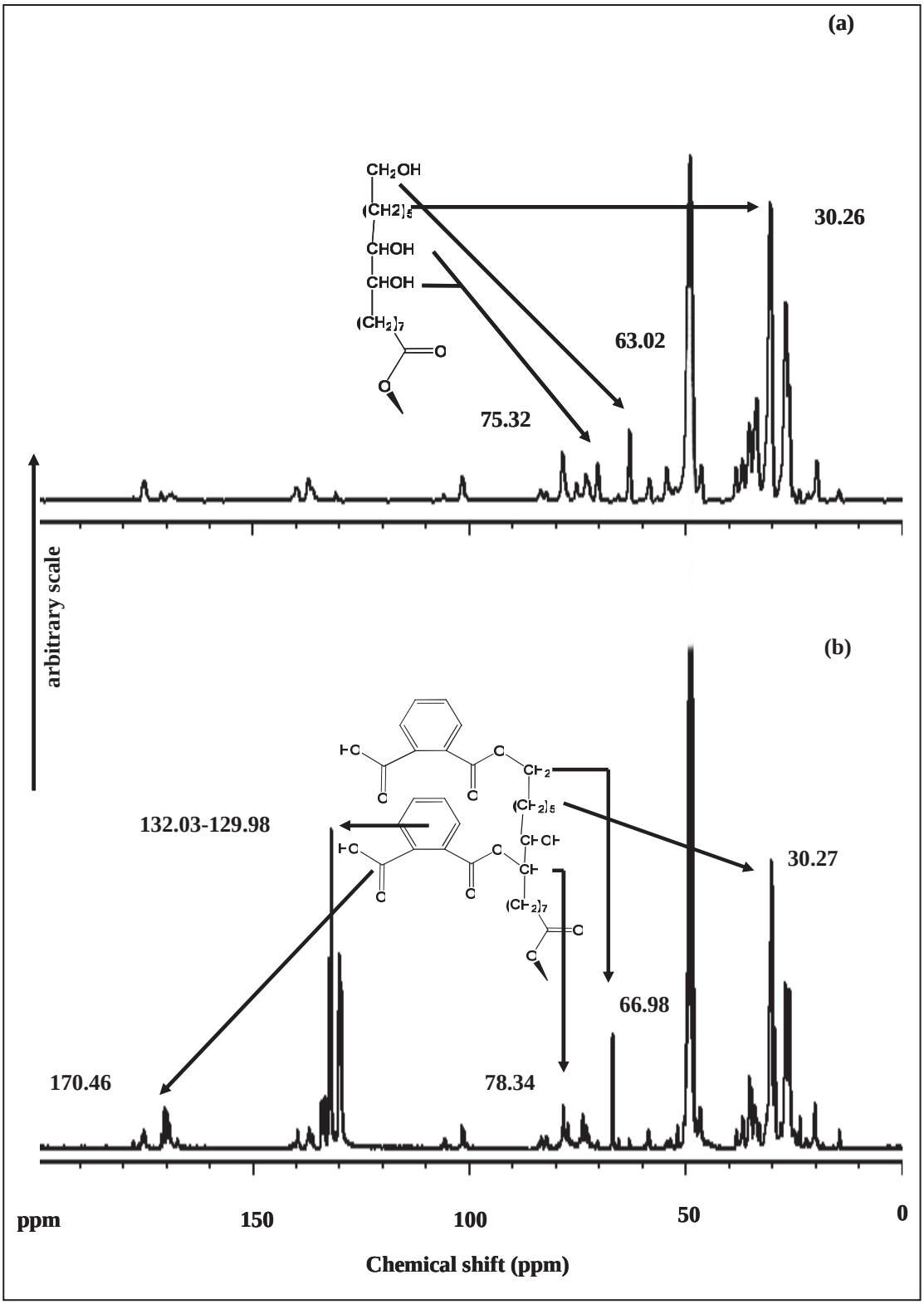


Figure 35 ^{13}C NMR spectra of SHL (a) and SHL-PHT 12 h AM (b)

2.2.2 Characterization of shellac phthalate film

SHL and SHL-PHT films were prepared by the same method as described in SHL-SUC systems and then comparatively evaluated for mechanical properties, pH solubility profile and water vapor permeability as described above.

2.2.2.1 Physical appearance

All films of SHL-PHT were clear transparent, dark yellow or brown in color and were not clearly different as compared to that prepared from SHL and SHL-SUC. The SHL-PHT films demonstrated more hydroscopic and soft characteristic at high humidity condition (75% RH), however, the film tended to more brittle if storage at lower humidity, as compared to the films of SHL and SHL-SUC.

2.2.2.2 Mechanical properties (puncture test)

Mechanical properties of SHL and SHL-PHT, including puncture strength, elongation, and modulus at puncture, are illustrates in Figure 36. The modulus of SHL-PHT films had a tendency to decrease as prolonged heat treatment time, especially after 1 h and the values were lower as compared to SHL and SHL-SUC (Figure 36a). The low modulus of SHL-PHT film was resulted from the low puncture strength and the high percent elongation. The puncture strength and percent elongation of SHL films showed the least value, indicating the brittle characteristic. With the increment of annealing time, the puncture strength of SHL-PHT film was decreased and the percent elongation of film was increased. The results were in good agreement with those observed in SHL-SUC, suggesting the increased plasticization effect by substituted moieties. In addition, SHL-SUC showed the more pronounced effect as compared to SHL-PHT. The more molecular mobility of

succinate moieties might provide more strong interaction with shellac polymer chain and thus reduced the SHL-SHL chain entanglement.

The data suggested that the hardness and flexibility of the film was changed after phthalate formation. The change of mechanical properties might be due to the higher polarity of shellac phthalate that was easily to react with vapor in the atmosphere. In addition, the steric effect of phthalate moiety caused the isolation of shellac polymer chain, resulting in the alteration of mechanical properties.

2.2.2.3 Water vapor permeability

Moisture protection is one of excellent characteristic properties of SHL. Since the esterification of SHL with PHTA might affect on this property, the water vapor permeability (WVP) of SHL-PHT was comparatively investigated. The WVP coefficient of shellac samples is demonstrated in Figure 36. SHL-PHT film demonstrated higher moisture uptake as compared to SHL film, however, the value was not significantly deteriorated ($p>0.01$), indicating that moisture protection was not deteriorated after phthalate formation. In addition, the WVP coefficient of SHL-PHT film was not significantly changed as compared to that of SHL-SUC film ($p>0.01$), suggesting that the esterification with both anhydrides did not affect to moisture permeability of shellac film.

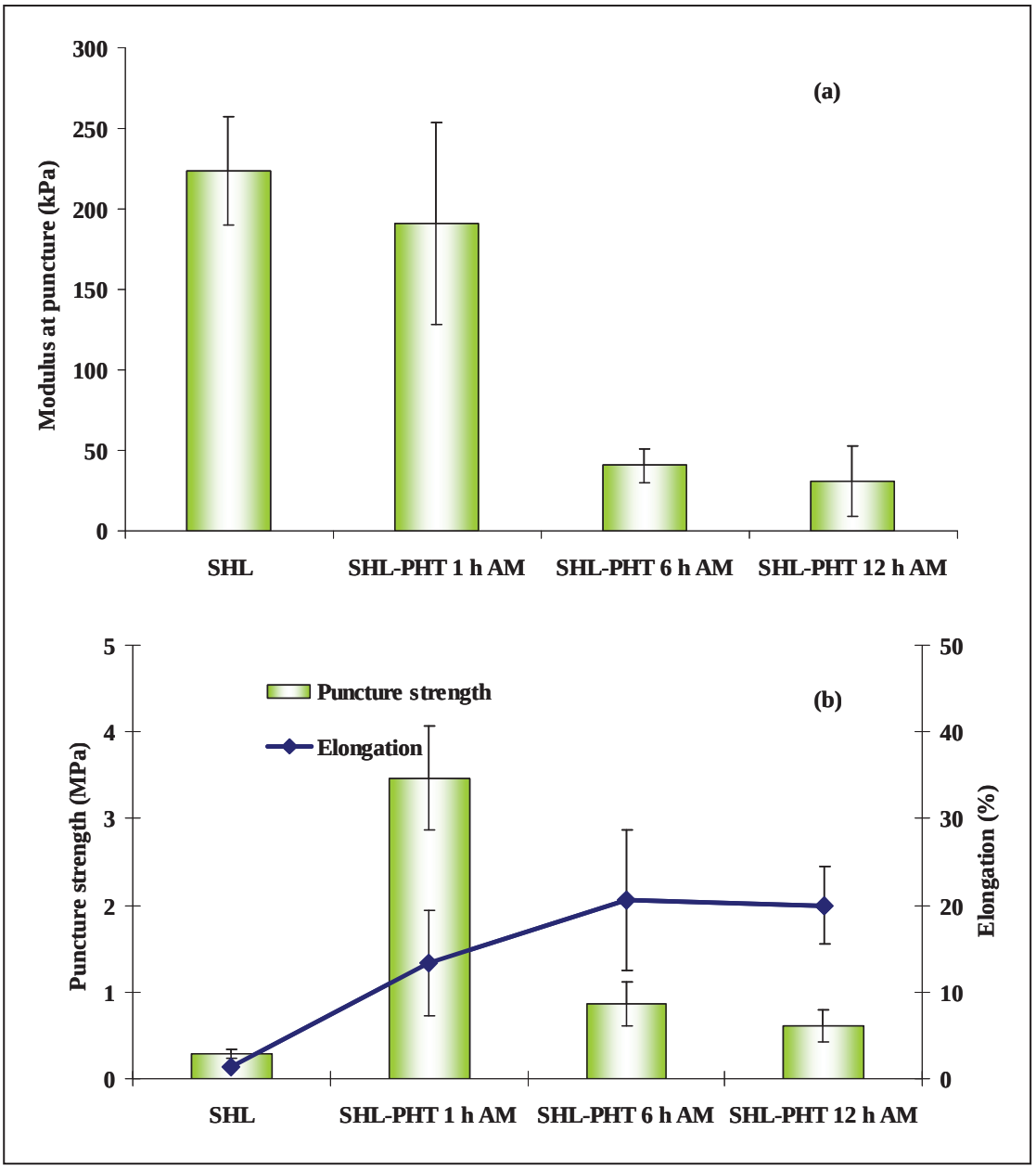


Figure 36 Mechanical properties of SHL-PHT AM systems: modulus at puncture (a), puncture strength and percentage of elongation (b)

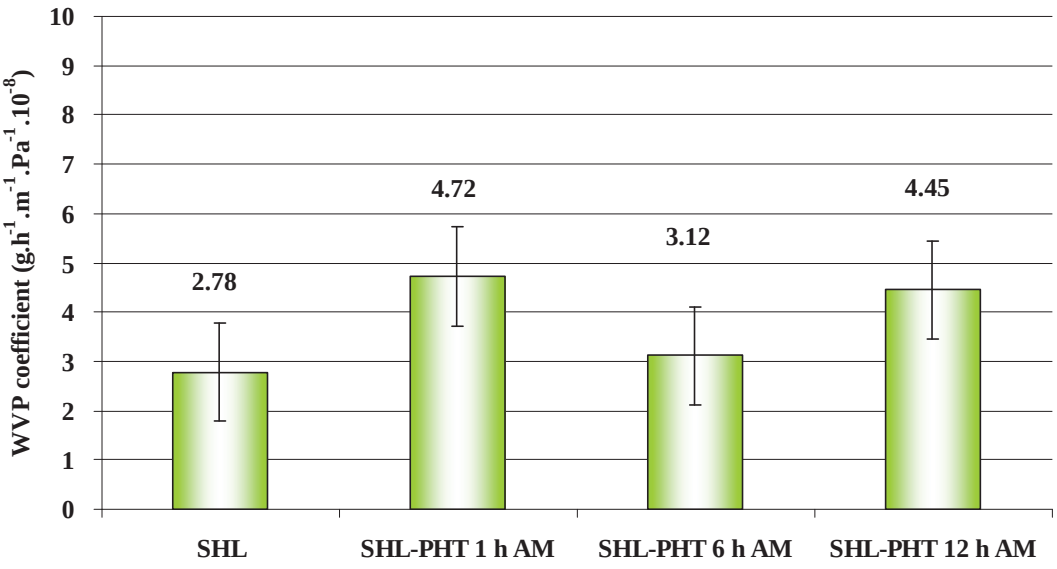


Figure 37 WVP coefficient of SHL and SHL-PHT AM systems

2.2.2.4 pH solubility profiles

One objective of oral dosage form is to achieve drug released at the highest therapeutic benefit site, so the important role of enteric film is to protect drug from acidic fluid or to decrease the irritant of drug and to dissolve immediately in the small intestine condition (Jones 2004). Although shellac has a good protection in gastric fluid, however shellac still has a limited solubility in intestinal fluid (Tarcha 1999: 39-66; Harianawala et al. 2002: 139-146; Sontaya Limmatvapirat et al. 2004: 41-49; Sontaya Limmatvapirat et al. 2007: 690-698; Liu et al. 2009: 119-124), thus SHL-PHT had to test for gastric resistance and solubility at the pH of intestine.

Figure 38 demonstrates percent dissolved of SHL and SHL-PHT after soaking in SGF. All SHL samples indicated low percent dissolved after soaking in SGF for 2 h. Although slightly increase of percent dissolved of SHL-

PHT was observed with extended annealing time, the values were still less than 5% w/w, confirming the gastric resistance of SHL-PHT AM.

Figure 39 shows pH solubility profile of shellac sample films. The result showed that the percent dissolved of all films was raised as increasing of pH of buffer solution. SHL and SHL-PHT 1 h AM films were completely dissolved at pH 7.0, while SHL-PHT 6 h AM and SHL-PHT 12 h AM began to completely dissolved at pH 6.4, indicating the more solubility of SHL-PHT at intestinal pH. However, the pH of completely film dissolved of SHL-PHT was higher as compared to that of SHL-SUC (SHL-SUC 24 h AM was completely dissolved at pH 6.1), supporting the more increment of carboxylic acid of SHL-SUC and consequently increased the solubility.

Figure 40 demonstrates the dissolution time at which all shellac films were completely dissolved (pH 7.0). The dissolving time of film was significantly declined as increasing phthalate formation ($p<0.01$). As compared to SHL, the dissolving time of all SHL-SUC and SHL-PHT at pH 7.0 dramatically declined ($p<0.01$). The result suggested the solubility enhancement due to the increasing carboxylic acid group and the higher ionization at lower pH of shellac esters (Nesbitt et al. 1985: 215-226; Bruce et al. 2003: 85-96; Marťnez-Gonzlez and Villafuerte-Robles 2003: 183-193; AlKhatib et al. 2008: 804-812).

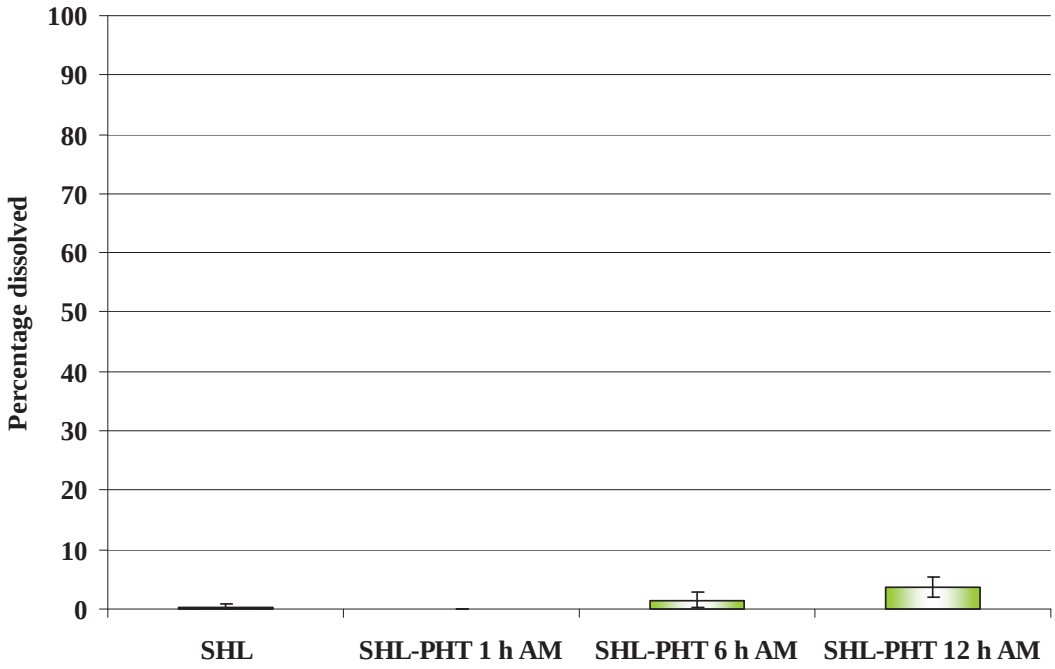


Figure 38 Percent dissolved of SHL and SHL-PHT AM in SGF

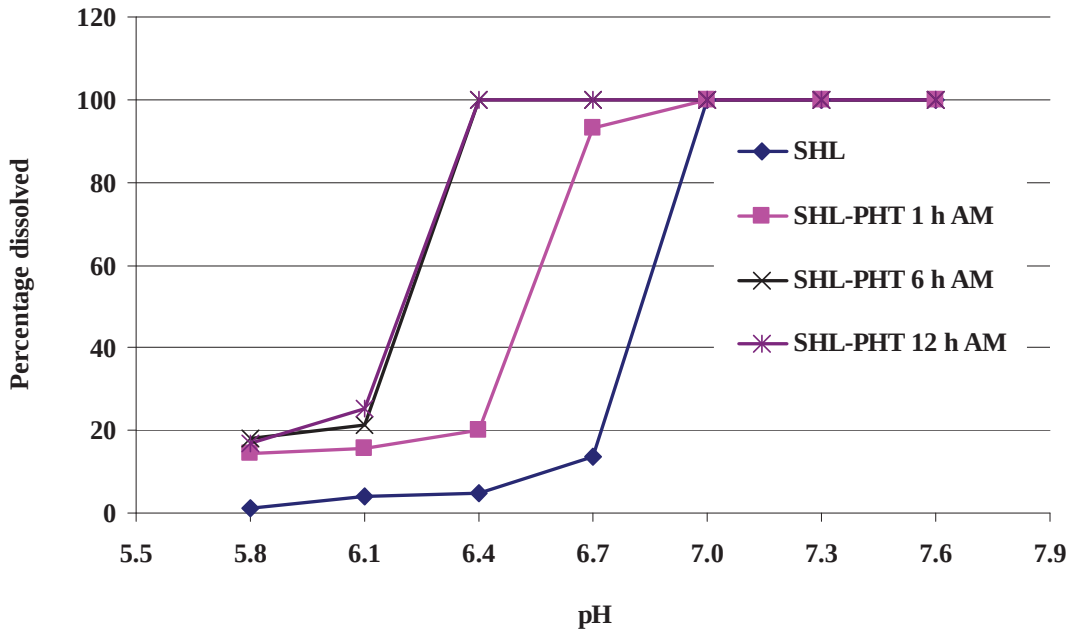


Figure 39 pH solubility profiles of SHL and SHL-PHT AM

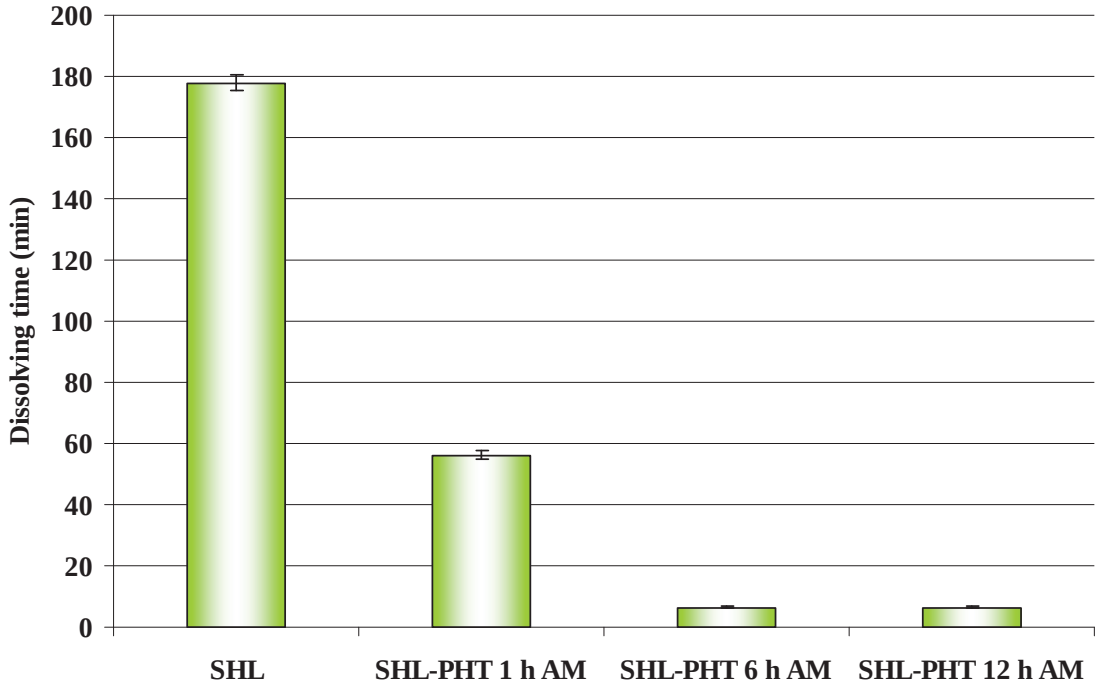


Figure 40 Dissolving time of SHL and SHL-PHT AM in pH 7.0 buffer

2.3 Characterization of shellac trimellitate

From the SHL-PHT formation, the esterification of SHL and PHTA was increased as increasing annealing temperature. Since the structure of TMTA was similar to that of PHTA, the SHL-TMT formation at various annealing temperatures was further investigated and compared with previously described in SHL-SUC and SHL-PHT systems.

2.3.1 Acid value and percentage of insoluble solid

Figure 41 shows the effect of annealing temperature on acid value (bar chart) and percent insoluble solid (line chart) of SHL-TMT. The results demonstrated that the acid value of SHL-TMT was gradually increased as increasing annealing temperature for 12 h. The acid value of SHL-TMT after annealing at 100 ºC (100 ºC AM) was approximately 1.5 times as compared to that of SHL. However,

SHL-TMT 100 ºC AM showed lower acid value as compared to SHL-PHT. Since acid value represents amount of carboxyl group per molecule, the lower acid value suggested the lower substitution of trimellitate moiety into the shellac molecule. The larger molecules of TMTA might impede the esterification at hydroxyl group of SHL molecule.

Since the melting point of TMTA was highest (161.0-163.5 ºC), the lowest acid value of SHL-TMT at low annealing temperature (60 ºC) was observed as compared to SUCA and PHTA. The high melting point and the steric effect of larger molecule of TMTA might cause the low degree of esterification between SHL and TMTA.

In order to investigate the stability of SHL-TMT during heat treatment, the percentage of insoluble solid was monitored. As illustrated in Figure 41 (line chart), the percent insoluble solids of SHL-TMT was dramatically raised as increased annealing temperature, indicating that SHL-TMT was not stable during the annealing process. For SHL-TMT, the number of substituted trimellitate group might not sufficient to protect the reactive hydroxyl group of SHL molecule. In this case, SHL molecule might polymerize before the esterification was fully occurred.

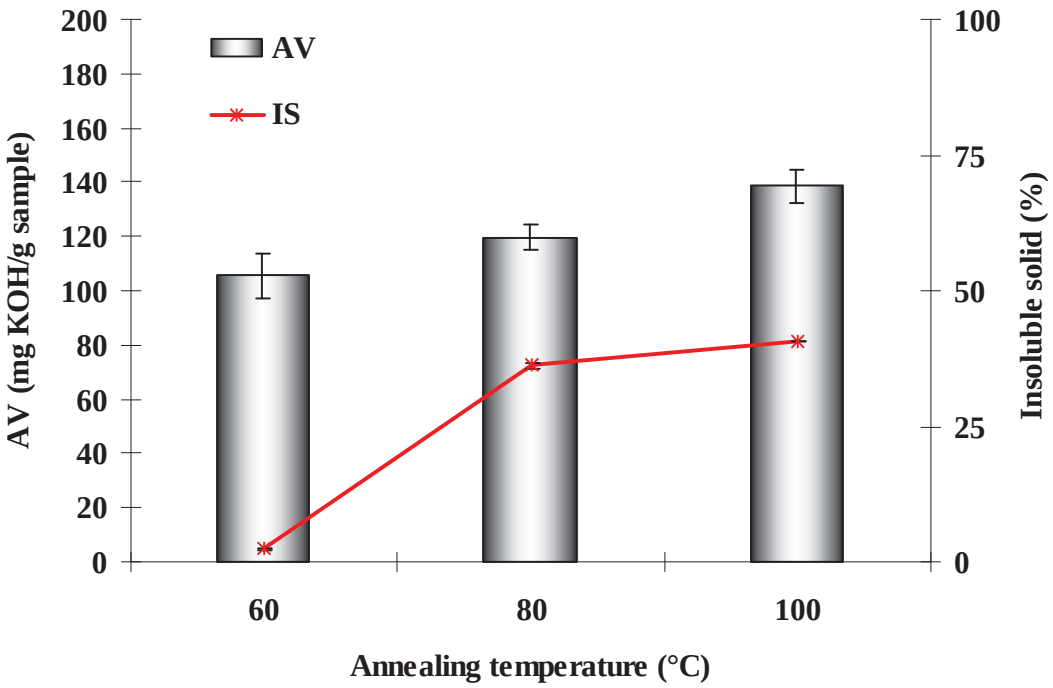


Figure 41 Acid value and insoluble solid of SHL-TMT after annealing at various temperatures for 12 h

2.3.2 FTIR spectroscopy

As illustrated in Figure 42, after removing excess TMTA, the new peaks of C-O stretching, the aromatic C-H in-plane bending and C-H out-of-plane bending of attached trimellitate moiety at 1252, 1067 and 760 cm^{-1} were increased as increased annealing temperature. The increment of relative intensity of carbonyl stretching at 1717 cm^{-1} was also observed, indicating that the SHL-TMT formation could take place after heat treatment at higher temperature. However, the intensities of new peaks were not clearly increased as compared to SHL-SUC and SHL-PHT systems, especially at low annealing temperature. The results were well agreed with that of AV increment, supporting the lower degree of esterification by TMTA.

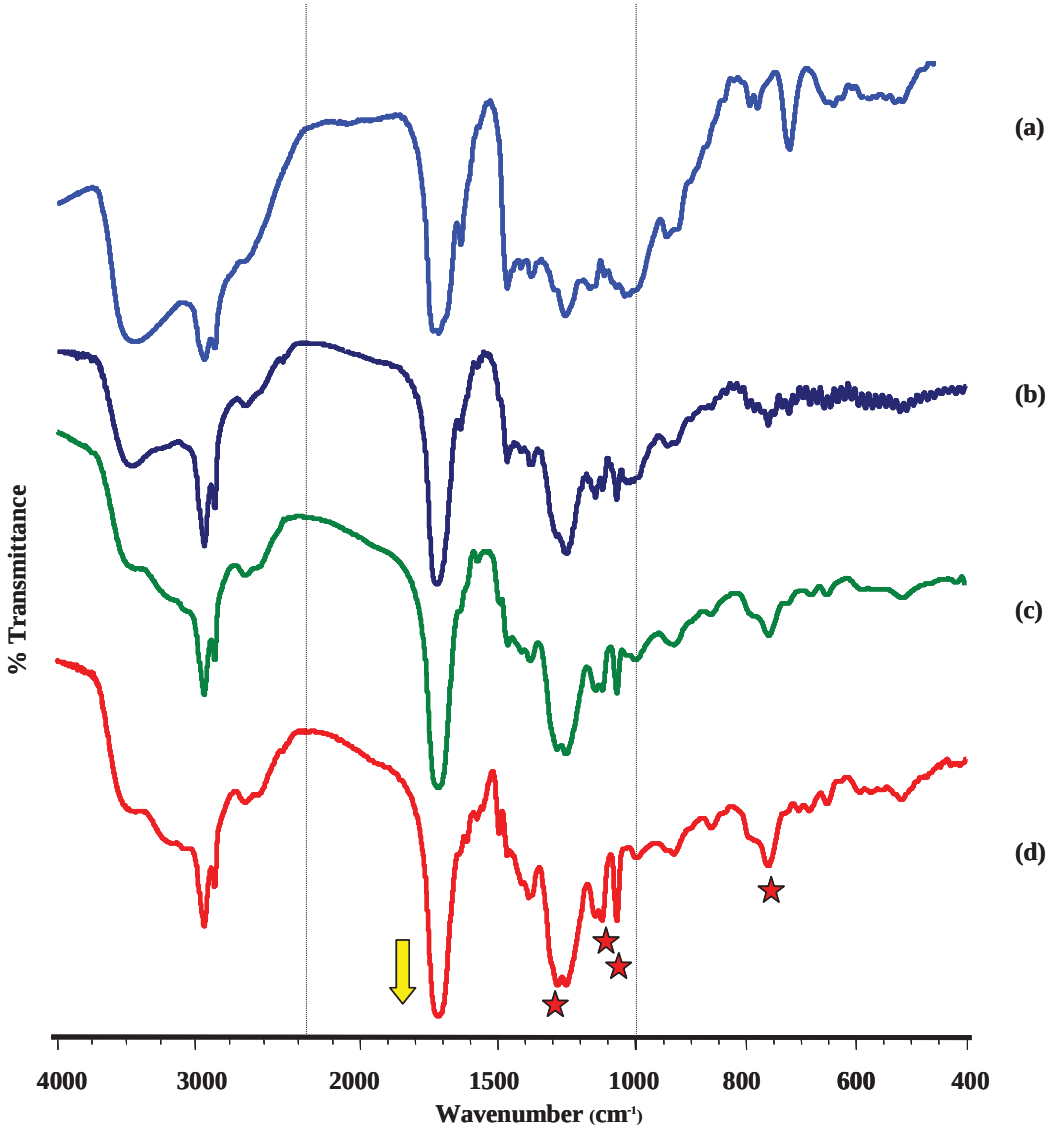


Figure 42 FTIR spectra of SHL-TMT AM systems; SHL (a), SHL-TMT 60 ºC AM (b), SHL-TMT 80 ºC AM (c) and SHL-TMT 100 ºC AM (d)

2.3.3 Differential scanning calorimetry

After heat treatment, the change of endothermic peak of SHL-TMT AM was not clearly observed (data not shown). However, the washed SHL-TMT AM (after washing) (12 h annealing) showed different thermogram as compared to SHL (Figure 43). The result suggested that the change of SHL structure might occur after trimellitate formation, however, the change was not obviously observed as compared to SHL-SUC and SHL-PHT.

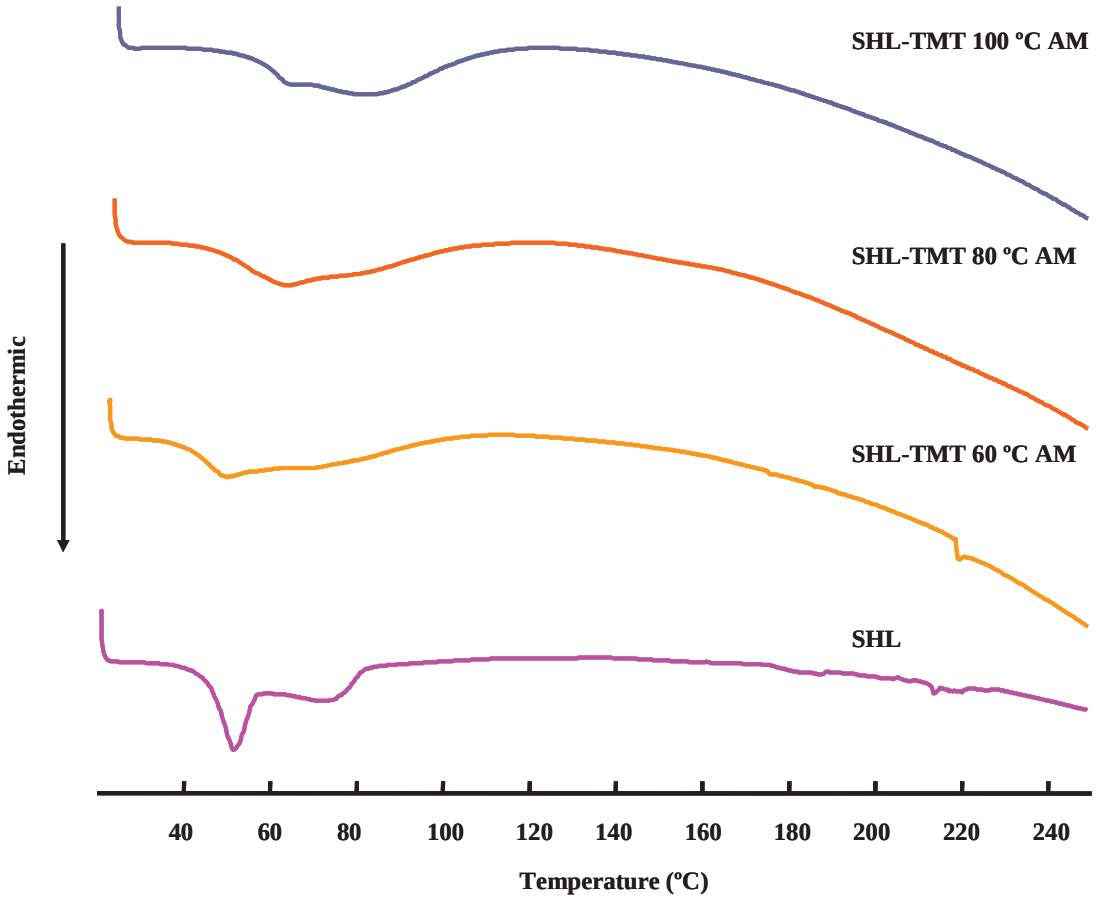


Figure 43 DSC curves of SHL, SHL-TMT AM (after washing) (12 h annealing)

2.3.4 Powder X-ray diffraction

Figure 44 demonstrates the PXRD patterns of SHL and SHL-TMT (before washing). An amorphous phase of SHL showed a halo pattern (Figure

44a). The PXRD patterns of SHL-TMT PM (Figure 44c) and SHL-TMT GM (Figure 44d) were the superimposed pattern of SHL and TMTA. As raised the temperature, the intensity of diffraction peaks of TMTA gradually declined (Figure 44e-44g), indicating that TMTA was used for SHL-TMT formation during annealing process. However, the intensity of diffraction peaks assigned to TMTA was relatively higher as compared to that of PHTA in SHL-PHT systems, suggesting that the low amount of TMTA was consumed to reaction. The result correlated with the lower AV and FTIR spectrum of SHL-TMT AM. The PXRD patterns of SHL-TMT AM after removing excess TMTA was demonstrated in Figure 45. The halo pattern of all SHL-TMT AM was observed, suggesting the amorphous state of SHL-TMT.

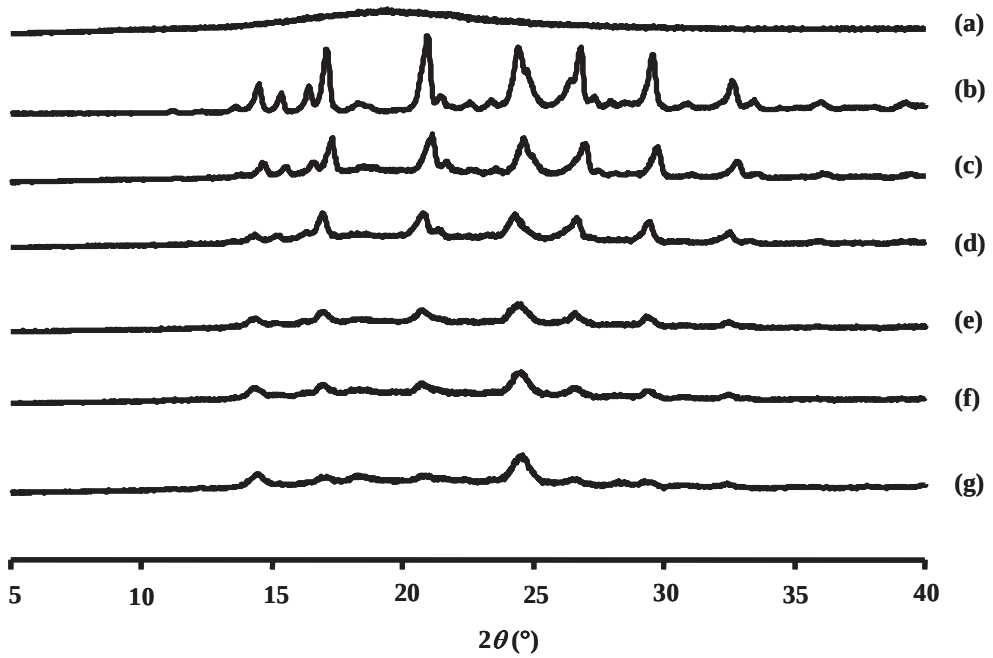


Figure 44 Powder X-ray diffraction patterns of SHL-TMT systems before washing: SHL (a), TMTA (b), SHL-TMT PM (c), SHL-TMT GM (d), SHL-TMT 60 ºC AM (e), SHL-TMT 80 ºC AM (f) and SHL-TMT 100 ºC AM (g)

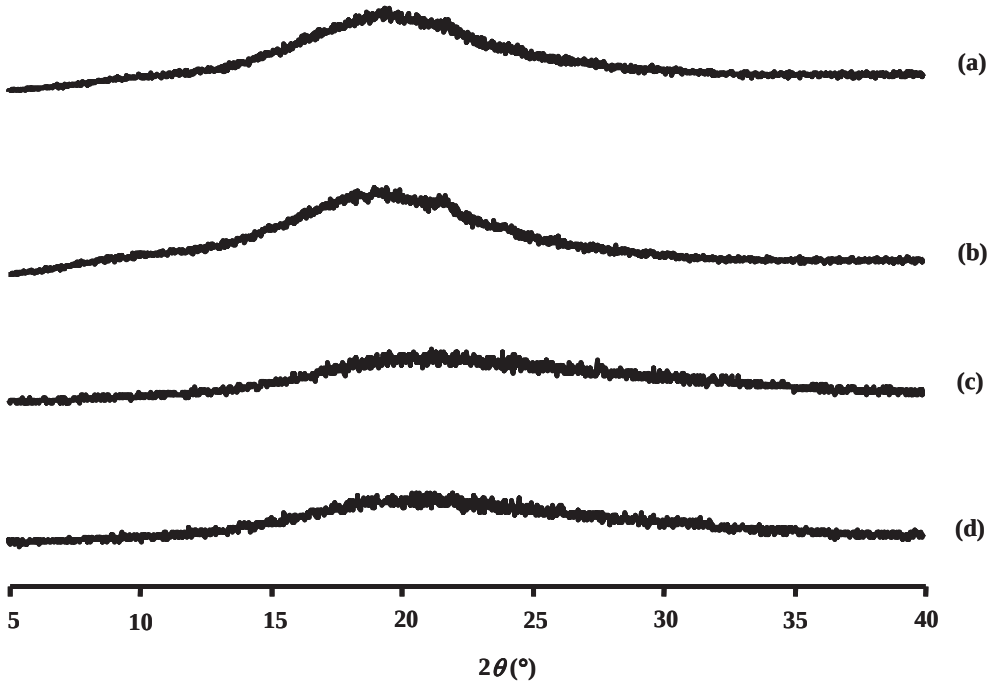


Figure 45 Powder X-ray diffraction patterns of SHL-TMT systems after washing: SHL (a), SHL-TMT 60 ºC AM (b), SHL-TMT 80 ºC AM (c) and SHL-TMT 100 ºC AM (d)

2.3.5 NMR spectroscopy

In order to confirm the SHL-TMTA esterification, the ^{1}H and ^{13}C NMR spectra of SHL-TMT 80 ºC AM were comparatively analyzed with those of SHL. Figure 46 illustrated the ^{1}H NMR spectrum of SHL-TMT systems. The relative resonance of methylene group (-CH_2-OH) and methine group (-CH-OH) of SHL-TMT (Figure 46c) at 3.49 and 3.25 ppm, respectively, were slightly decreased as compared to those observed in SHL. The new small signal at 4.13 ppm corresponding to an oxygenated methylene of SHL-TMT was also observed. In addition, the small signal of peaks assigned as aryl proton of trimellitate moiety was observed in the range of 8.03-8.26 ppm. The result suggested the formation of SHL-TMT although the degree

of esterification was relatively low as compared to SHL-PHT and SHL-SUC. ^{13}C NMR was used for confirming the formation (Figure 47). The presence of peaks assigned as aromatic carbon on the spectrum of SHL-TMT (Figure 47b) was observed in the range of 130-140 ppm. The declining of the relative resonance at 75.33 and 63.03 ppm, corresponding to methine group (-CH-OH) and methylene group (-CH_2-OH) of SHL, respectively, and the resonance of addition trimellitate moieties at 72.74 and 67.23 ppm, confirmed the present of trimellitate group. However, as compared with SHL-SUC and SHL-PHT, the intensity of all new signals and the reduction of characteristic resonance of SHL were much lower.

After trimellitation, percent substituted at hydroxyl group of SHL-TMT was increased to 31%, indicating the esterification between SHL and TMTA. However the less increment of percent substituted at hydroxyl group of SHL-TMT was observed as compared with SHL-SUC and SHL-PHT, supporting the lower degree of esterification by TMTA.

The results were well agree with the previous results such as acid value, insoluble solid and FTIR spectrum, indicating that the large molecular size of TMTA might cause the low degree of ester formation in which the polymerization between the SHL chains, could not be protected.

Therefore SHL-TMT was not chosen to further study of film and coating product.

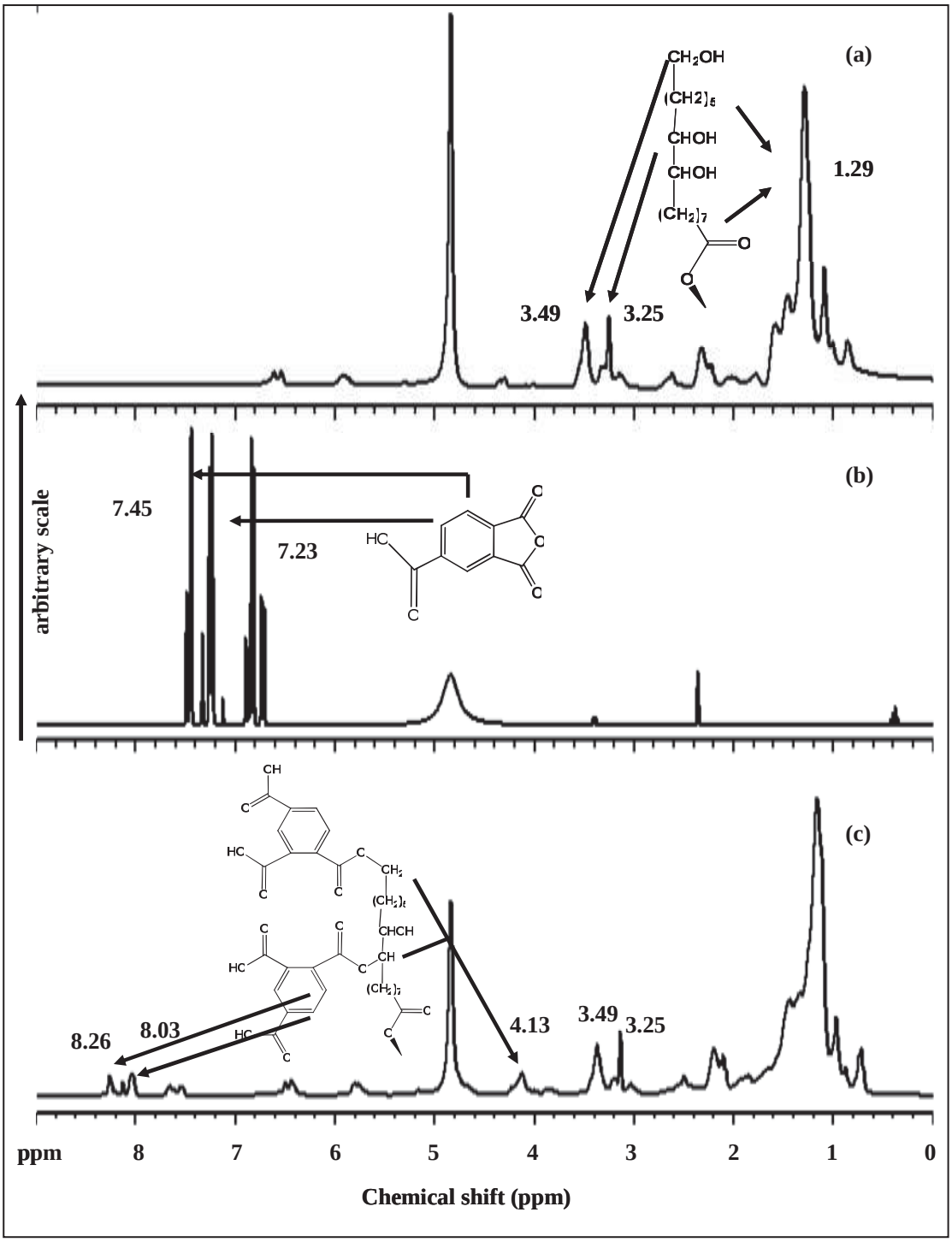


Figure 46 ^{1}H NMR spectra of SHL (a), TMTA (b) and SHL-TMT 80 ºC AM (c)

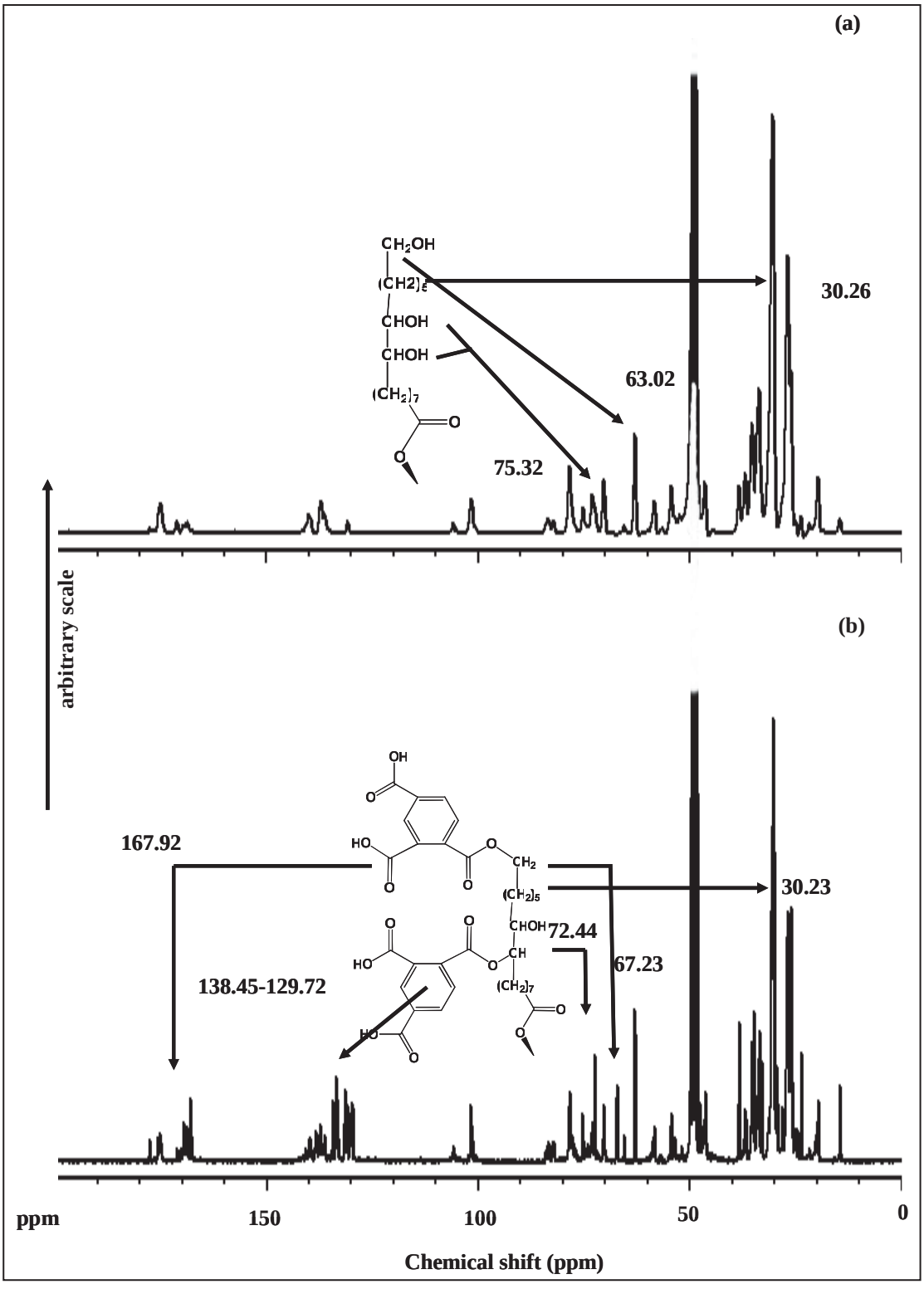


Figure 47 ^{13}C NMR spectra of SHL (a) and SHL-TMT 80 ºC AM (b)

3. Characterizations of coated tablets

In order to study enteric properties of shellac ester coated tablets, the SHL and shellac esters coated tablets were prepared. Each coating solution was prepared by dissolving SHL or shellac ester in ammonium solution. The core tablets which contained paracetamol 100 mg were coated with different coating solutions and then comparatively evaluated.

3.1 Characterization of SHL-SUC AM coated tablets

The enteric coating solution (8% w/w) was prepared by dissolving shellac sample in ammonia solution and then plasticized with PEG 400. Talcum, which enhanced lubricity and minimized stickiness during the drying step (Porter 2005), was also added to solution.

Properties of SHL and SHL-SUC AM coated tablets at different coating levels such as weight, thickness, hardness, tablet disintegration and dissolution profile were comparatively evaluated as shown in Table 2-5. As increasing coating level, the change of tablet properties was observed, especially the disintegration time of coated tablets.

Table 2 demonstrates properties of shellac coated tablets at various coating levels. Weight and thickness of coated tablets gradually increased, while hardness was insignificant changed as increasing coating level. For tablet disintegration, uncoated tablets and coated tablets at low coating level (0.5 and 1 mg/cm^2) were completely disintegrated in 0.1 HCl, level of coating at 2-3 mg/cm^2, some coated tablets could resist disintegration in 0.1 N HCl for 2 h while complete gastric protection was achieved at a coating level of 4 mg/cm^2 or more. The increment

of DT in buffer pH 6.8 was also observed as increase coating level. The DT in buffer pH 6.8 was dramatically increased especially at the coating level of 6.0 mg/cm^2.

Table 2 Properties of SHL coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	0.999±0.006	8.87±0.09	14.4±2.4	< 1.0	-
1	1.006±0.009	8.86±0.07	13.9±2.5	< 1.0	-
2	1.016±0.009	8.93±0.05	13.8±1.0	> 2 h*	19.2±18.0
3	1.026±0.008	8.97±0.05	14.4±1.4	> 2 h**	43.5±2.1
4	1.032±0.008	8.97±0.07	15.5±1.0	> 2 h	41.0±0.8
5	1.044±0.011	9.03±0.06	17.0±0.7	> 2 h	58.6±9.3
6	1.047±0.009	9.03±0.06	16.9±2.0	> 2 h	134.3±23.4

* 2 of 6 tablets disintegrated in 1.0 min;** 1 of 6 tablets disintegrated in 2.0 min

The properties of SHL-SUC 1 h AM, SHL-SUC 6 h AM and SHL-SUC 24 h AM coated tablets are shown in Table 3-5, respectively. The results were similar to that observed in SHL coated tablets. The coating level directly affected the tablet properties of all SHL-SUC AM coated tablets. SHL-SUC 1 h AM coated tablets could be protected from acid condition at the coating level of 4 mg/cm^2 which was the same as SHL coated tablets. However, SHL-SUC 1 h AM coated tablets showed significant rapid disintegration in buffer pH 6.8, as compared to SHL coated tablets (p<0.01). For SHL-SUC 6 h AM and SHL-SUC 24 h AM, the picking of coated tablets was observed during coating process. Thus, the increase of coating level was necessary for the 2 h-gastric resistance. The coating level of 8 mg/cm^2 could prolong disintegration time in 0.1 N HCl of SHL-SUC 6 h AM and SHL-SUC 24 h AM coated tablets to more than 2 h. However, the disintegration time in buffer pH 6.8 was decreased, as increase succinate formation. The results were good agreement with the solubility of SHL-SUC film (Figure 23-25). The succinate formation, therefore, could

increase the solubility of film in small intestine, which the gastric protection was still in acceptance level if the coating level was sufficient.

Table 3 Properties of SHL-SUC 1 h AM coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	0.999±0.006	8.87±0.09	14.4±2.4	< 1.0	-
1	1.001±0.012	8.79±0.13	10.0±2.0	34.0±18.0	-
2	1.013±0.008	8.86±0.06	9.0±1.0	> 2 h*	3.3±0.7
3	1.020±0.009	8.89±0.06	9.3±0.6	> 2 h**	6.0±2.8
4	1.029±0.008	8.93±0.05	10.6±0.8	> 2 h	7.4±2.3
5	1.039±0.012	8.98±0.08	13.9±2.0	> 2 h	9.7±0.1
6	1.041±0.009	8.99±0.11	11.4±0.6	> 2 h	12.3±0.5

* 4 of 6 tablets disintegrated in 111.7±11.3 min; ** 6 tablets swelled but not disintegrated

Table 4 Properties of SHL-SUC 6 h AM coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	0.999±0.006	8.87±0.09	14.4±2.4	< 1.0	-
1	1.001±0.012	8.85±0.05	10.6±4.4	< 1.0	-
2	1.012±0.008	8.91±0.04	10.0±1.3	5.7±0.9	-
3	1.017±0.009	8.94±0.06	9.3±1.0	31.9±4.9	-
4	1.026±0.009	8.97±0.05	13.3±1.8	> 2 h*	2.4±0.7
5	1.032±0.007	8.96±0.06	14.8±2.2	> 2 h**	4.2±0.2
6	1.042±0.009	9.05±0.06	15.9±1.5	> 2 h***	4.5±0.3
7	1.045±0.009	9.07±0.05	16.3±1.7	> 2 h****	4.7±1.4
8	1.054±0.008	9.10±0.06	14.2±2.0	> 2 h	6.5±0.3
9	1.058±0.010	9.16±0.05	13.1±1.8	> 2 h	7.0±0.2

*4 of 6 tablets disintegrated in 84.7±35.0 min; **2 of 6 tablets disintegrated in 43.1±9.8 min;
*** 1 of 6 tablets disintegrated in 81.6 min; **** 1 of 6 tablets disintegrated in 118.9 min

Table 5 Properties of SHL-SUC 24 h AM coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	0.999±0.006	8.89±0.09	14.4±2.4	< 1.0	-
1	1.003±0.006	8.82±0.07	16.6±1.5	< 1.0	-
2	1.007±0.010	8.83±0.01	14.5±1.4	< 1.0	-
3	1.015±0.007	8.92±0.05	14.6±0.8	1.1±0.3	-
4	1.024±0.014	8.97±0.08	15.0±0.8	15.1±14.7	-
5	1.030±0.010	9.00±0.09	15.3±1.9	42.2±40.6	-
6	1.034±0.008	9.02±0.05	16.4±1.8	> 2 h*	2.2±0.8
7	1.047±0.010	9.06±0.06	17.4±0.9	> 2 h**	1.3±0.3
8	1.050±0.008	9.10±0.06	18.4±0.4	> 2 h	2.4±0.9
9	1.056±0.007	9.09±0.08	18.6±2.0	> 2 h	4.1±0.3

*4 of 6 tablets disintegrated in 19.9±26.1 min; ** 1 of 6 tablets disintegrated in 107.9 min

After oral administration, coated tablets undergo transit from the stomach to the small intestine. Dosage form must be stable in gastric condition, but the medication should be immediately released in the small intestine. Hence the dissolution profile of coated tablets at various coating levels was also evaluated. Figure 48 demonstrates the dissolution profile of SHL coated tablets. SHL coated tablets did not dissolve and release paracetamol in 0.1N HCl for 2 h even the coating level of 4 mg/cm^2. However, the drug released in buffer 6.8 was significantly decreased as the coating level was increased. The result was correlated with the disintegration time (Table 2). For example, the time for 80% drug released of the coating level of 4 mg/cm^2 was approximately 20 min, while that of 6 mg/cm^2 was up to 80 min; indicating that coating level of SHL had a great influence on percent dissolved in small intestine. As illustrated in Figure 49-51, all SHL-SUC AM coated tablets could inhibit drug dissolved in gastric condition for 2 h, similar to SHL coated tablets. However, SHL-SUC AM demonstrated faster drug release in buffer pH 6.8.

The time for 80% drug released of SHL-SUC AM coating was less than 20 min. In addition, the drug release in buffer pH 6.8 of SHL-SUC AM coated tablets was insignificantly changed with increased coating level, confirming the better enteric properties of SHL-SUC AM.

The time for 50% drug released (T_{50}) of SHL and SHL-SUC AM coated tablets is shown in Figure 52. T_{50} of SHL coated tablets significantly increased as increasing coating level (p<0.01), which was due to the low solubility of SHL in small intestine. On the other hand, T_{50} of SHL-SUC AM coated tablets was 10-15 min and did not significantly changed as increasing coating level, especially for SHL-SUC 6 h AM and SHL-SUC 24 h AM. The results were in good agreement with the excellent solubility of SHL-SUC film (Figure 24), confirming the enhanced enteric properties of SHL by succinate formation.

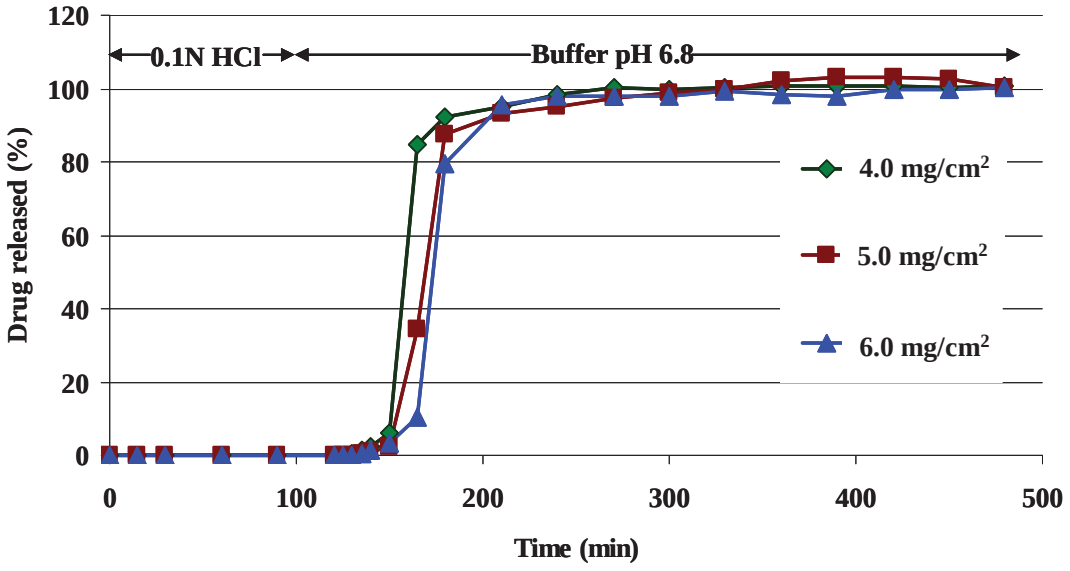


Figure 48 Dissolution profile of shellac coated tablets

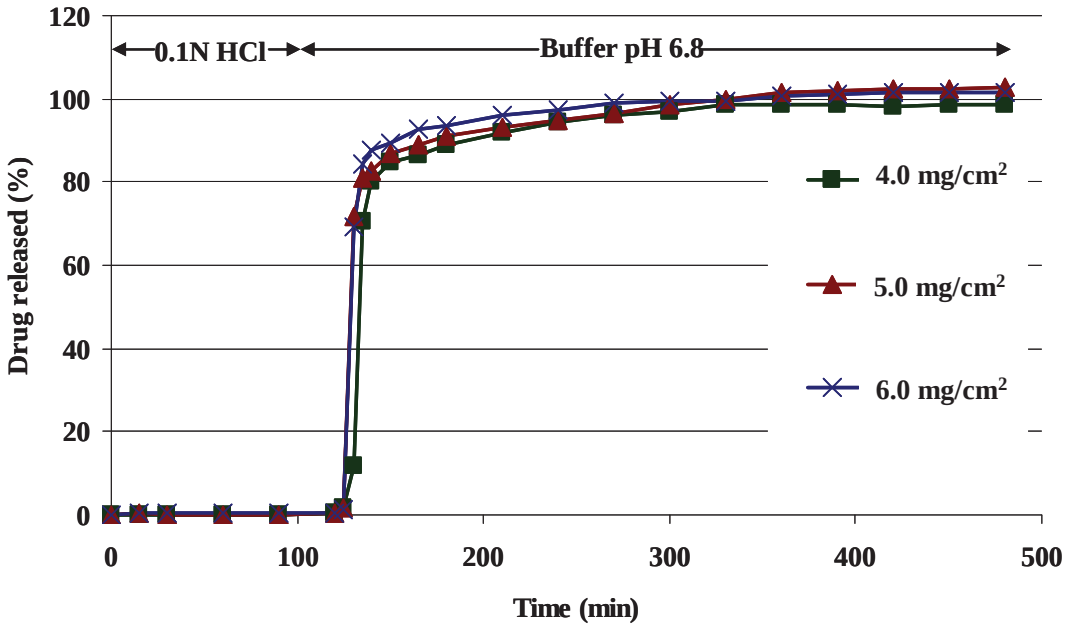


Figure 49 Dissolution profile of SHL-SUC 1 h AM coated tablets

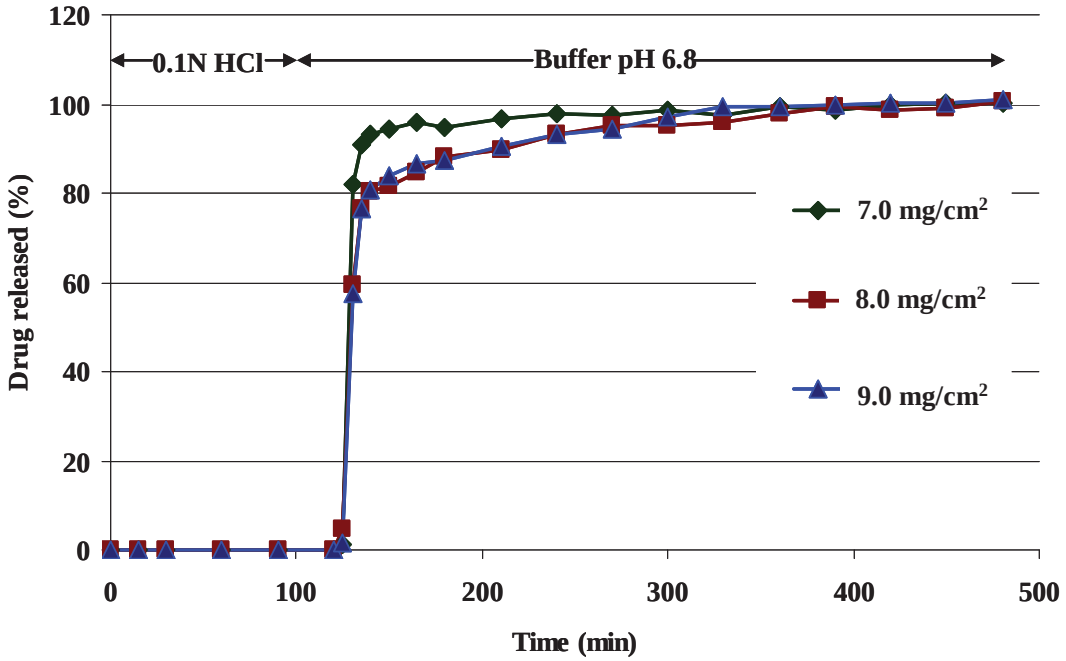


Figure 50 Dissolution profile of SHL-SUC 6 h AM coated tablets

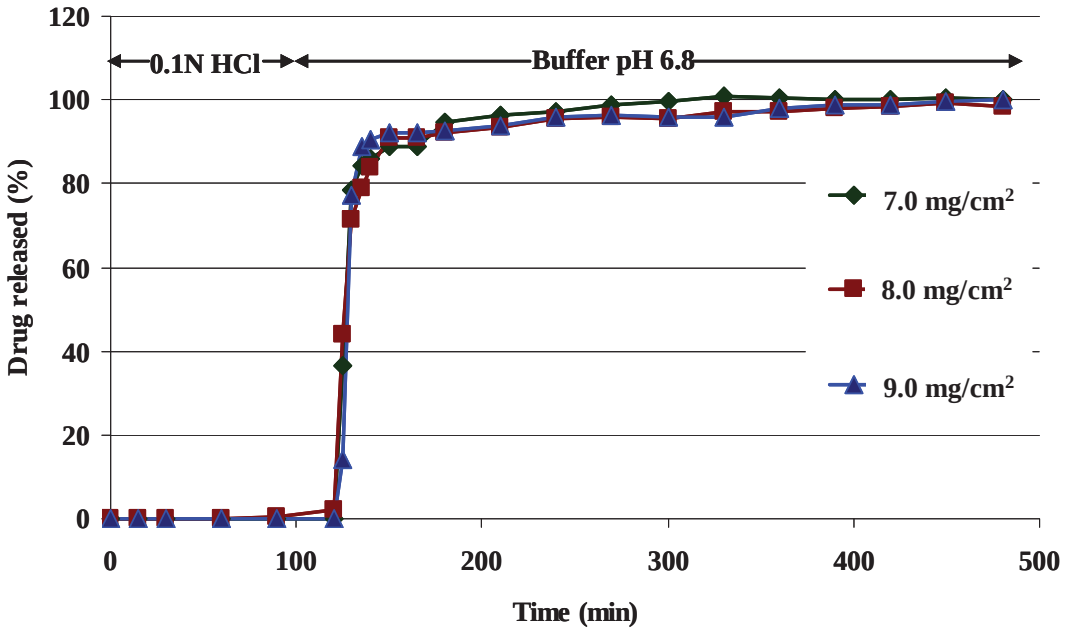


Figure 51 Dissolution profile of SHL-SUC 24 h AM coated tablets

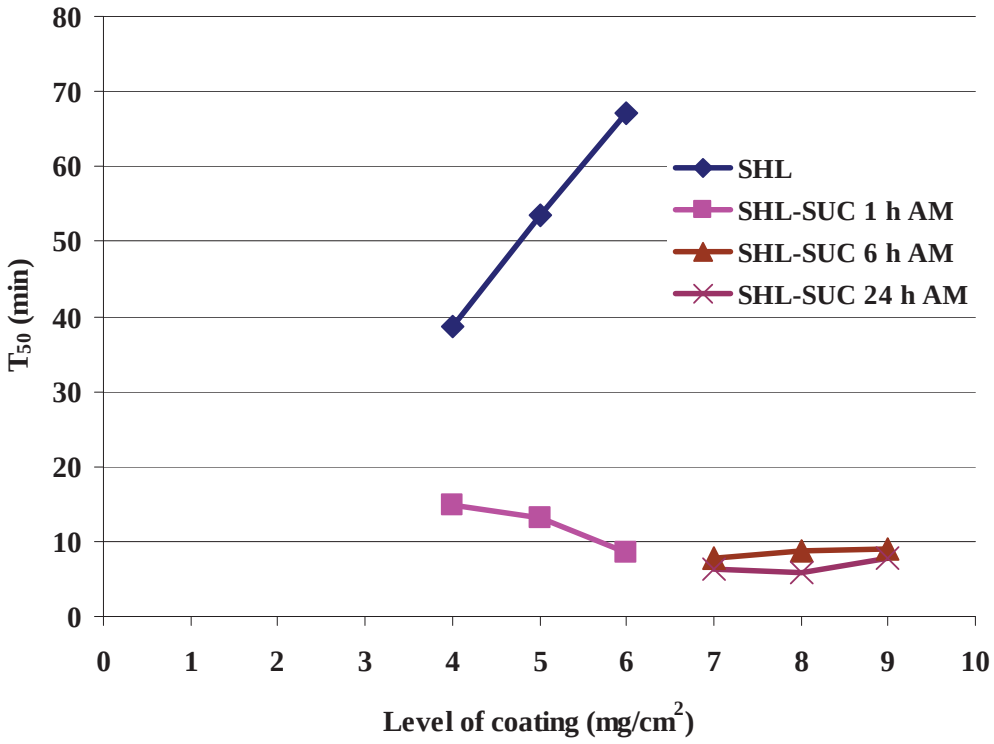


Figure 52 Time for 50% drug released (T_{50}) of SHL and SHL-SUC AM coated tablets with various coating levels

3.2 Characterization of SHL-PHT AM coated tablets

As SHL-PHT showed lower film strength and more fragility as compared to SHL-SUC. SHL-PHT could not be used as single film forms for coating. From preliminary study, the SHL-PHT coated tablets demonstrated cracking during coating. To solve the problem, HPMC was also added in the coating solution. The core tablets were then coated by the same process as described in SHL-SUC systems.

Table 6 demonstrates properties of SHL coated tablets at various coating levels. Weight and thickness of coated tablets increased progressively, while hardness was not significantly changed as increasing of coating level. For tablet disintegration, uncoated tablets and coated tablets at low coating level (0.5 and 1 mg/cm^2) were disintegrated in 0.1 N HCl although the DT was increased. The disintegration time in 0.1 N HCl was extended to more than 2 h at the coating level of 2 mg/cm^2 or more, however some coated tablets of the coating level of 2-6 mg/cm^2 still swelled in 0.1 N HCl, resulted in incomplete gastro resistance. The complete gastro resistance was observed at a coating level of 7 mg/cm^2, but the disintegration time in buffer 6.8 was more than 3 h, indicating the poor solubility of SHL coated tablets in the small intestine condition.

The properties of SHL-PHT coated tablets are demonstrated in Table 7-9. The effect of coating level on tablet hardness, weight and thickness was similar to that of SHL coated tablets. The complete acid resistance of SHL-PHT 1 h AM, SHL-PHT 6 h AM and SHL-PHT 12 h AM coated tablets was observed at the coating level of 5, 10, 11 mg/cm^2, respectively. As SHL-PHT 6 h AM and SHL-PHT 12 h AM were brittle and low elasticity, the cracking and peeling of coated tablets was occurred during coating process, so the coating level was increased to obtain sufficient gastric

resistance. However, SHL-PHT AM coated tablets still demonstrated fast disintegration in buffer solution even at the high coating level. As compared to SHL coated tablets, SHL-PHT AM coated tablets showed shorter disintegration time in buffer pH 6.8. The disintegration time of SHL-PHT 6 h AM and SHL-PHT 12 h AM coated tablets was less than 15 min at all of coating level (Table 8-9).

Table 6 Properties of SHL coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	1.006±0.011	8.65±0.12	37.2±3.8	< 1.0	-
1	1.012±0.041	8.60±0.14	38.3±3.9	44.5±3.6	-
2	1.025±0.037	8.64±0.28	39.1±4.1	> 2h*	23.0±9.9
3	1.029±0.039	8.65±0.14	37.5±7.9	> 2h*	59.6±30.7
4	1.037±0.041	8.67±0.14	38.4±3.4	> 2h*	> 3h
5	1.043±0.044	8.69±0.09	39.4±1.7	> 2h*	> 3h
6	1.049±0.042	8.72±0.17	38.9±4.0	> 2h*	> 3h
7	1.060±0.027	8.79±0.10	39.3±4.8	> 2h	> 3h

* swell in 0.1N HCl

Table 7 Properties of SHL-PHT 1 h AM coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	1.006±0.011	8.65±0.12	37.2±3.8	< 1.0	-
1	1.023±0.026	8.61±0.13	37.3±3.6	12.7±2.3	-
2	1.029±0.031	8.63±0.06	37.5±4.8	69.3±9.1	-
3	1.035±0.035	8.72±0.13	38.4±3.6	84.0±23.9	-
4	1.047±0.032	8.74±0.09	38.6±3.1	> 2h*	7.5±0.6
5	1.054±0.026	8.76±0.07	39.1±2.6	> 2h	14.8±5.3
6	1.060±0.045	8.77±0.23	38.4±4.9	> 2h	25.2±12.5
7	1.068±0.038	8.82±0.17	37.8±4.8	> 2h	71.9±11.1
8	1.076±0.024	8.84±0.11	39.7±6.0	> 2h	91.2±4.5

* 2 of 6 tablets disintegrated in 89.5±30.41 min

Table 8 Properties of SHL-PHT 6 h AM coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	1.006±0.011	8.65±0.12	37.2±3.8	< 1.0	-
1	1.014±0.033	8.67±0.10	37.4±4.9	6.08±0.98	-
2	1.015±0.020	8.68±0.06	38.6±3.2	13.60±1.92	-
3	1.023±0.035	8.71±0.13	38.3±4.2	23.61±0.77	-
4	1.031±0.033	8.73±0.10	37.3±2.2	70.95±13.90	-
5	1.040±0.040	8.74±0.08	39.8±2.6	100.33±7.51	-
6	1.055±0.029	8.77±0.06	37.5±3.7	> 2h*	5.55±1.27
7	1.059±0.009	8.80±0.03	38.6±1.3	> 2h**	4.43±0.97
8	1.063±0.026	8.82±0.19	37.8±1.0	> 2h***	4.35±0.42
9	1.072±0.014	8.82±0.11	41.4±2.7	> 2h****	4.63±0.45
10	1.085±0.32	8.83±0.13	36.8±2.3	> 2h	6.96±1.58
11	1.089±0.014	8.86±0.11	39.9±3.3	> 2h	9.51±1.53
12	1.093±0.034	8.87±0.03	39.4±2.9	> 2h	9.69±3.80
13	1.109±0.019	8.89±0.12	39.8±3.0	> 2h	10.39±1.79
14	1.114±0.022	8.92±0.33	38.6±3.8	> 2h	10.64±1.84
15	1.117±0.037	9.02±0.04	40.5±6.3	> 2h	13.00±1.60

* 1 of 6 tablets disintegrated in 110 min; ** swell in 0.1N HCl; *** 1 of 6 tablets disintegrated in 109 min; **** 1 of 6 tablets disintegrated in 73 min

Table 9 Properties of SHL-PHT 12 h AM coated tablets at various coating levels

Level of coating (mg/cm^2)	Tablet Properties				
	Weight (g)	Thickness (mm)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Core tablet	1.006±0.011	8.65±0.12	37.2±3.8	< 1.0	-
1	0.990±0.043	8.64±0.13	40.2±1.9	8.33±1.55	-
2	1.016±0.034	8.64±0.18	39.1±2.1	10.44±1.54	-
3	1.027±0.026	8.66±0.09	37.5±2.9	34.41±5.81	-
4	1.034±0.035	8.68±0.09	40.0±2.2	37.66±5.36	-
5	1.035±0.043	8.70±0.25	37.3±3.0	48.59±5.79	-
6	1.046±0.023	8.71±0.14	37.6±3.4	> 2h*	5.02±1.08
7	1.053±0.020	8.73±0.11	37.8±4.1	> 2h**	5.78±0.38
8	1.058±0.025	8.75±0.07	38.7±5.2	> 2h**	6.73±0.48
9	1.064±0.033	8.76±0.04	38.4±5.4	> 2h**	7.86±1.54
10	1.077±0.037	8.78±0.20	39.9±3.0	> 2h**	13.15±3.31
11	1.082±0.027	8.80±0.10	38.8±3.5	> 2h	13.27±0.43
12	1.086±0.010	8.84±0.09	40.4±2.0	> 2h	13.57±0.38
13	1.094±0.029	8.88±0.12	39.2±1.8	> 2h	13.84±2.48
14	1.104±0.031	8.89±0.03	39.7±2.4	> 2h	14.42±1.63
15	1.111±0.032	8.92±0.07	40.4±2.7	> 2h	14.85±2.23

* 1 of 6 tablets disintegrated in 95 min; ** swell in 0.1N HCl

Figure 53 shows the dissolution profile of SHL coated tablets at various coating levels. SHL coated tablets demonstrated good resistance to the acid medium, as indication by very low drug release in 0.1 N HCl. However, the slow drug released was observed in buffer pH 6.8. The drug dissolution from coated tablets was decreased as increasing coating level. For example, the time for 80% drug released at the coating level of 5, 6 and 7 mg/cm^2 were about 100, 140 and 170 min, respectively.

Drug release profiles of tablet coated with SHL-PHT AM are shown in Figure 54-56. All enteric coated formulations demonstrated low drug release in 0.1 N HCl, indicating excellent gastro resistance. Percent of drug release in buffer of SHL-PHT 1 h AM coated tablets was inversely proportional to the coating level especially

at the initial period (with 1 h) in buffer. The time for 80% drug released was approximately 50 min at the coating level of 8 mg/cm^2 (Figure 54). For SHL-PHT 6 h AM and SHL-PHT 12 h AM, the time for 80% drug released was below 20 min and the dissolution profile was not significantly changed as increasing coating level (Figure 55-56). Moreover, as compared to SHL coating, SHL-PHT AM coating showed the better enteric properties and solubility in the small intestine.

Figure 57 illustrates the time for 50% drug released (T_{50}) of shellac and shellac phthalate coated tablets. As shellac has low solubility in the pH of small intestine (Tarcha 1999: 39-66), thus the increment of coating level caused the significant increasing T_{50} of SHL coated tablets (p<0.01). On the contrary, T_{50} of SHL-PHT coating was low value. For example, T_{50} of SHL-PHT 1 h AM, 6 h AM and 12 h AM was less than 40, 20 and 20 min, respectively. The results confirmed the enhancement of solubility of shellac phthalate in the intestinal condition.

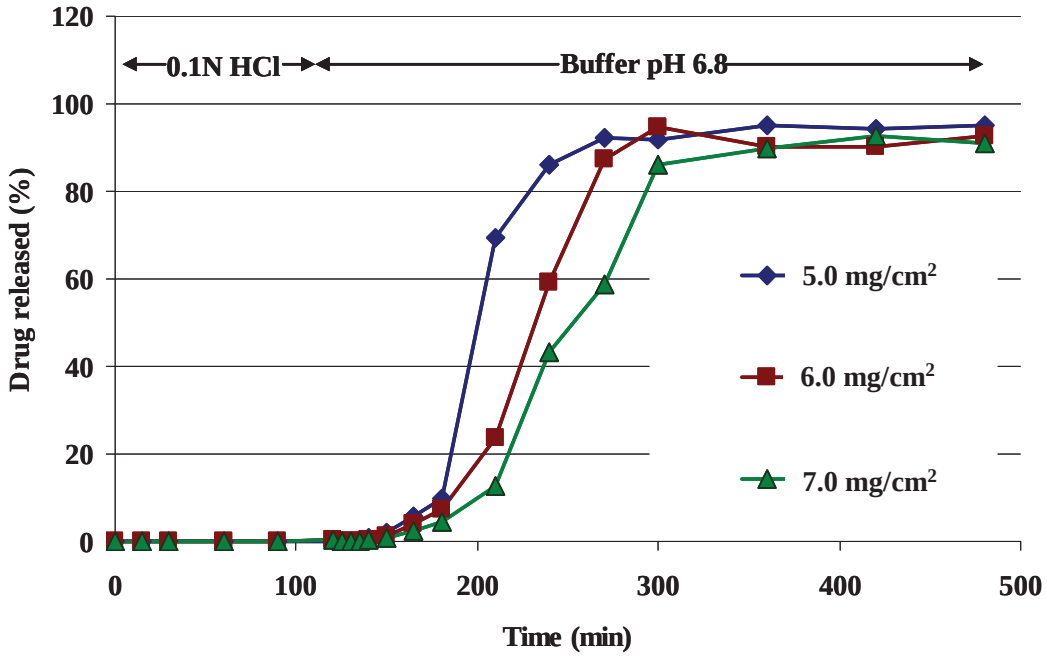


Figure 53 Dissolution profile of SHL coated tablets

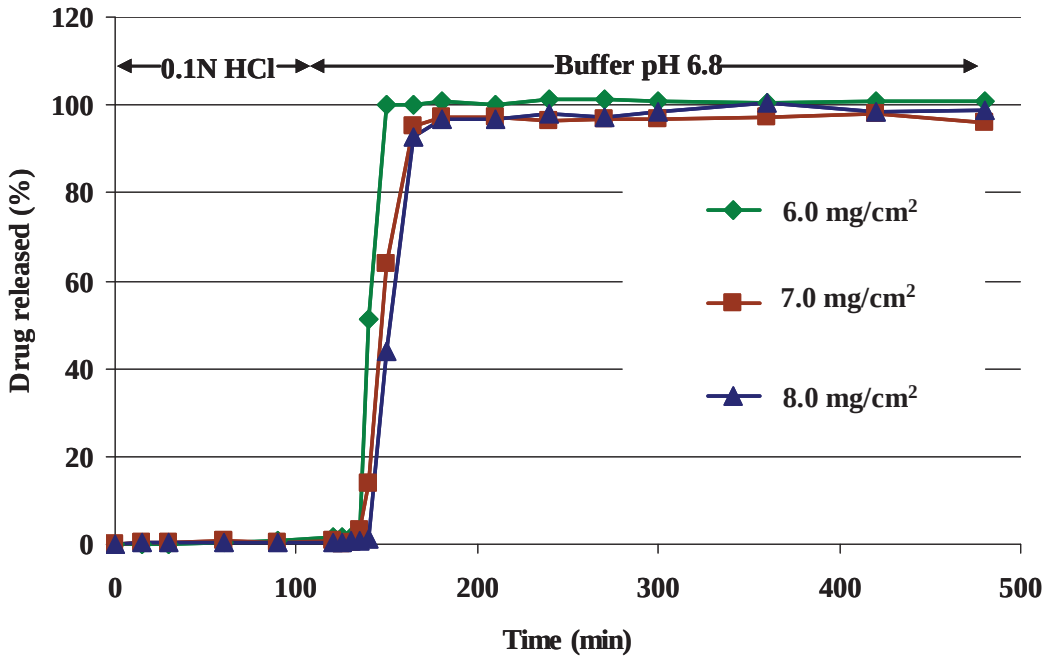


Figure 54 Dissolution profile of SHL-PHT 1 h AM coated tablets

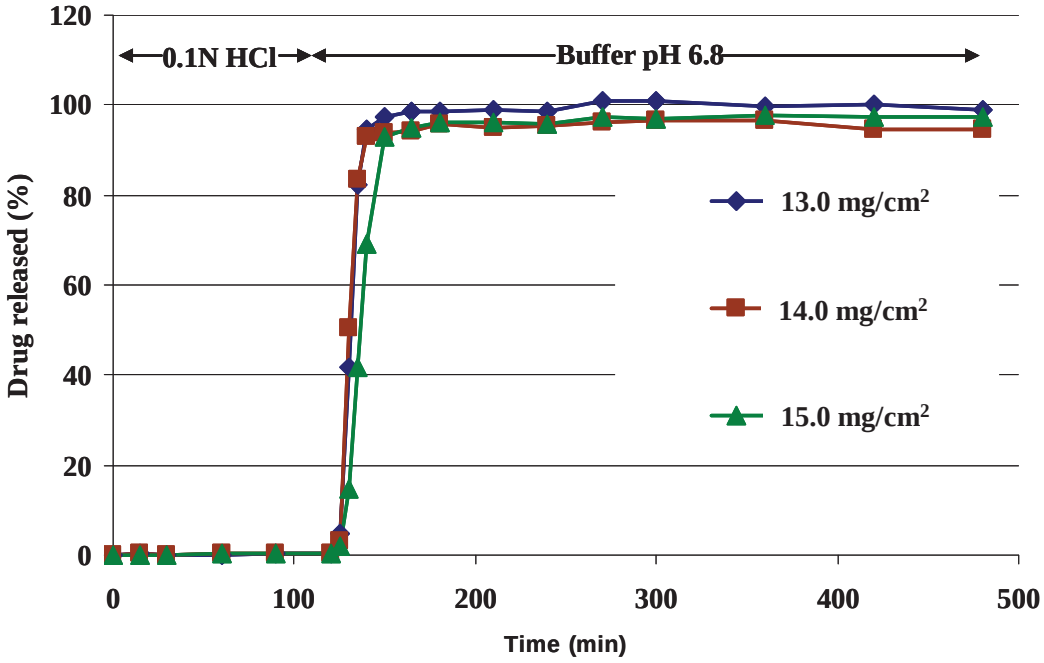


Figure 55 Dissolution profile of SHL-PHT 6 h AM coated tablets

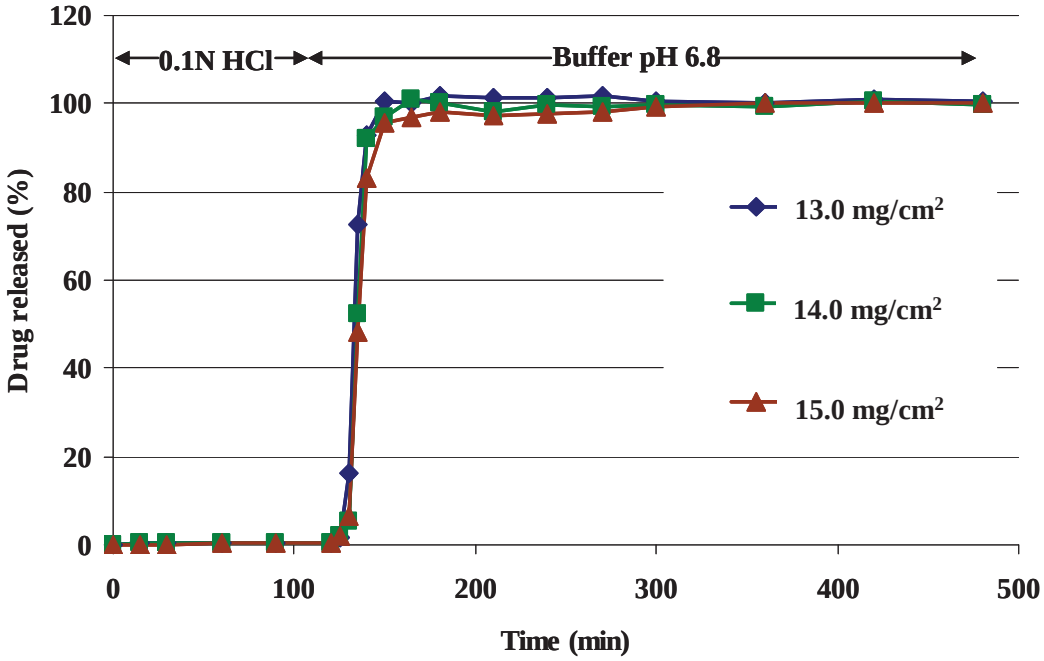


Figure 56 Dissolution profile of SHL-PHT 12 h AM coated tablets

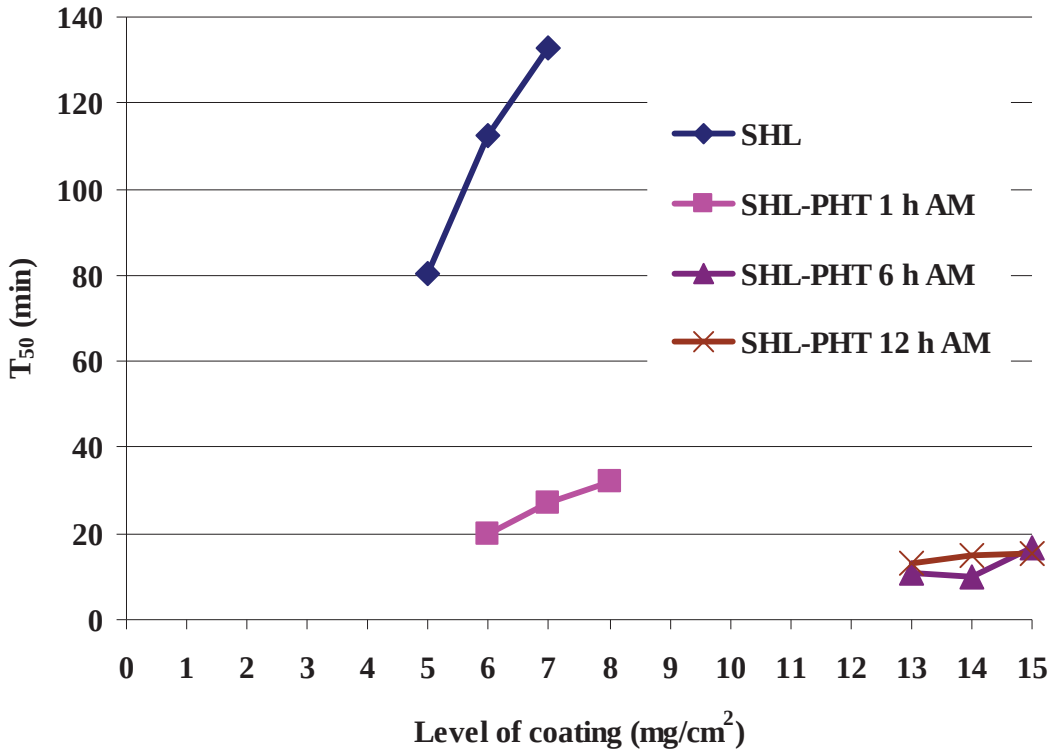


Figure 57 Time for 50% drug released (T_{50}) from SHL and SHL-PHT AM coated tablets with various coating levels

4. Cytotoxicity

One of the important requirements for polymer should be non-toxic. Although shellac is biodegradable and approved by the FDA as a food additive and a coating polymer for many fields (Berger and Sicker 2009; Herren 2009), the toxicity of shellac derivatives should be comparatively evaluated. The cytotoxicity of shellac sample was determined using the MTT test on Caco-2 cell. According to the MTT assay results, all shellac polymers incubated for 24 h showed concentration-dependent cytotoxicity Caco-2 cell. The IC_{50} of SHL, SHL-SUC 24 h AM and SHL-PHT 12 h AM was 1.80, 0.30 and 0.35 mg/mL (as shown in Figure 91-93 of Appendix),

suggesting that additional succinate and phthalate moieties slightly increased the cytotoxicity. Although shellac esters had a higher toxic, however the IC_{50} was still in a low value as compared to other polymers e.g. chitosan and its salts (0.22-0.72 mg/ml) (Kean and Thanou 2009: 3-11; Baldrick 2010: 290-299).

5. Stability study

5.1 Stability study of shellac esters film

5.1.1 Stability study of shellac succinate film

The shellac films were prepared in ammonium salt form using the casting/solvent evaporation technique, were subjected to stability testing under 40 ºC, 75 % RH for up to 6 months. Samples were taken at predetermined time intervals and then comparatively evaluated by the previously described method.

Alcohol insoluble solid is one of the parameters that point the aging effect of shellac. After storage, the polymer chains of shellac were inter-esterification among the chain and thus produced cross-linked product which could not dissolve in ethanol (Sontaya Limmatvapirat et al. 2007: 690-698). Figure 58 presents the percentage of insoluble solid of SHL and SHL-SUC film after storage. The percentage of insoluble solid of all shellac films was increased as prolonged storage time. The obvious increase of insoluble solid of SHL was seen after storage for 60 days ($p<0.01$). However, after succinate formation, the percentage of insoluble solid of SHL-SUC 1 h AM, 6 h AM and 24 h AM was increased after 30, 15 and 15 days, respectively. These results indicated that the polymerization was not protected due to succinate formation. As the mechanical properties of SHL-SUC film were high flexibility that might be due to the free movement of aliphatic chain (succinate

moiety). The esterification between free hydroxyl and carboxylic group of SHL-SUC was easily occurred, resulting in the poor stability.

Acid value is an importance indicator of free carboxylic acid in substance structure as measured by the milligrams of potassium hydroxide needed to neutralize it. The acid value of shellac film after storage is illustrated in Figure 59. The acid value of all shellac samples was declined after kept for a period of six months. The reduction of acid value was related to the decrease of free carboxylic acid amount, so these results suggested the aging effect led to a polymerization of free carboxylic group and hydroxyl group (Sontaya Limmatvapirat et al. 2005: 41-46).

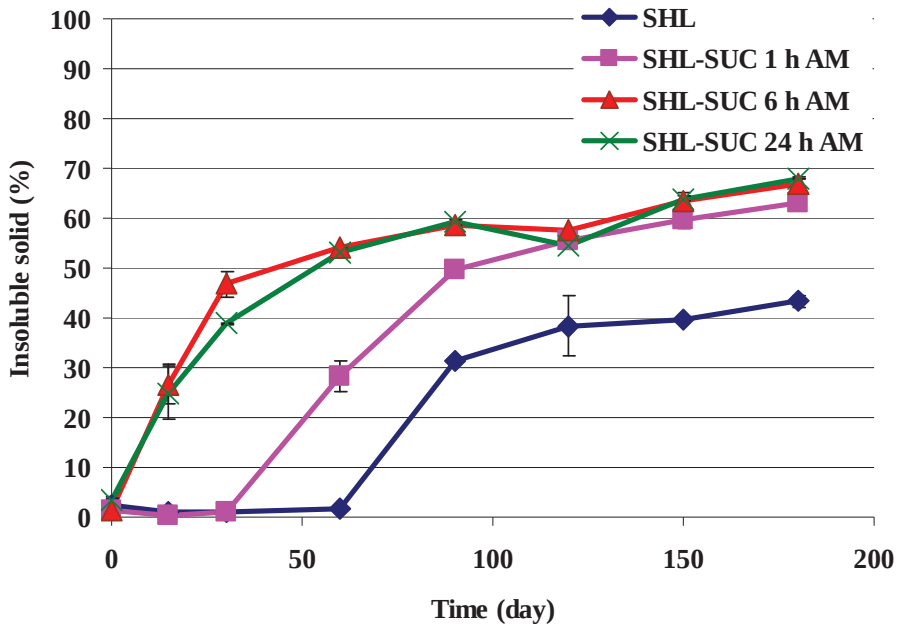


Figure 58 Change of percentage of insoluble solid of SHL and SHL-SUC films after Storage at 40 °C, 75% RH for 6 months

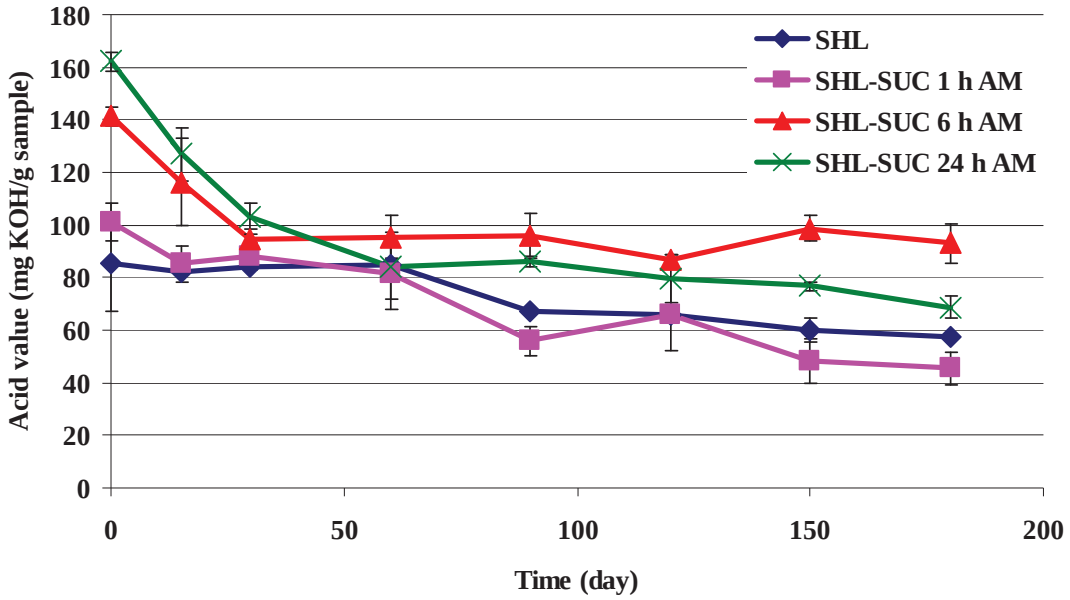


Figure 59 Change of acid value of SHL and SHL-SUC films after storage at 40 °C, 75% RH for 6 months.

The stability of SHL and SHL-SUC film by monitoring the change of percent dissolved at pH 6.8 was also studied. The percent dissolved of SHL films was low value (10.28 %) while that of all SHL-SUC films was completely dissolved (100%) at the initial day. After storage, the percent dissolved of SHL film gradually decreased until completely undissolved at 90 days. SHL-SUC films showed extremely reduction after 15 days (Figure 60). SHL-SUC also demonstrated the lowering of percentage film dissolved as prolonged storage time. However, the solubility of SHL-SUC films was higher as compared to SHL, especially for SHL-SUC 6 h and 24 h AM. The result was well agreed with the percent insoluble solid, suggesting the solubility enhancement although the polymerization was not completely protected by succinate moieties.

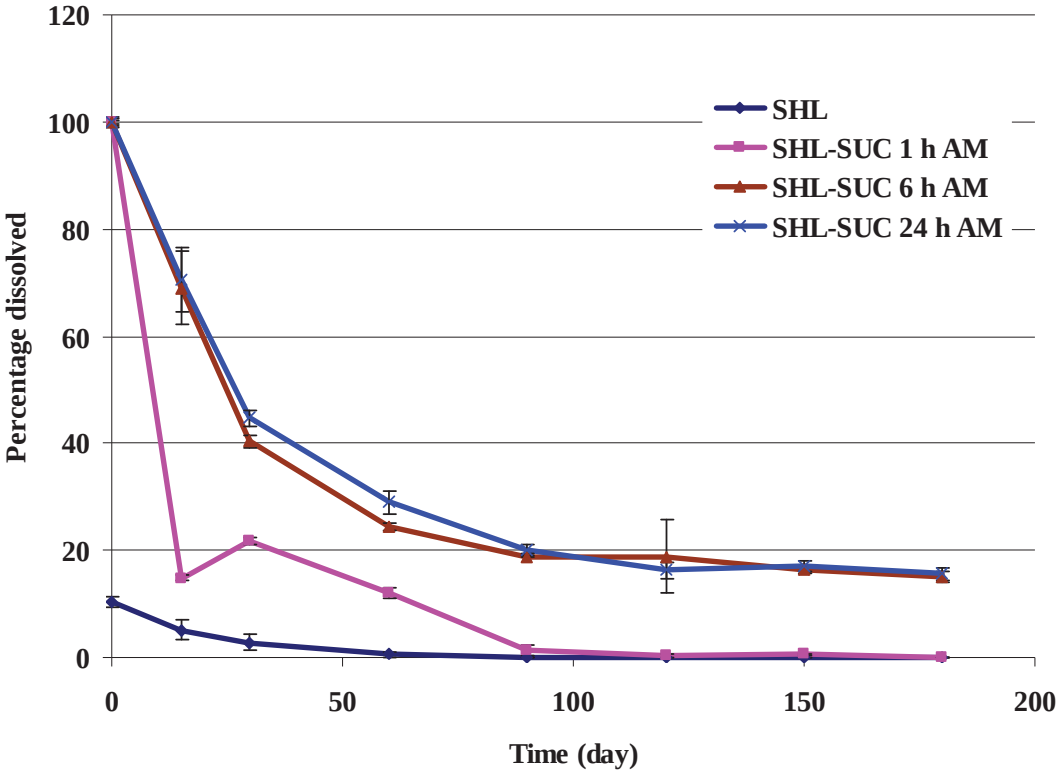


Figure 60 Change of percent dissolved of SHL and SHL-SUC films in buffer pH 6.8 after storage at 40 °C, 75% RH for 6 months

In order to further investigate the stability of SHL-SUC, the FTIR spectra of all shellac films were comparatively tested for 6 months. The ammonium salt of SHL and SHL-SUC AM showed an appearance of asymmetric and symmetric C=O stretching of carboxylate at 1556 and 1385 cm^{-1}, respectively, and the peak at 1716 cm^{-1} due to C=O stretching of carboxylic acid. The conversion of carboxylic acid to carboxylate was occurred after salt formation (Figure 61). The relative absorbance ratios of the FTIR peaks assigned to C=O stretching of carboxylate and carboxylic acid (ABS_{1556}/ABS_{1716}) of all shellac films after storage are shown in Figure 62. For SHL-SUC 6 h AM and 24 h AM film, the absorbance

ratios were fast decreased after 15 days. The results suggested that ammonium salt might detach from the carboxylate binding site and the carboxylate consequently conversed to carboxylic acid. Hence the polymerization of free carboxylic acid and hydroxyl group was occurred, confirming rapid aging effect after storage of SHL-SUC 6 h AM and 24 h AM film. However, the absorbance ratios of SHL and SHL-SUC 1 h AM film were gradually declined and were higher than that of SHL-SUC 6 h AM and SHL-SUC 24 h AM after keep in the stability chamber, therefore, the slower polymerization might related with the higher ratio of carboxylate form.

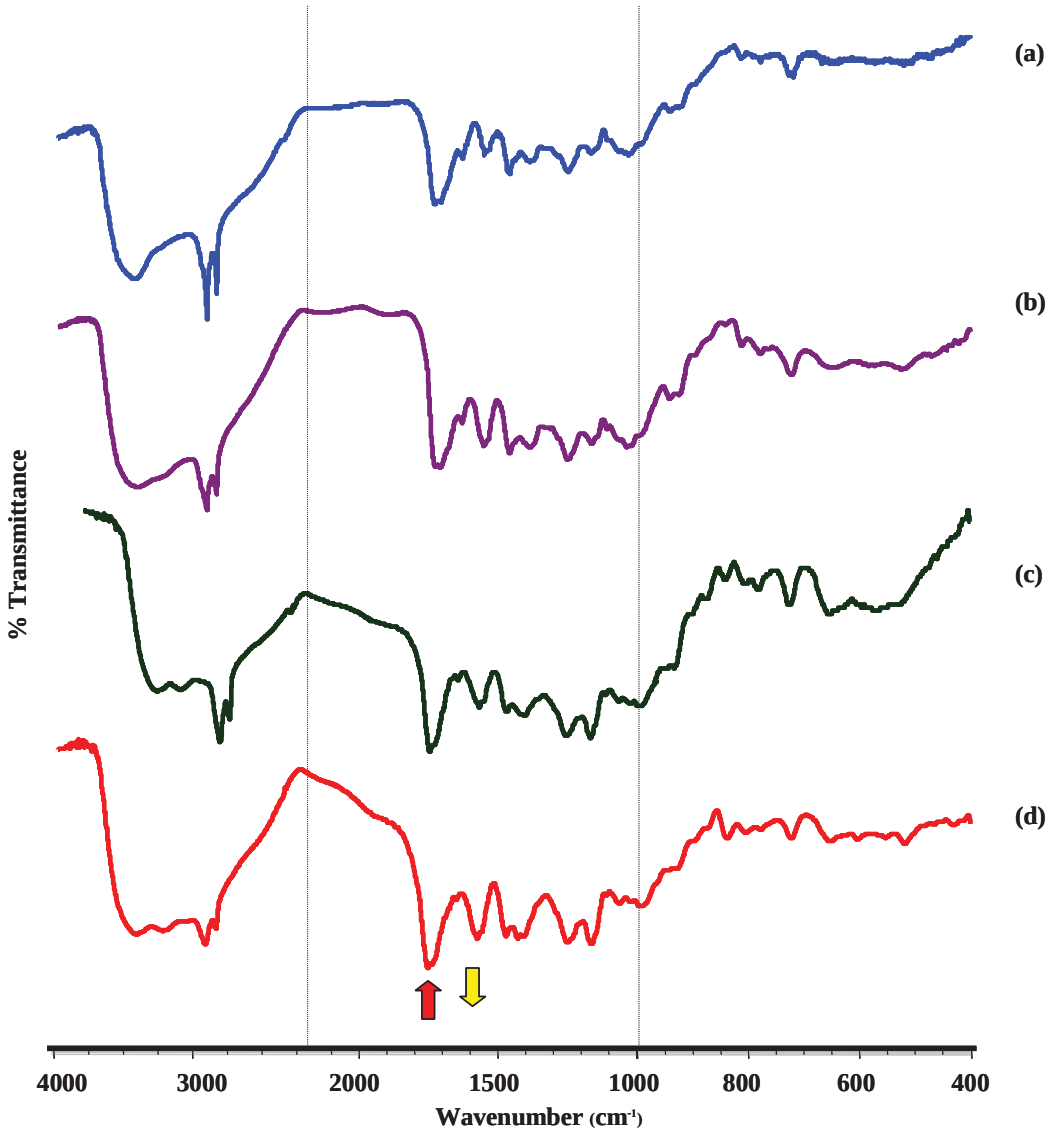


Figure 61 FTIR spectra of SHL and SHL-SUC films at initial; SHL (a), SHL-SUC 1 h AM (b), SHL-SUC 6 h AM (c) and SHL-SUC 24 h AM (d)

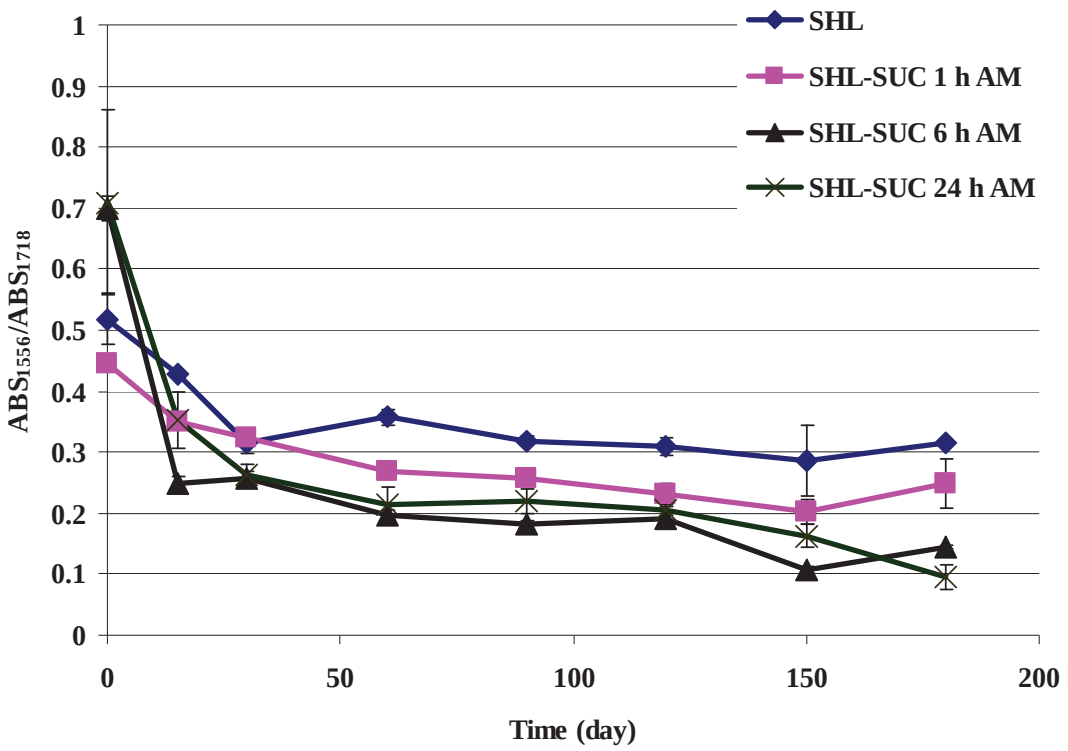


Figure 62 Change of ABS_{1556}/ABS_{1716} of SHL and SHL-SUC films after storage at 40 ºC, 75% RH for 6 months

To evaluate the thermal behavior of shellac film after storage at 40 ºC, 75 % RH for 6 months, DSC was utilized for thermal analysis. DSC curves of SHL and SHL-SUC film are demonstrated in Figure 63-66. All films showed small endothermic peak at the low temperature that might be due to water was loss from their films. Although the insoluble solid of SHL film was observed during stability study, the thermal change and the endothermic peak of all SHL did not clearly demonstrate. For SHL-SUC film, the DSC curves of them were similar to SHL film. However, the added endothermic peak, presumably assigned as decomposition peak, around 150 ºC of SHL-SUC 6 h AM and SHL-SUC 24 h AM film was observed throughout storage, while that of SHL and SHL-SUC 1 h AM film did not clearly

observe. The endothermic peak was not clearly changed as increasing the storage time. The results suggested that DSC might not be the suitable tool for monitoring stability of shellac film.

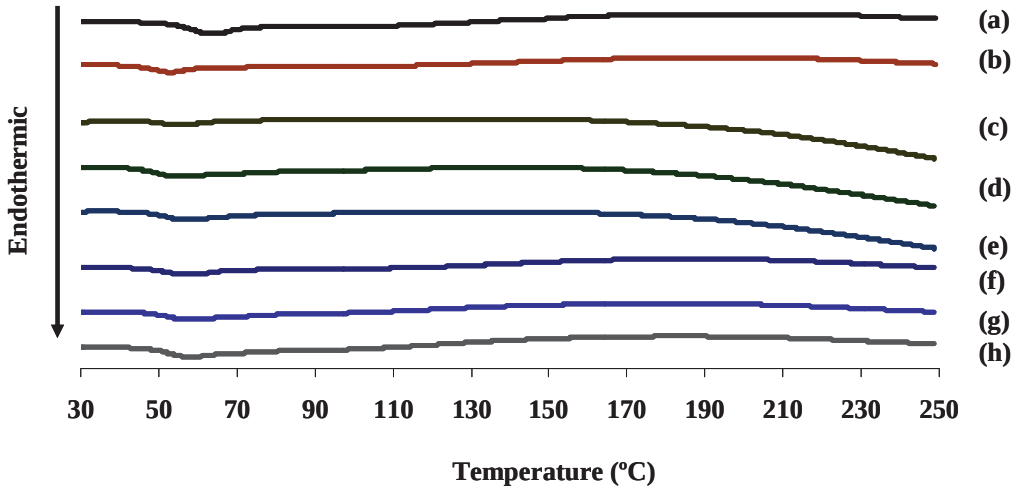


Figure 63 DSC curves of SHL films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

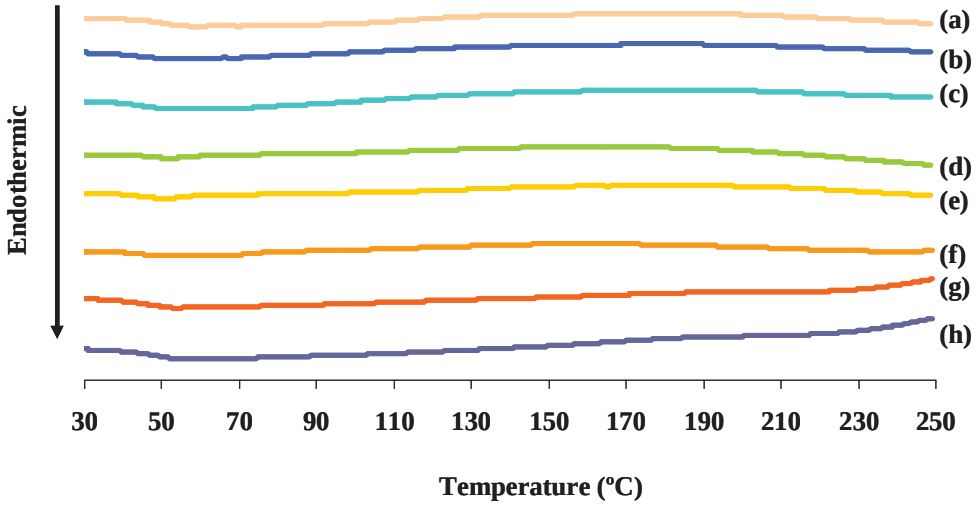


Figure 64 DSC curves of SHL-SUC 1 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

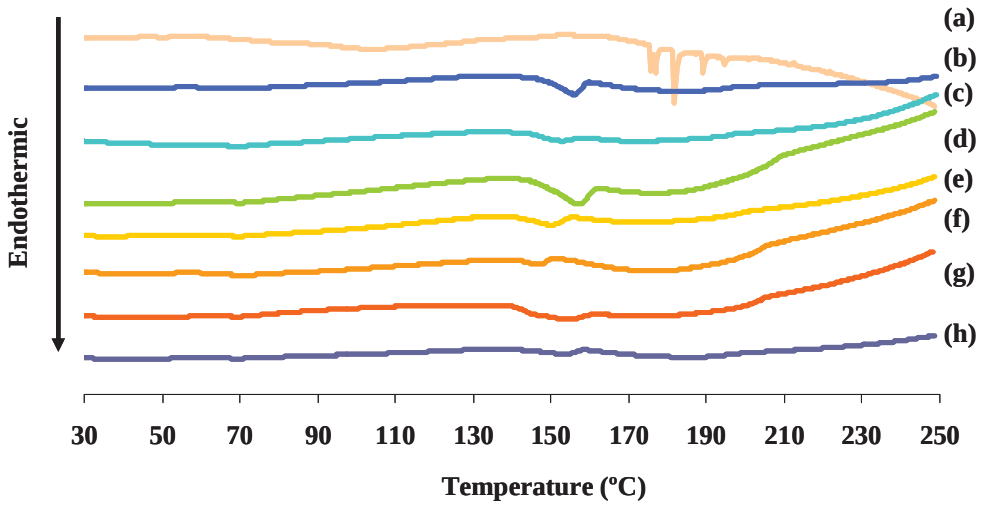


Figure 65 DSC curves of SHL-SUC 6 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

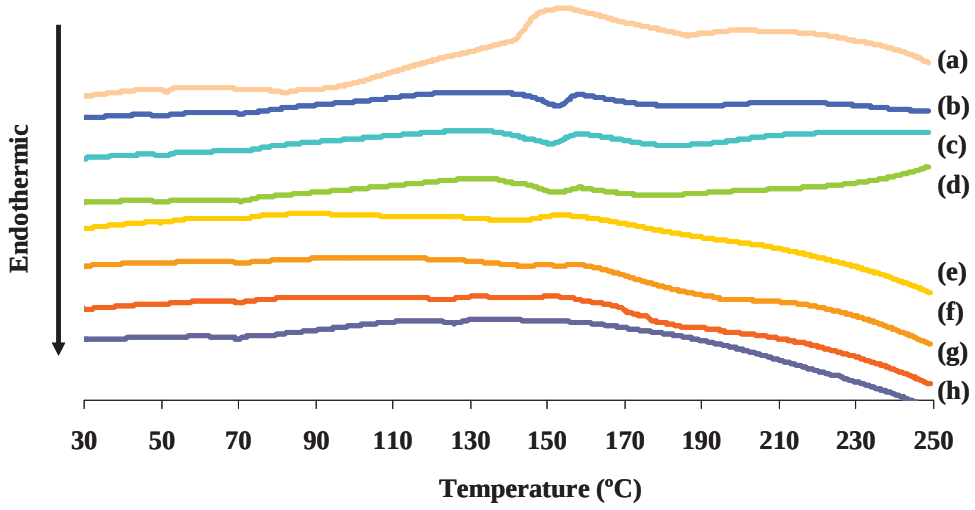


Figure 66 DSC curves of SHL-SUC 24 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

Powder X-ray diffraction (PXRD) was employed for monitoring change of crystalline phase during storage. The PXRD patterns of SHL film after storage is shown in Figure 68. SHL demonstrated similar halo pattern for all storage time although the insoluble solid was dramatically increased. The result suggested the amorphous state of SHL as well as degradation product of SHL. The result was later confirmed by PXRD measurement of polymerize SHL (Figure 67 i). Figure 68-70 show the PXRD pattern of SHL-SUC film after storage. The crystalline peaks of succinic acid (SUC) were gradually appeared with increased storage time; however the diffract peaks were relatively small. The results indicated that a minor part of succinate moiety was hydrolyzed and conversed to free succinic acid. As a result, the free -OH group on polymer chain of SHL was subsequently polymerized with carboxyl group, resulting in the increment of insoluble solid after storage.

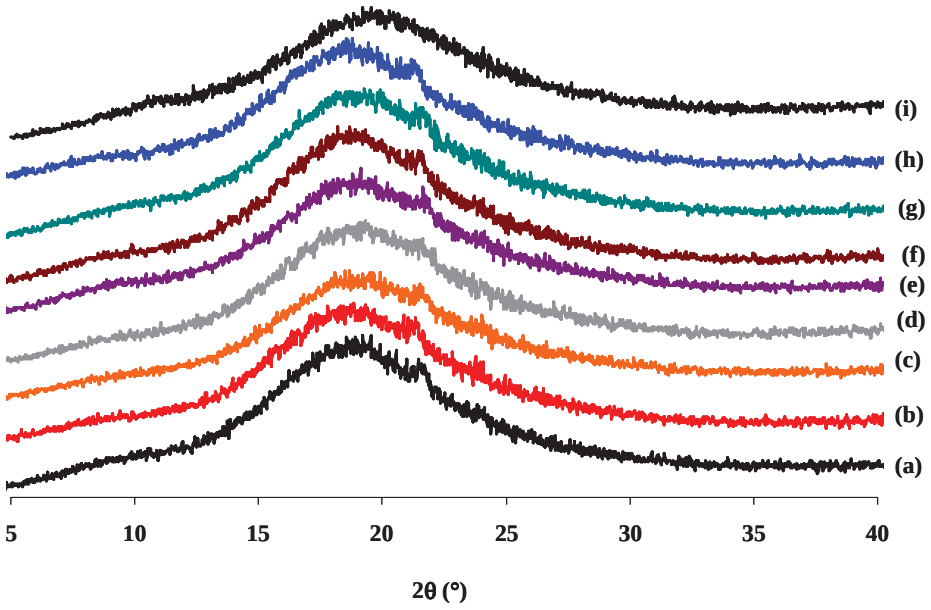


Figure 67 Powder X-ray diffraction patterns of SHL films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g), 180 days (h) and polymerize SHL (i)

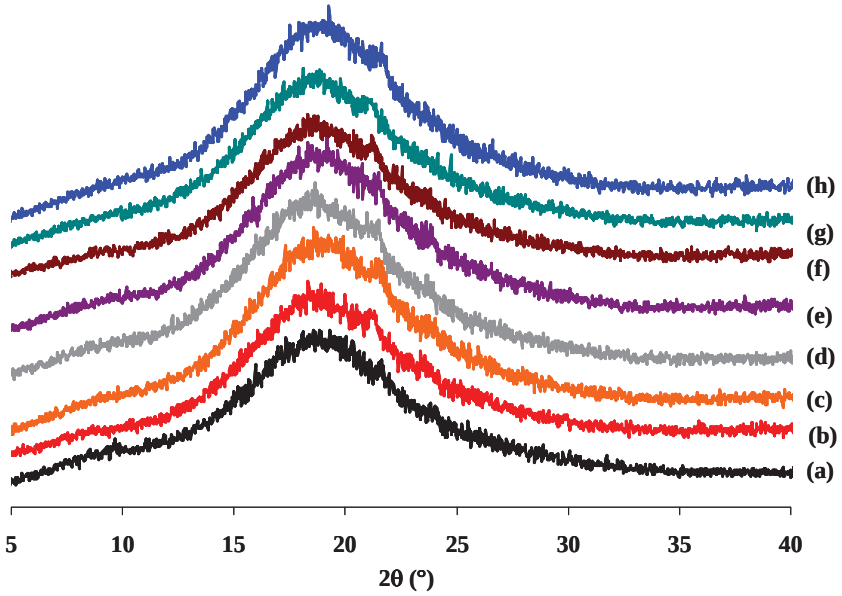


Figure 68 Powder X-ray diffraction patterns of SHL-SUC 1 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

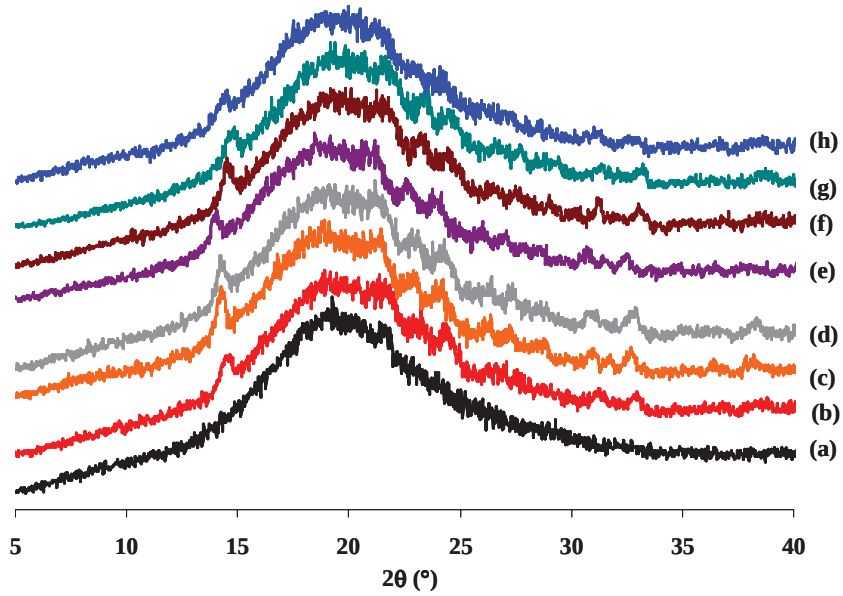


Figure 69 Powder X-ray diffraction patterns of SHL-SUC 6 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

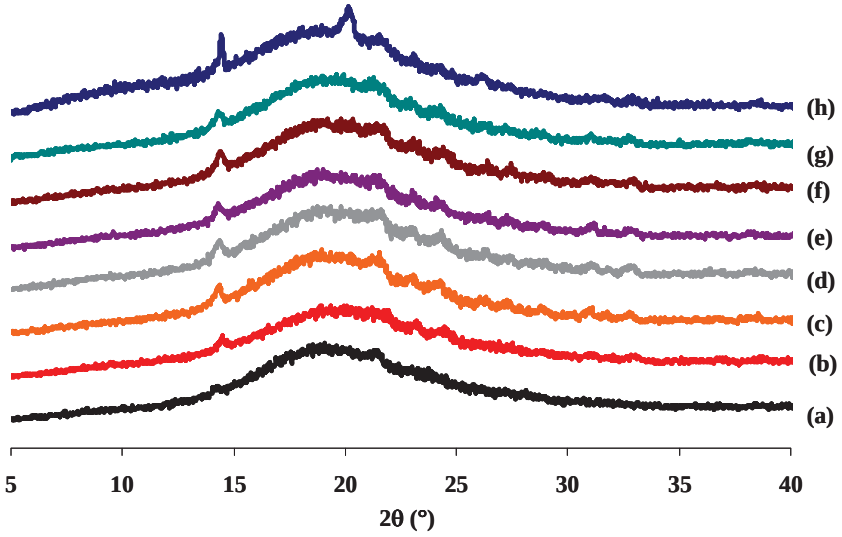


Figure 70 Powder X-ray diffraction patterns of SHL-SUC 24 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

5.1.2 Stability study of shellac phthalate film

Figure 71 illustrates the percentage of insoluble solid of SHL and SHL-PHT films after storage at 40 ºC, 75 % RH for 6 months. The insoluble solid of SHL was observed after storage for 2 months while that of SHL-PHT 1 h AM, SHL-PHT 6 h AM and SHL-PHT 12 h AM was increased after storage for 2, 3 and 5 months, respectively. Although the polymerization of SHL-PHT AM might occur, the percentage of insoluble solid was relatively low as compared with SHL. the insoluble solid after 6-month storage time of SHL-PHT 1 h AM film was approximately 25 % and that of the SHL-PHT 6 h AM and SHL-PHT 12 h AM film was less than 10 %. The results indicated that the aging was protected by the addition of phthalate moieties and the better stability of SHL-PHT film was observed.

Besides the changes of insoluble solid, the increment of acid value (AV) during storage also indicated a degree of polymerization. The decreasing AV was correlated with the increment of the amount of insoluble solid (Sontaya Limmatvapirat et al. 2005: 41-46). The AV of SHL and SHL-PHT film upon storage is shown in Figure 72. The AV of SHL and SHL-PHT 1 h AM film was more decreased than that of SHL-PHT 6 h AM and SHL-PHT 12 h AM, supporting the higher insoluble solid (Figure 71). Since the decline of acid value represented the decreasing free carboxylic acid, the results indicated that the higher phthalate formation could more prevent the polymerization between free carboxylic acid and hydroxyl group in shellac structure.

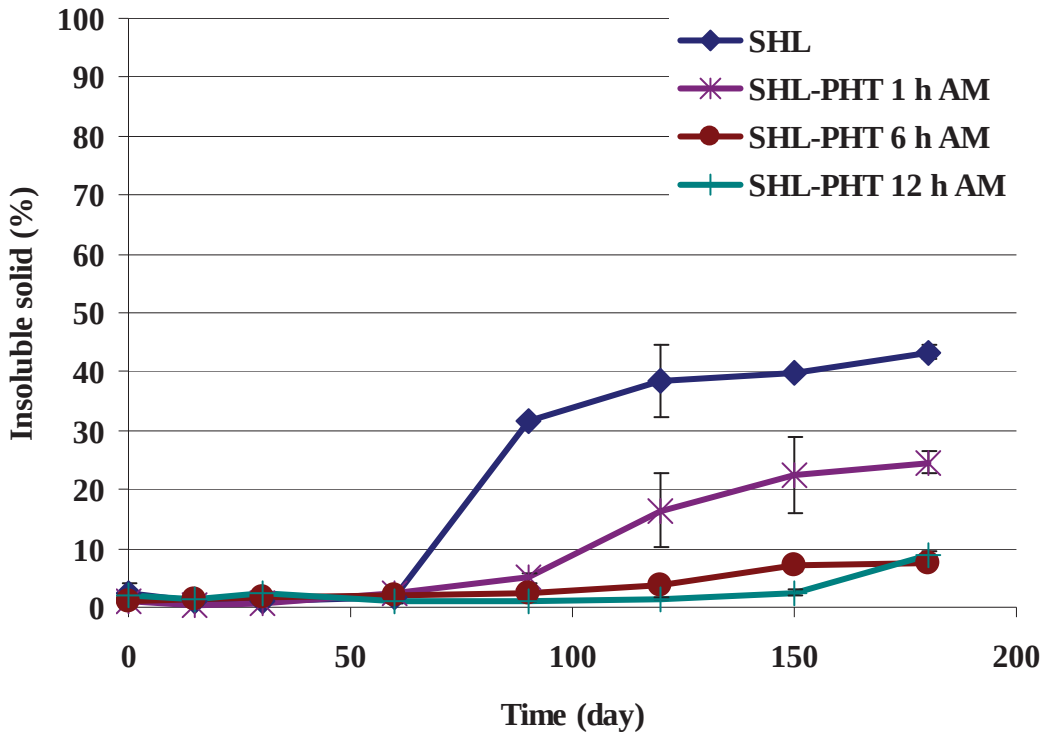


Figure 71 Change of percentage of insoluble solid of SHL and SHL-PHT films after storage at 40 °C, 75% RH for 6 months

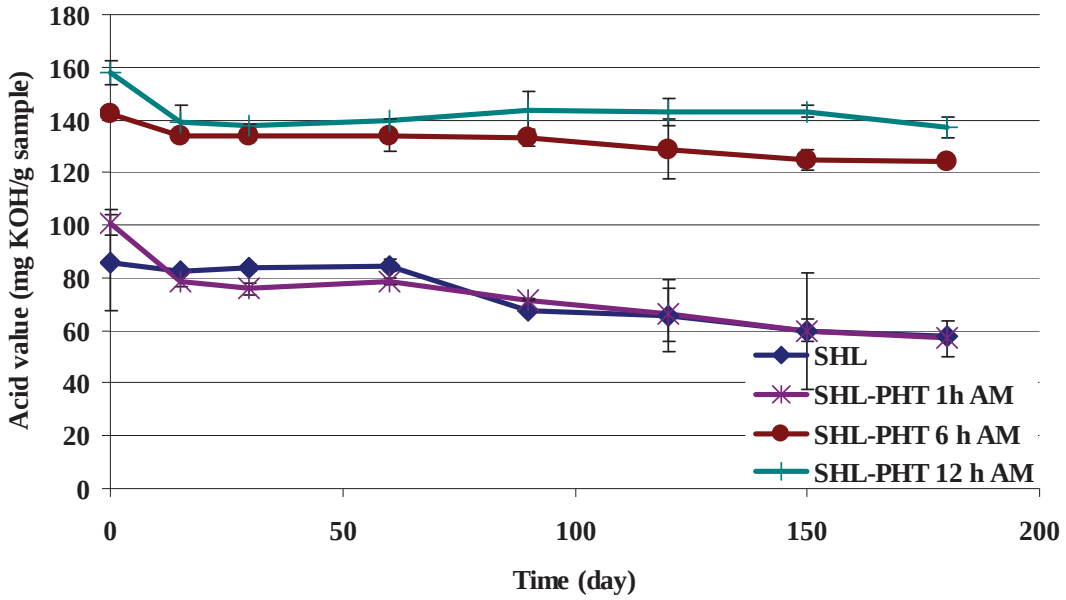


Figure 72 Change of acid value of SHL and SHL-PHT films after storage at 40 °C, 75% RH for 6 months

Figure 73 demonstrates the change of percent dissolved of SHL and SHL-PHT film at pH 6.8 during storage. The percent dissolved of SHL-PHT 1 h AM, SHL-PHT 6 h AM and SHL-PHT 12 h AM film gradually decreased after 90, 120, 180 days, respectively, while that of SHL film reduced after 15 days. The result well agreed with insoluble solid, confirming that the phthalate formation should improve the stability of SHL. In addition, the stability of SHL-PHT films was relatively better as compared to SHL-SUC since the percent dissolved was higher and the extended time, in which solubility decreased, was observed.

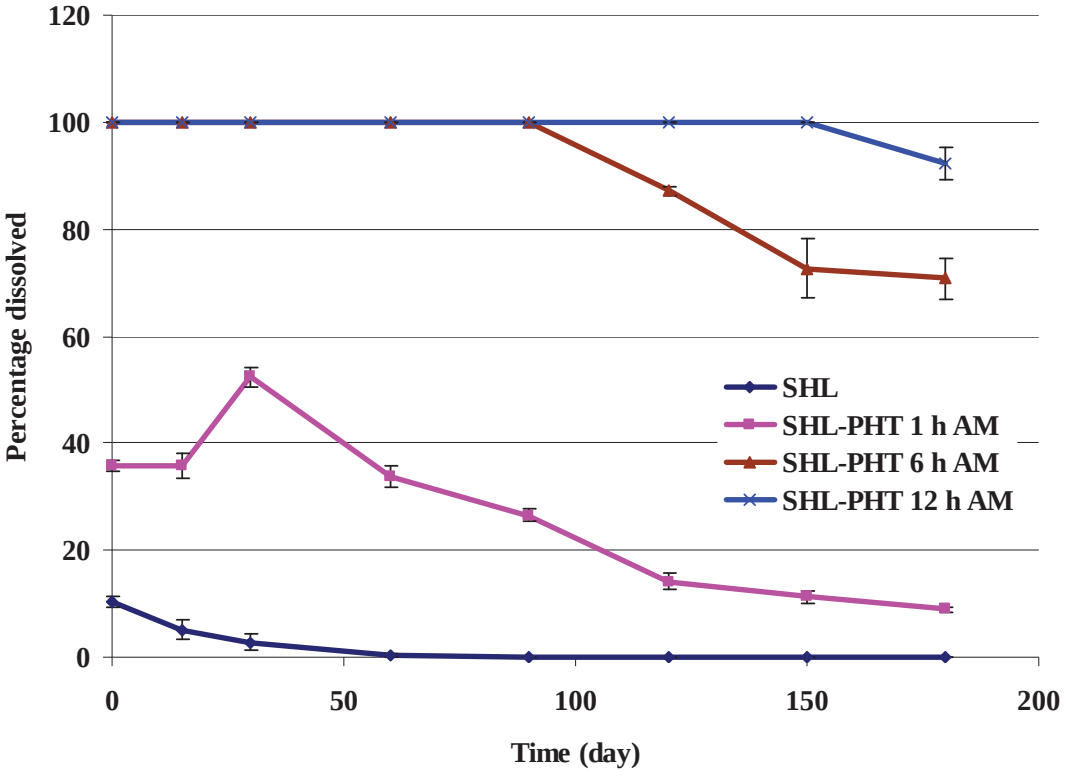


Figure 73 Change of percent dissolved of SHL and SHL-PHT films in buffer pH 6.8 after storage at 40 °C, 75% RH for 6 months

In order to examine the change of structure of SHL-PHT film during storage, FTIR spectroscopy was used for characterization. The FTIR spectra of SHL and SHL-PHT films are illustrated in Figure 74. The ammonium salt of SHL and SHL-PHT showed the carbonyl stretching peak of carboxylic acid at 1716 cm^{-1}. The peaks at 1556 and 1385 cm^{-1} were assigned as the asymmetric and symmetric carbonyl stretching of carboxylate, respectively. The relative absorbance ratios of carbonyl stretching of carboxylate and carboxylic acid (ABS_{1556}/ABS_{1716}) of shellac samples during storage are demonstrated in Figure 75. All shellac films displayed the decline of ABS_{1556}/ABS_{1716}, but the relative absorbance ratio of SHL-PHT film was higher, as compared with that of SHL film. Similar to SHL-SUC film, the carboxylate

of ammonium salt could easily converse to carboxylic acid. In spite of decreasing carboxylate, SHL-PHT film showed the excellent stability. The better stability of SHL-PHT might correlate with the molecular conformation of phthalate group. Since the molecular sizes of phthalate were larger as compared to succinate group, the phthalate group could more separate the polymer chain of SHL and thus preventing the cross-linking between polymer chains. In addition, the molecular movement of substituted group was assumed to affect the cross-linking process. Although succinate moiety could separate the polymer chain of SHL at initial period but the conformation of succinate group was aliphatic chain that was freely movement as compared to rigid phthalate group. Therefore, the succinate group might change the conformation or collapse during storage until the cross-linking process between SHL polymer chains could initiate.

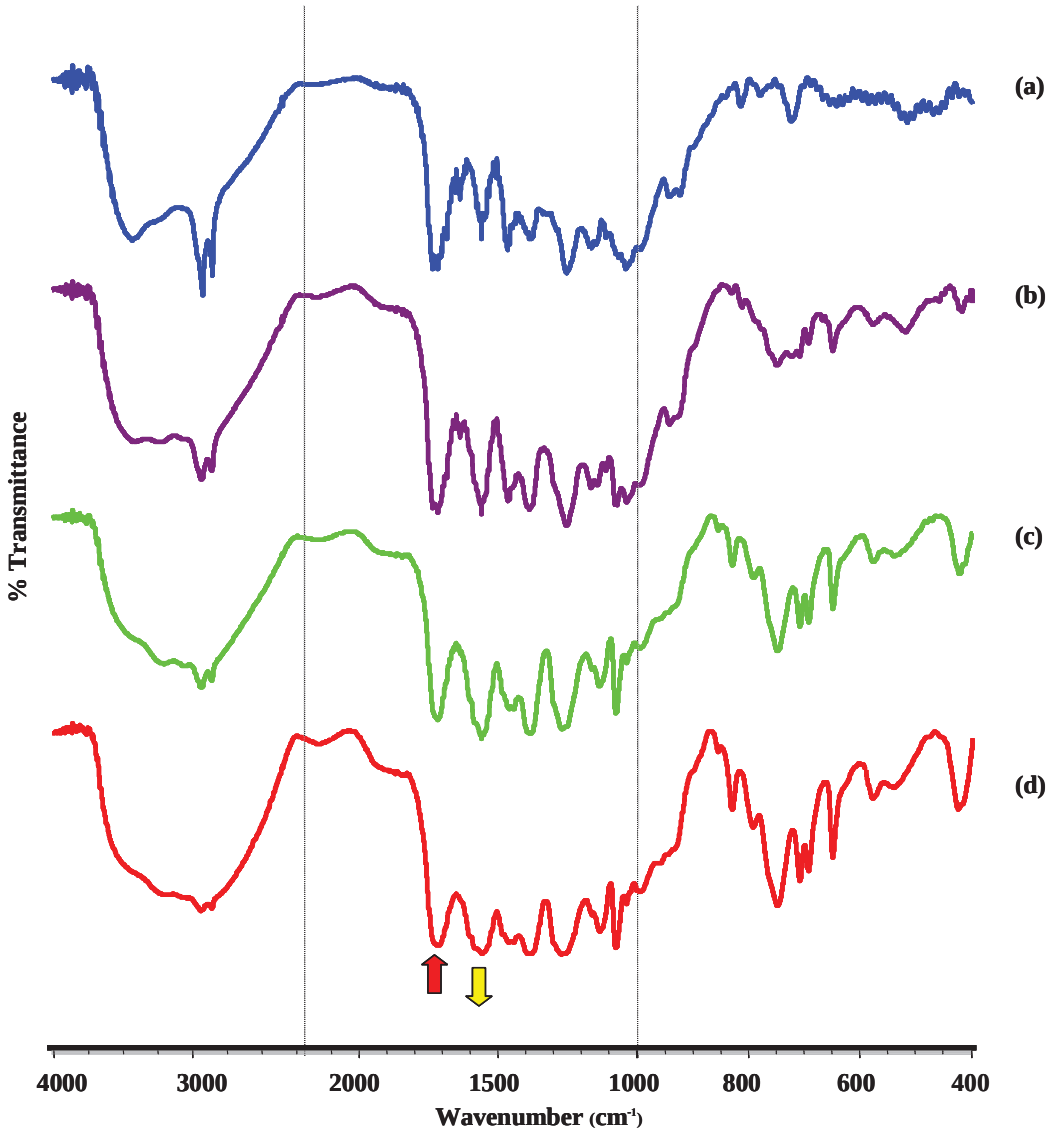


Figure 74 FTIR spectra of SHL and SHL-PHT films at initial; SHL (a), SHL-PHT 1 h AM (b), SHL-PHT 6 h AM (c) and SHL-PHT 12 h AM (d)

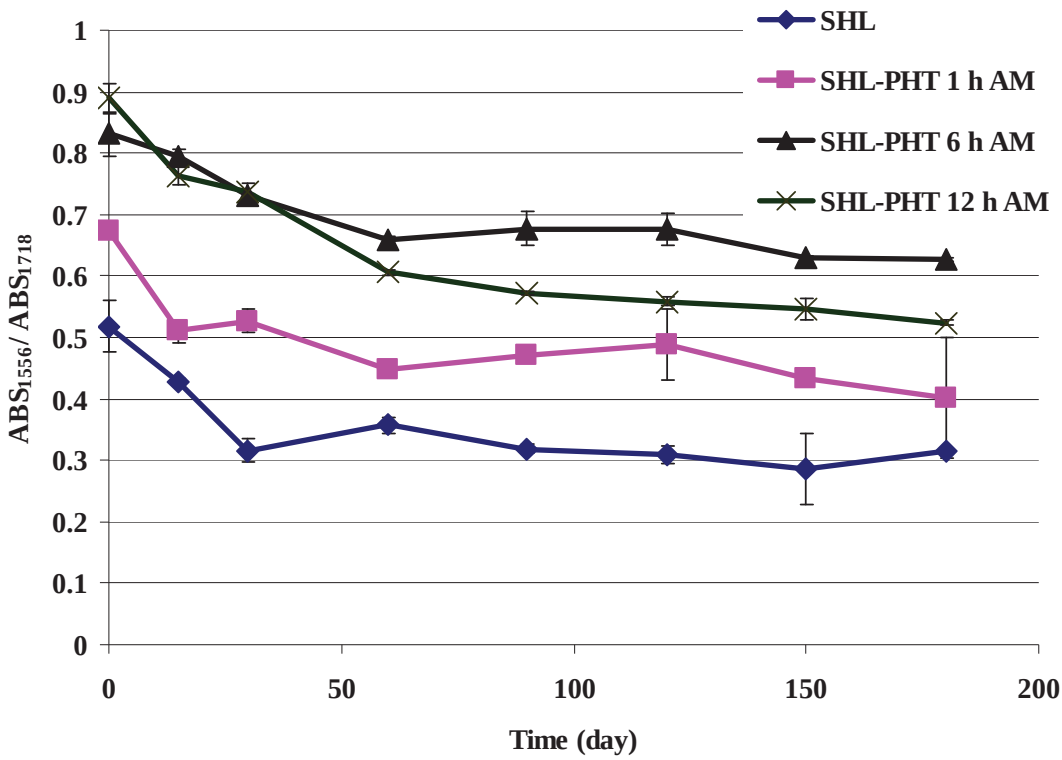


Figure 75 Change of ABS_{1556}/ABS_{1716} of SHL and SHL-PHT films after storage at 40 ºC, 75% RH for 6 months

The thermal behavior of SHL-PHT film upon storage at 40 ºC, 75 % RH for 6 months was investigated by DSC. Figure 76-78 illustrate DSC curves of SHL-PHT films. SHL-PHT films demonstrated small broad endothermic peak of the water loss in the range of 60-80 ºC similar to the DSC curves of SHL and SHL-SUC. In addition, the endothermic peak assigned as decomposition peak of SHL-PHT was clearly observed at higher temperature as compared to SHL-SUC film. The result was well agreed with the more stability of SHL-PHT. However, the change of thermal behavior and endothermic peak of SHL-PHT films did not observed during storage. The results indicated that the change of SHL-PHT films during stability study could not clearly monitor by DSC.

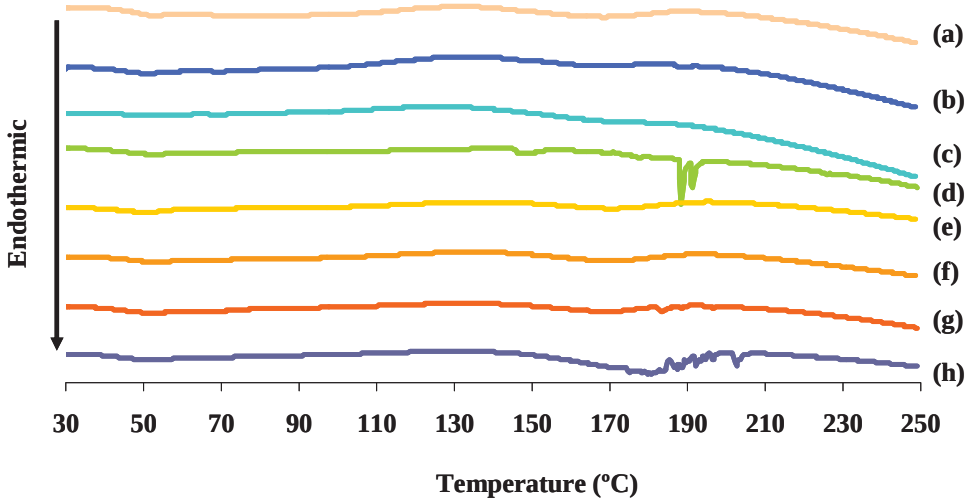


Figure 76 DSC curves of SHL-PHT 1 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

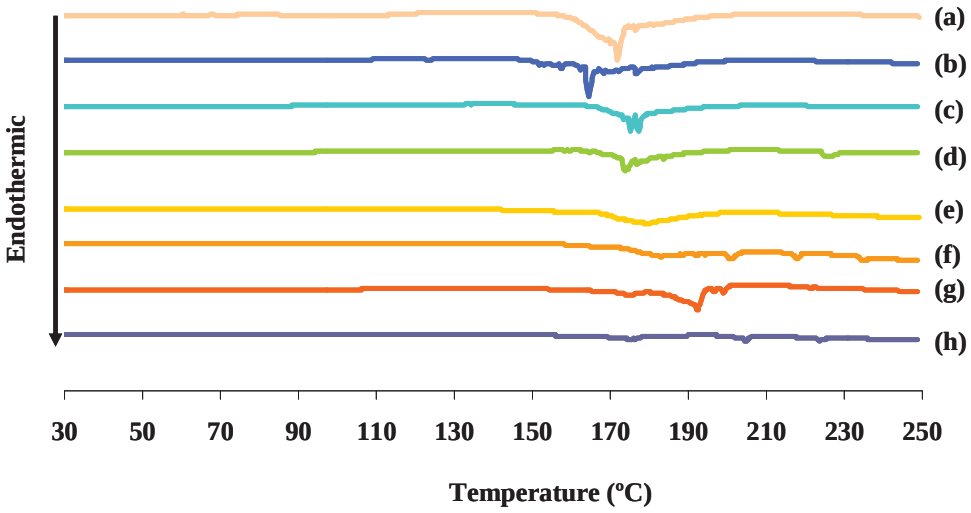


Figure 77 DSC curves of SHL-PHT 6 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

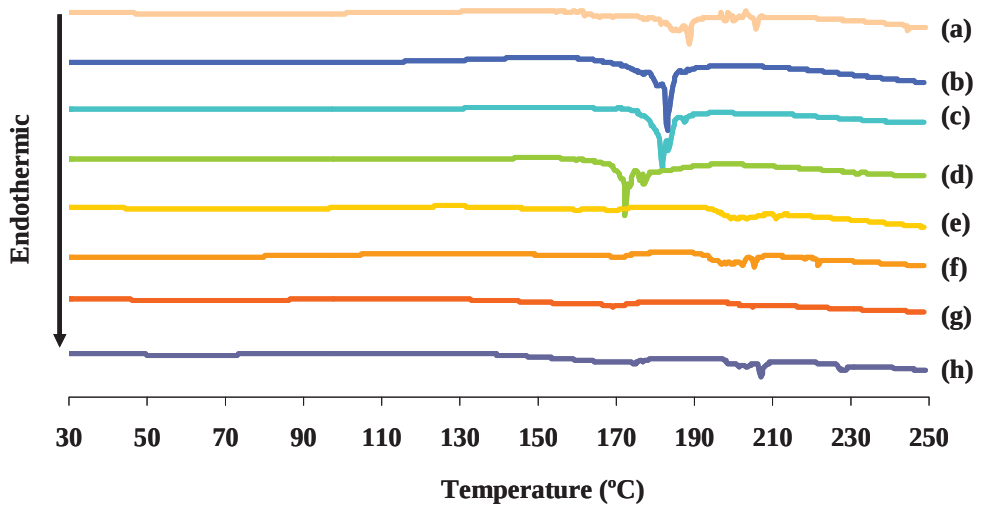


Figure 78 DSC curves of SHL-PHT 12 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

Figure 79 shows the powder X-ray diffractogram of SHL-PHT film after storage. For SHL-PHT 1 h AM film, the halo pattern was seen after storage up to 150 days and the small crystalline peaks of phthalic acid (PHT) was observed at 180-days storage, indicating the hydrolysis of phthalate group from shellac structure. As indicated by insoluble solid of SHL-PHT 1 h AM, the increment of insoluble solid was observed after 60 days. The result indicated that degradation process of SHL-PHT 1 AM did not correlate with the hydrolysis. In addition, SHL-PHT 6 h AM and 12 h AM (Figure 80-81), the peak of phthalic acid was gradually observed after 60-days storage. Despite the appearance of phthalic acid, the films of SHL-PHT 6 h AM and SHL-PHT 12 h AM did not obviously show the increment of insoluble solid (Figure 71). The result suggested that films prepared from SHL-PHT, especially at higher degree of esterification, was stable although partial hydrolysis of SHL-PHT

was observed. The polymerization of SHL-PHT would not occur if the sufficient phthalate was still attached at the hydroxyl group of SHL molecule.

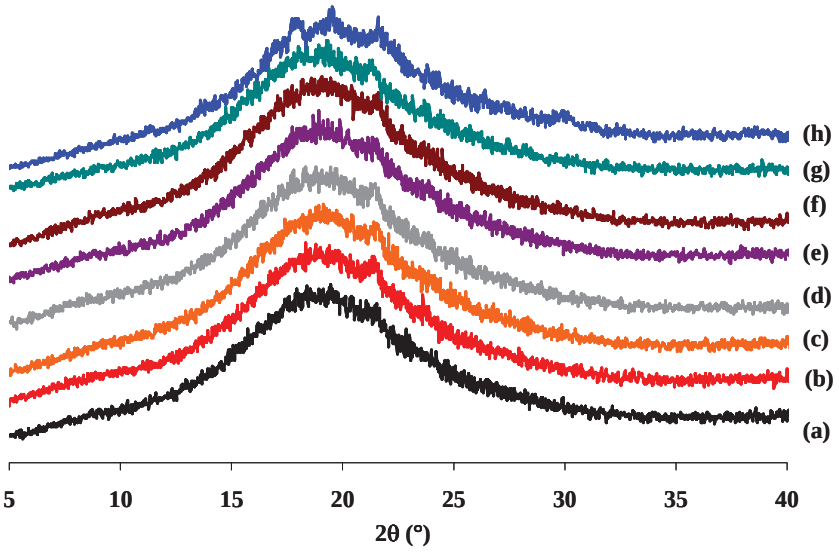


Figure 79 Powder X-ray diffraction patterns of SHL-PHT 1 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 day (g) and 180 days (h)

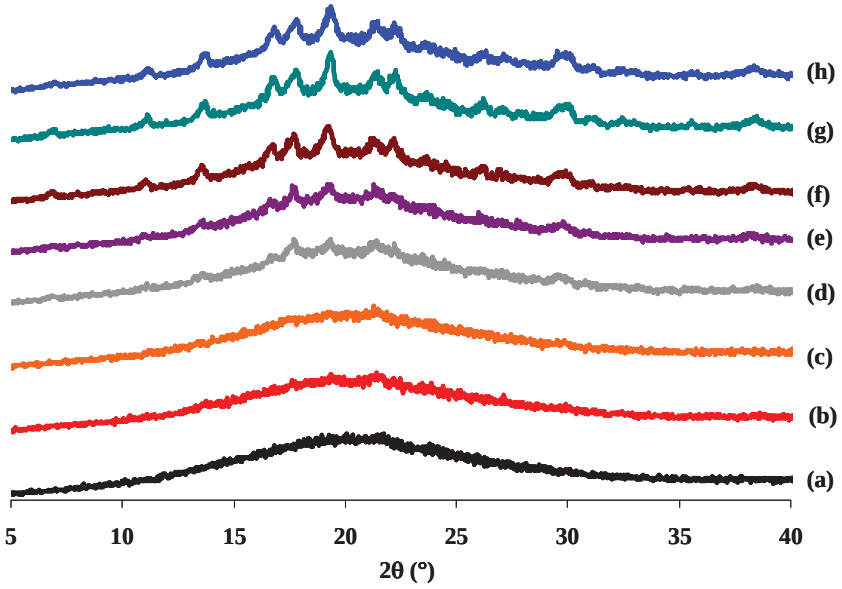


Figure 80 Powder X-ray diffraction patterns of SHL-PHT 6 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 day (g) and 180 days (h)

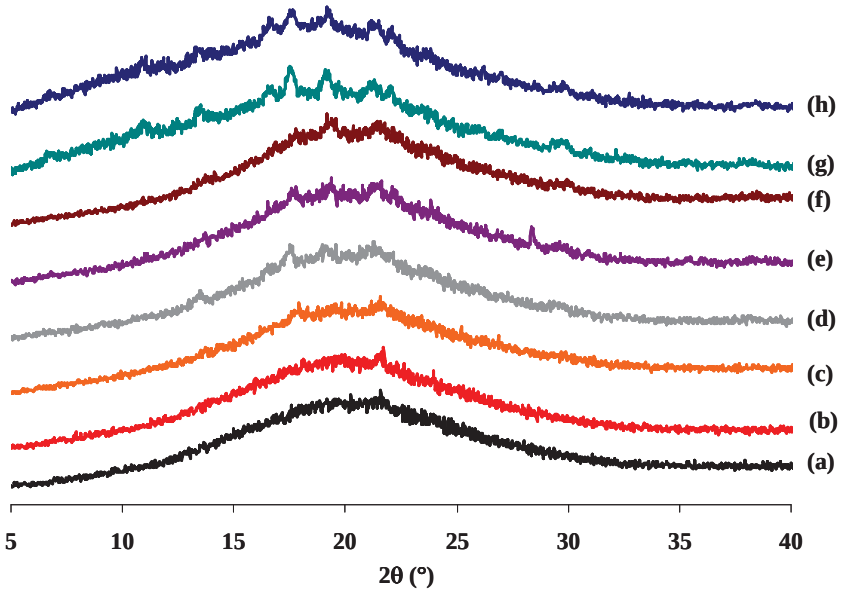


Figure 81 Powder X-ray diffraction patterns of SHL-PHT 12 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 day (g) and 180 days (h)

5.2 Stability study of shellac and shellac ester coated tablets

The stability testing of coated tablets was carried out under stress condition of tropical areas. Shellac coated tablets were kept in a stability chamber at 40 ºC, 75 % RH for up to 6 months. Samples of coated tablets were periodically withdrawn and then evaluated for tablet properties.

5.2.1 Stability study of shellac succinate coated tablets

The SHL and SHL-SUC 1 h AM coated tablets with the coating level of 6 mg/cm^2 and SHL-SUC 6 h AM and SHL-SUC 24 h AM coated tablets with the coating level of 9 mg/cm^2, were subjected to stability study at 40 ºC with 75% RH for 6 months and comparatively characterized

The properties of SHL coated tablets after storage are shown in Table 10. The change of tablet weight and hardness was not observed during storage time. In addition, all coated tablets did not disintegrate after immersion for first 2 h in 0.1 HCl even storage up to 180 days. However, the disintegration time in phosphate buffer pH 6.8 was dramatically increased, especially storage 30 days. The result suggested that SHL coated tablets was not stable during storage. Degradation process did not affect the gastric resistance but directly influenced on solubility at intestinal pH.

As illustrated in Table 11-13, weight and hardness of SHL-SUC coated tablets were not significantly changed after kept in stability chamber. For disintegration results, the SHL-SUC coated tablets showed similar result as SHL coated tablets. However, the disintegration time in phosphate buffer was relatively shorter especially for SHL-SUC 24 h AM. The result suggested that the polymerization of SHL-SUC occurred during storage.

Table 10 Properties of SHL coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.047±0.009	16.9±2.0	> 2 h	112.7±13.6
15	1.058±0.008	13.2±0.3	> 2 h	101.4±2.3
30	1.051±0.007	13.0±0.6	> 2 h	157.1±12.4
60	1.058±0.008	12.6±0.6	> 2 h	> 3 h
90	1.058±0.009	12.9±1.7	> 2 h	> 3 h
120	1.054±0.009	13.6±1.0	> 2 h	> 3 h
150	1.053±0.008	12.7±1.7	> 2 h	> 3 h
180	1.057±0.008	14.6±0.7	> 2 h	> 3 h

Table 11 Properties of SHL-SUC 1 h AM coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.041±0.009	11.4±0.6	> 2 h	12.3±0.5
15	1.037±0.008	11.0±1.1	> 2 h	33.1±13.5
30	1.035±0.011	11.3±0.7	> 2 h	58.6±16.2
60	1.039±0.012	11.0±1.4	> 2 h	> 3 h
90	1.040±0.010	10.8±0.6	> 2 h	> 3 h
120	1.037±0.008	10.8±1.2	> 2 h	> 3 h
150	1.036±0.009	10.9±0.2	> 2 h	> 3 h
180	1.038±0.009	11.0±2.3	> 2 h	> 3 h

Table 12 Properties of SHL-SUC 6 h AM coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.058±0.010	13.1±1.8	> 2 h	7.0±0.2
15	1.032±0.008	16.4±0.5	> 2 h	12.4±1.2
30	1.063±0.011	18.9±1.0	> 2 h	85.6±28.9
60	1.061±0.012	16.4±1.6	> 2 h	> 3 h
90	1.058±0.011	14.5±2.1	> 2 h	> 3 h
120	1.062±0.008	13.0±2.3	> 2 h	> 3 h
150	1.062±0.012	16.9±1.3	> 2 h	> 3 h
180	1.061±0.010	15.1±2.6	> 2 h	> 3 h

Table 13 Properties of SHL-SUC 24 h AM coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.056±0.007	18.6±2.0	> 2 h	4.1±0.3
15	1.069±0.015	12.5±1.1	> 2 h	3.9±0.4
30	1.059±0.007	12.2±0.8	> 2 h	7.4±0.5
60	1.070±0.009	13.0±0.6	> 2 h	23.5±7.9
90	1.068±0.008	10.4±0.9	> 2 h	167.8±9.1
120	1.067±0.009	11.1±1.0	> 2 h	> 3 h*
180	1.067±0.008	9.5±0.5	> 2 h	> 3 h

* 2 of 6 tablets dissolved within 3 h

The enteric coated tablets are designed to dissolve in the small intestine after it leaves the stomach. All shellac coated tablets could resist in 0.1 HCL for 2 h. The dissolution profiles of SHL coated tablets are demonstrated in Figure 82. The percent drug released in phosphate buffer pH 6.8 of SHL coated tablets was

gradually decreased with increasing storage time up to 180 days. The 180-days storage, paracetamol was released below 10 %, supporting the poor stability of SHL.

Figure 83-85 demonstrate the dissolution profile of SHL-SUC coated tablets upon storage. The drug released of SHL-SUC 1 h AM, 6 h AM and 24 h AM coated tablets was remarkably declined after storage up to 15, 30 and 60 days, respectively. The results confirmed the polymerization of SHL-SUC, but SHL-SUC 24 h AM coated tablets showed better stability (> 40% drug released) when storage up to 180 days, as compared to SHL and other SHL-SUC coated tablets (< 10% drug released).

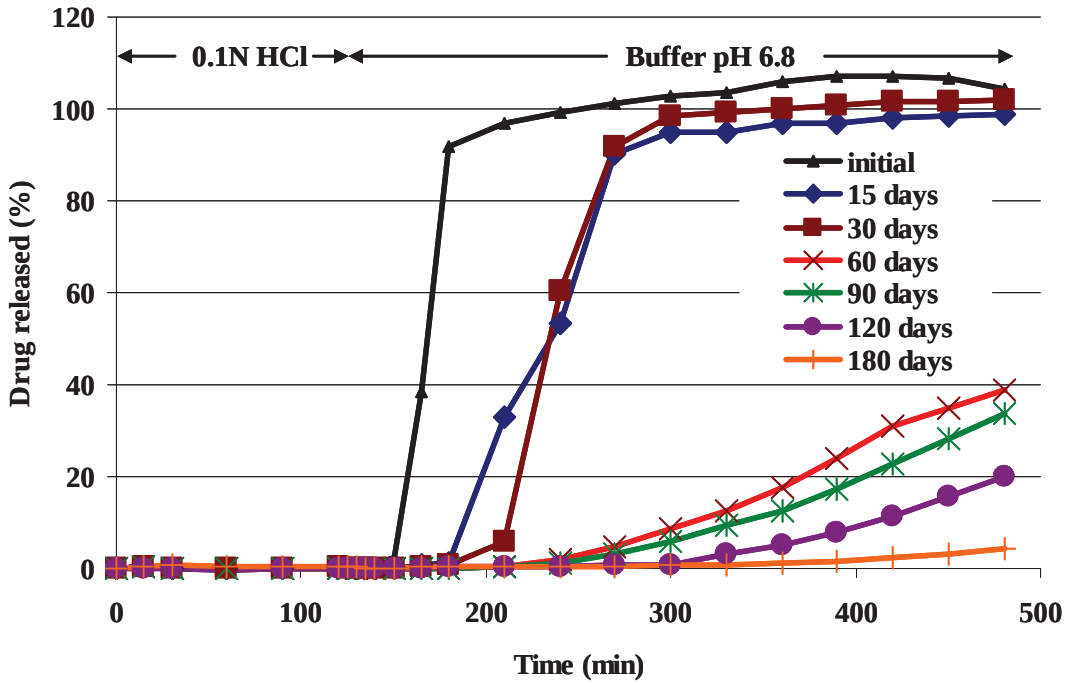


Figure 82 Dissolution profile of SHL coated tablets after storage

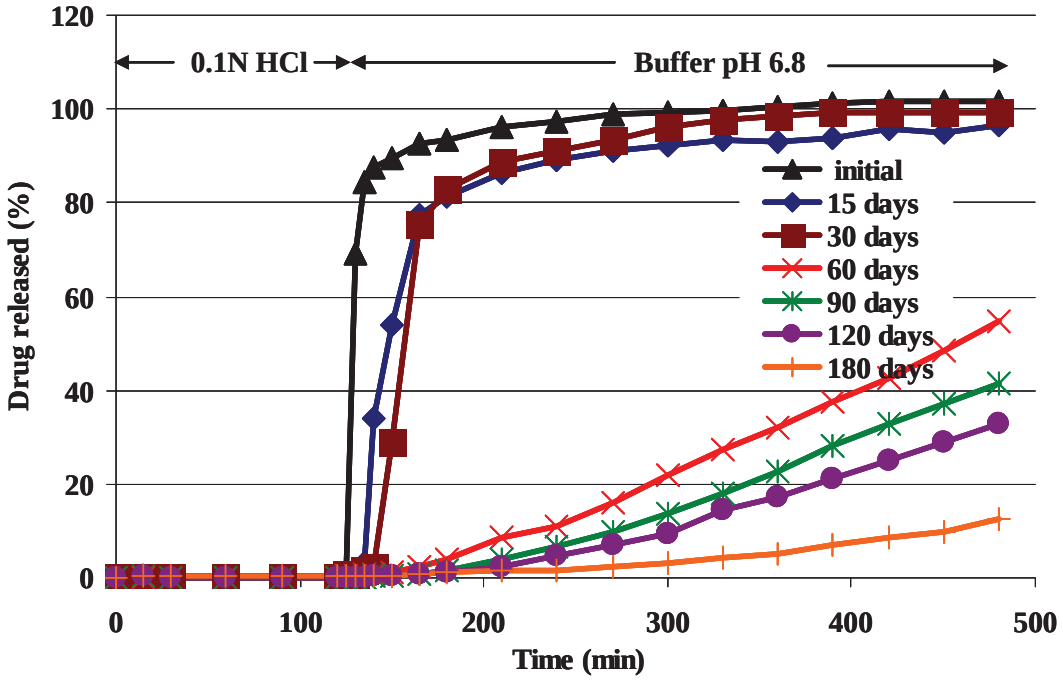


Figure 83 Dissolution profile of SHL-SUC 1 h AM coated tablets after storage

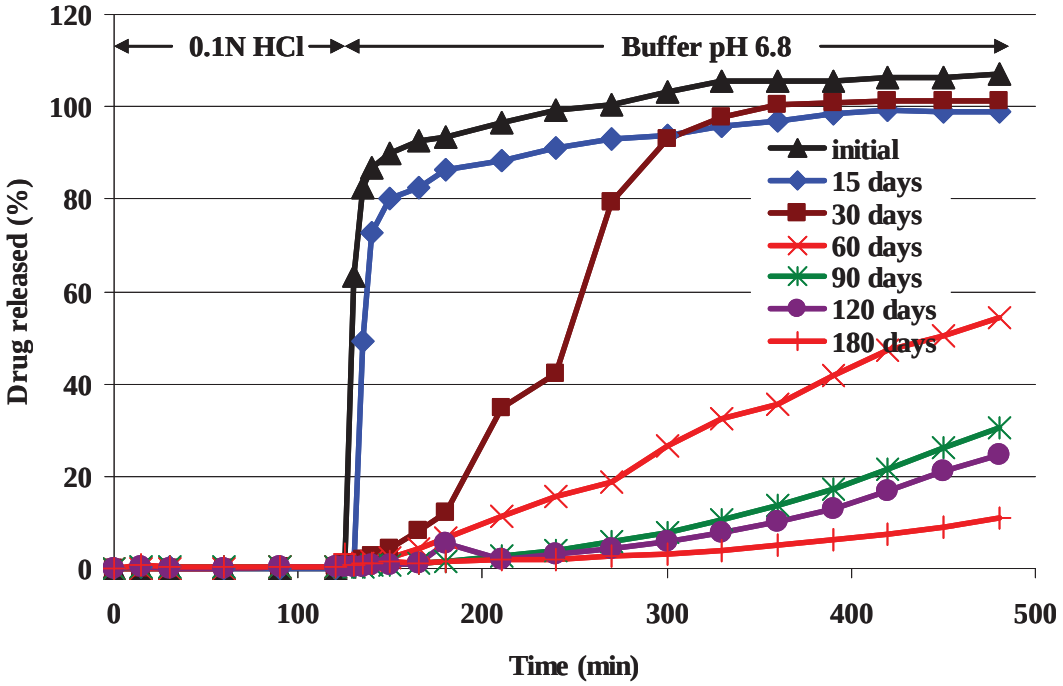


Figure 84 Dissolution profile of SHL-SUC 6 h AM coated tablets after storage

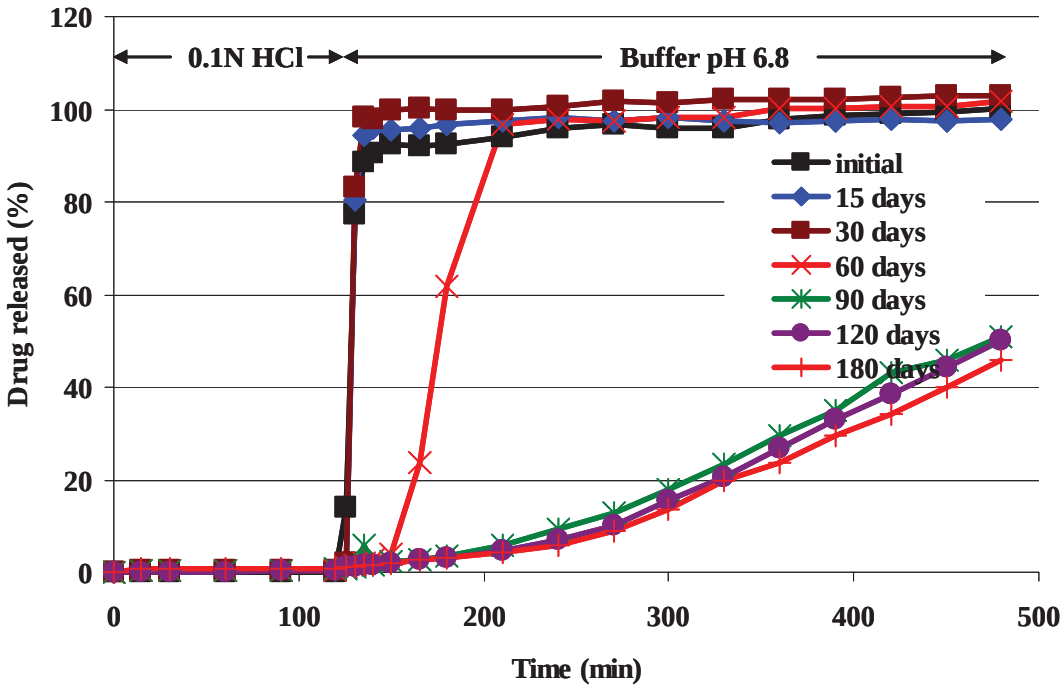


Figure 85 Dissolution profile of SHL-SUC 24 h AM coated tablets after storage

5.2.2 Stability study of shellac phthalate coated tablets

The SHL, SHL-PHT 1 h AM, SHL-PHT 6 h AM and SHL-PHT 12 h AM coated tablets at coating level of 7, 8, 15 and 15 mg/cm^2 respectively were kept under storage condition (40 ºC, 75% RH) for up to 6 months. Samples of the enteric coated tablets were periodically withdrawn and comparatively assessed.

The properties of SHL coated tablets after storage are demonstrated in Table 14. The change of weight, hardness and disintegration time of enteric coated tablets was not observed. For disintegration time, the initial value of disintegration time was high so the increment of DT might not observed after prolonged storage.

Table 15-17 show the tablet of SHL-PHT AM coated tablets during storage. For SHL-PHT 1 h AM, the disintegration time of the coated tablets in buffer 6.8 was more than 3 h after storage for 15 days, indicating the instability of SHL-PHT 1 h AM. On the other hand, SHL-PHT 6 h AM and SHL-PHT 12 h AM coated tablets demonstrated better stability. The coated tablets could completely disintegrate in buffer pH 6.8 after storage up to 90 days, although the disintegration time was increased as increasing storage time. In addition, the stability of SHL-PHT coated tablets was relatively higher as compared to SHL-SUC coated tablets.

Table 14 Properties of SHL coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.049±0.042	38.9±4.0	> 2 h	> 3h
15	1.050±0.047	37.7±4.5	> 2 h	> 3h
30	1.049±0.050	39.3±6.5	> 2 h	> 3h
60	1.051±0.043	37.5±2.4	> 2 h	> 3h
90	1.050±0.014	39.0±1.3	> 2 h	> 3h
120	1.048±0.032	36.4±4.4	> 2 h	> 3h
150	1.050±0.022	38.5±2.5	> 2 h	> 3h
180	1.050±0.055	39.0±2.4	> 2 h	> 3h

Table 15 Properties of SHL-PHT 1 h AM coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.076±0.024	39.7±6.0	> 2 h	91.2±4.5
15	1.079±0.019	40.8±3.9	> 2 h	> 3h*
30	1.078±0.027	40.8±5.8	> 2 h	> 3h**
60	1.074±0.039	40.9±1.2	> 2 h	> 3h
90	1.075±0.022	40.3±1.8	> 2 h	> 3h
120	1.076±0.031	41.5±5.7	> 2 h	> 3h
150	1.079±0.025	40.9±3.2	> 2 h	> 3h
180	1.079±0.032	37.9±2.8	> 2 h	> 3h

* 1 of 6 tablets dissolved in 70 min, ** 1 of 6 tablets dissolved in 165 min

Table 16 Properties of SHL-PHT 6 h AM coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.117±0.037	40.5±6.3	> 2 h	13.0±1.6
15	1.116±0.036	40.6±2.8	> 2 h	31.1±1.1
30	1.117±0.029	39.7±1.9	> 2 h	37.0±7.3
60	1.116±0.018	40.6±2.7	> 2 h	153.5±13.1*
90	1.118±0.027	40.1±2.2	> 2 h	172.4±40.3**
120	1.118±0.024	40.8±2.3	> 2 h	> 3 h
150	1.117±0.029	39.4±3.7	> 2 h	> 3 h
180	1.117±0.034	40.2±5.1	> 2 h	> 3 h

* 5 of 6 tablets dissolved within 3h, ** 3 of 6 tablets dissolved within 3h

Table 17 Properties of SHL-PHT 12 h AM coated tablets after storage

Storage time (days)	Tablet Properties			
	Weight (g)	Hardness (kp)	DT in 0.1 N HCl (min)	DT in pH 6.8 (min)
Initial	1.111±0.032	40.4±2.7	> 2 h	14.9±2.23
15	1.116±0.019	40.8±3.6	> 2 h	29.5±1.1
30	1.118±0.020	39.4±3.0	> 2 h	38.3±3.2
60	1.115±0.043	40.9±5.4	> 2 h	121.5±13.7
90	1.115±0.029	39.7±2.1	> 2 h	162.4±23.0
120	1.112±0.017	39.8±4.3	> 2 h	> 3 h
150	1.118±0.042	39.6±3.1	> 2 h	> 3 h
180	1.117±0.048	41.1±6.6	> 2 h	> 3 h

The dissolution profiles of SHL and SHL-PHT AM coated tablets after storage are shown in Figure 86-89. SHL and SHL-PHT AM coated tablets demonstrated gastric resistance ever storage up to 180 days, as indicated by lower 10 % of drug released in 0.1 N HCL for the first 2 h. However, the percent drug released of coated tablets was decreased as prolonged storage time. The remarkable decrease of drug released from SHL, SHL-PHT 1 h AM, SHL-PHT 6 h AM and SHL-PHT 12 h AM were obviously observed after storage up to 15, 30, 60 and 90 days, respectively. The results suggested the more stabilization as increasing phthalate formation. As compared to SHL-PHT film, SHL-PHT coated tablets showed faster polymerization, the aging effect might be accelerated with hot air during coating process. Although the instability of the SHL-PHT coated tablets was observed, the aging effect was slowly occurred as compare to SHL and SHL-SUC coating.

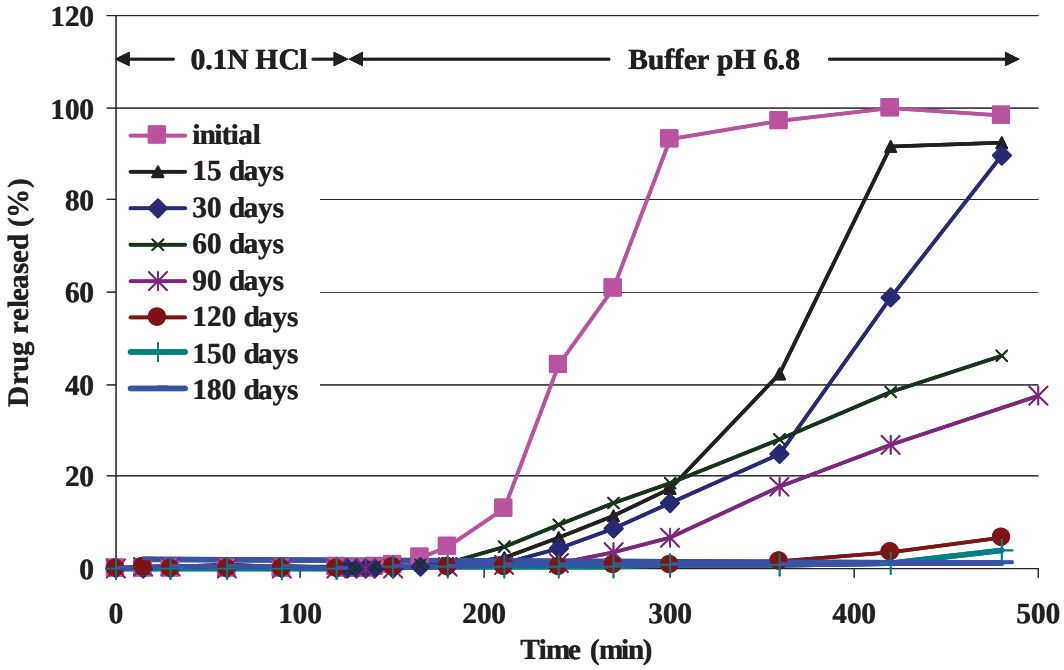


Figure 86 Dissolution profile of SHL coated tablets after storage

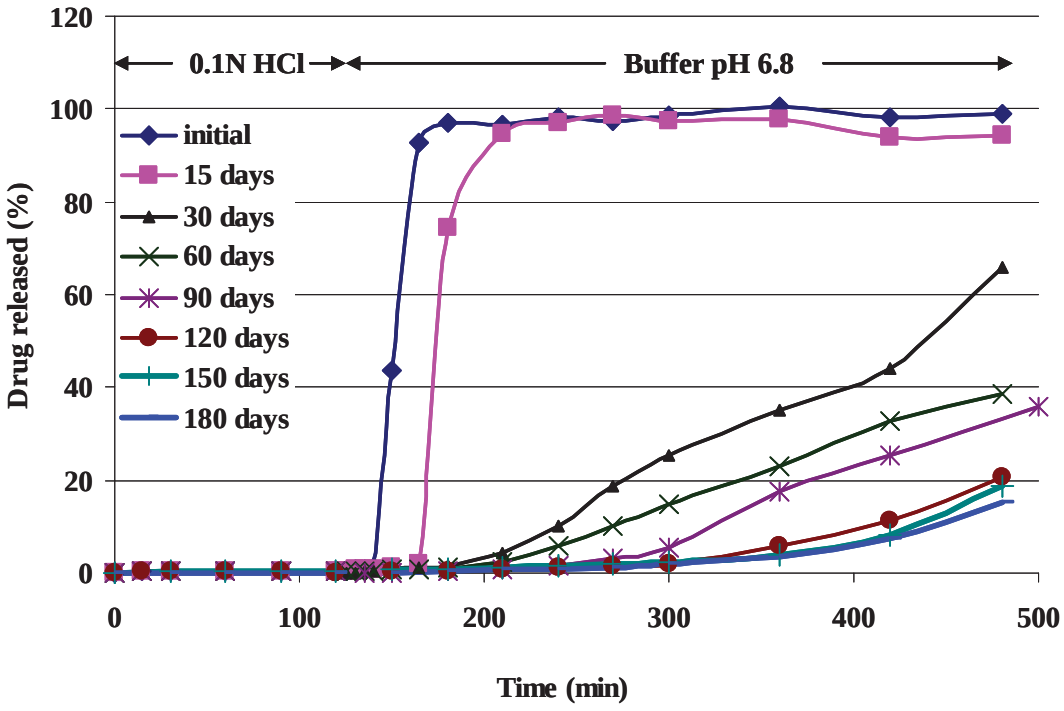


Figure 87 Dissolution profile of SHL-PHT 1 h AM coated tablets after storage

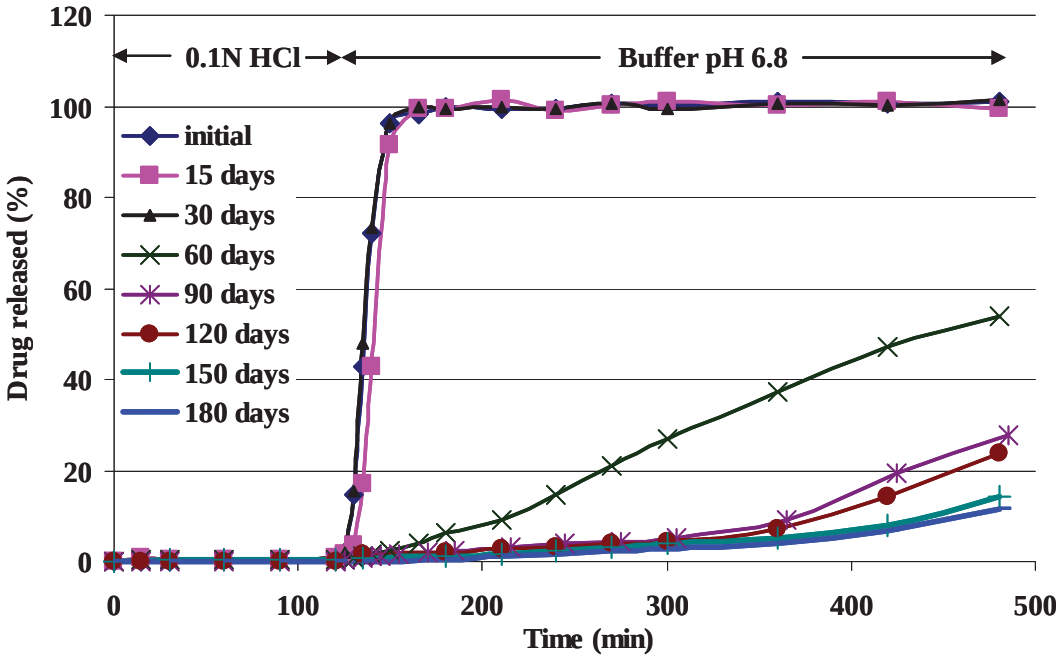


Figure 88 Dissolution profile of SHL-PHT 6 h AM coated tablets after storage

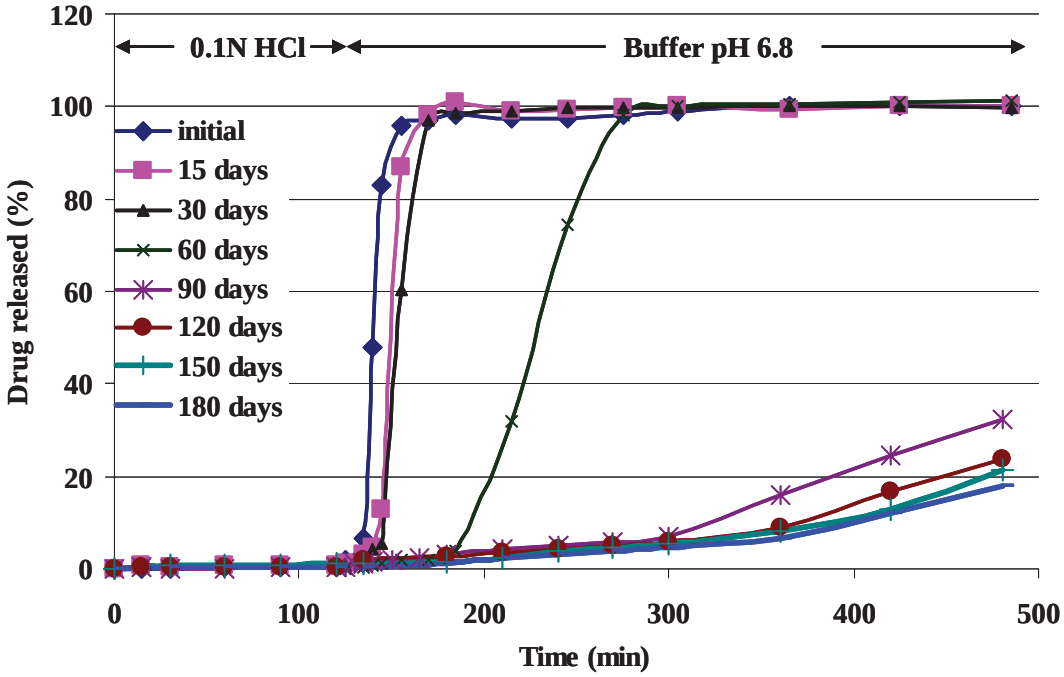


Figure 89 Dissolution profile of SHL-PHT 12 h AM coated tablets after storage

Figure 90 demonstrates the proposed diagram of shellac ester formation and aging mechanism of SHL and its ester forms. SHL was rapidly polymerized by esterification between hydroxyl and carboxyl groups of polymer chains of shellac after aging, resulting in increasing of the percentage of insoluble solid and lowering of the acid value. However, the polymerization could be protected by esterification with CAHs. The reduction of hydroxyl group and the increment of carboxylic group could enhance the solubility and stability of shellac. SUCA should easily react with the hydroxyl group of SHL, resulting in the highest acid value at low annealing temperature (Kim and Kim 2010: 170-176) and more ionization (good solubility) as compared to PHTA and TMTA which had larger molecular size. The mechanical properties of shellac esters were assumed to relate with the molecular conformation of substituted groups. The free movement of the attached succinate moieties at shellac polymer chain should promote the flexibility as compared to the more rigidity phthalate moiety. However, the flexibility of attached group might be one of the causes of instability as well. Although succinate moiety could separate the polymer chain of SHL at initial period but the conformation of succinate group was aliphatic chain that was more freely movement as compared to rigid aryl group. Therefore, the succinate group might change the conformation or collapse during storage until the cross-linking process between SHL polymer chains could initiate. In addition, the larger molecule of phthalate groups might provide more steric effect that separate the molecular chain of SHL and thus prevented the cross-linking process among hydroxyl groups and carboxylic group, resulting in the excellent stability. For SHL-TMT systems, TMTA demonstrated the largest molecule of CAHs in this finding. The AV of SHL-TMT AM was lower as compared to that of SHL-SUC AM

and SHL-PHT AM. Additionally, the insoluble solid was dramatically increased during heat treatment. The result suggested the lower substitution of trimellitate moiety into the shellac molecule. The larger molecules of TMTA might impede the esterification at hydroxyl group of SHL molecule. Therefore, shellac should degrade before the trimellitate formation was completed.

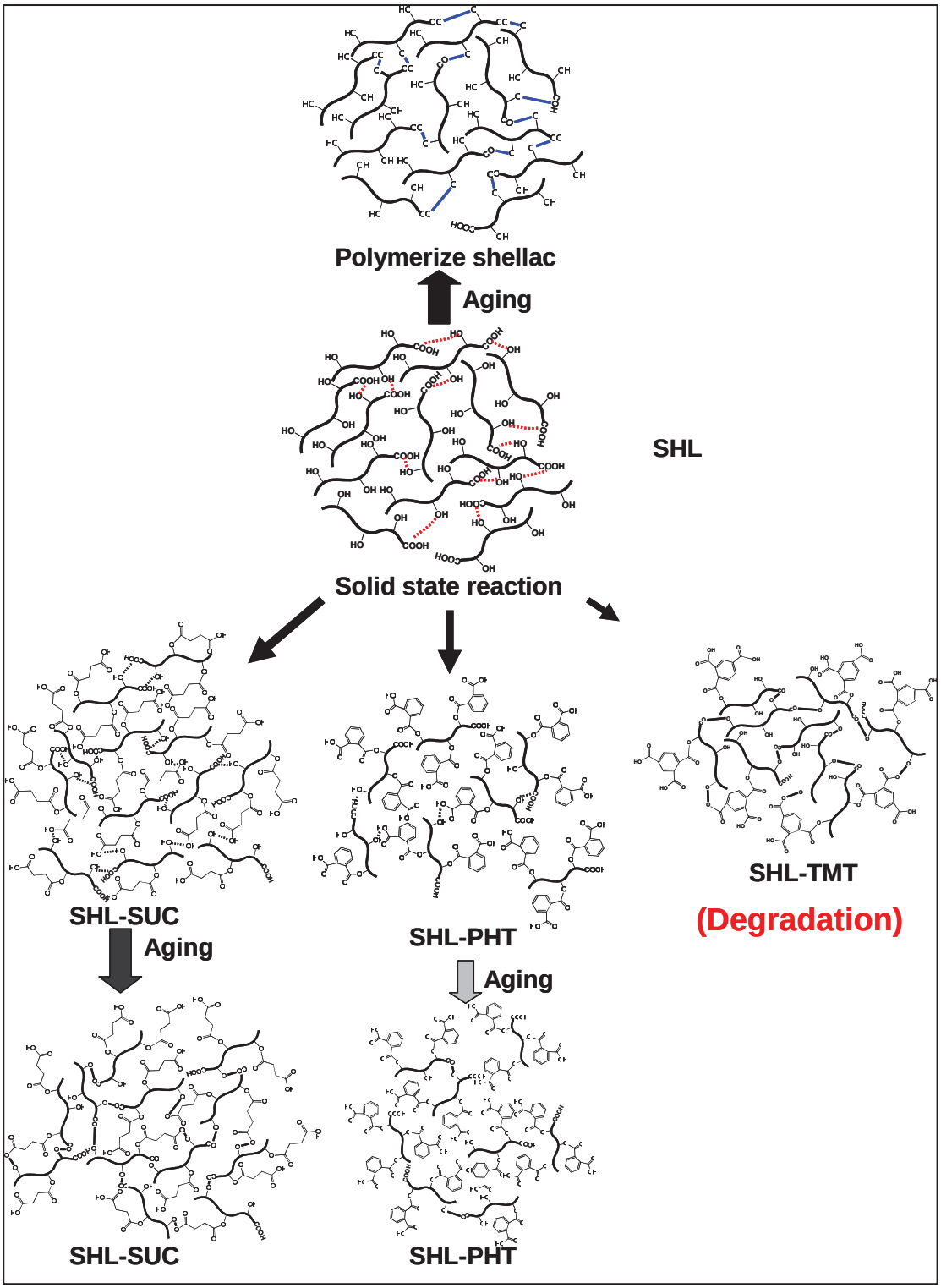


Figure 90 Proposed diagrams of shellac ester formation, aging effect and molecular arrangement of SHL and SHL-CAHs

CHAPTER 5

CONCLUSIONS

In the present study, shellac esters were successfully prepared by esterification SHL with CAHs i.e. SUCA, PHTA or TMTA through solid state reaction (grinding with heat treatment). Films and coated tablets prepared from SHL and shellac esters were then comparatively evaluated for enteric properties, stability and other related properties including, acid value, insoluble solid, pH solubility profile, disintegration and drug dissolution. The following results were obtained.

1. Formation of shellac esters was dependent on molecular size of CAHs. SUCA, the smallest molecule, could easily react with SHL under the low annealing temperature while PHTA and TMTA, larger molecule, demonstrated esterification with SHL at the higher temperature. In addition, the lower melting point of smaller molecule of CAHs might affect the reaction rate between SHL and CAHs. SUCA, having the lowest melting point, could easily change to more liquid and vapor state at the low heating temperature, as compared to PHTA and TMTA. Therefore the reaction between SHL and smaller CAHs was easily occurred. The results were in good agreed with previously reported. For SHL-SUC systems, the increment of acid value was observed as prolonging of annealing time at 60 ºC. SHL-PHT also showed similar result although the higher annealing temperature was used. For both systems, the increment of insoluble solid was not observed indicating that SHL-SUC and SHL-PHT were stable during the heat treatment. However, SHL-TMT demonstrated obvious increase of the insoluble solid, suggesting the instability of this system.

As compared to SHL, the acid value of SHL-SUC, SHL-PHT and SHL-TMT could increase up to 2, 2, 1.5 times, respectively, suggesting the addition of carboxylic group by esterification. The esterification was later confirmed by the increased relative intensity of carbonyl stretching and the new peak of ester linkage from FTIR spectroscopy. In addition, the formation of SHL-SUC and SHL-PHT was also confirmed by NMR spectroscopy. The characteristic resonance of SHL was loss while the new signals, referred to ester linkage, were remarkably observed. In addition, the increment of substituted OH group of shellac ester was observed. However, these signals and percent substituted at hydroxyl group were slightly changed in SHL-TMT systems. The results confirmed that larger molecule with more steric effect of TMTA was difficult to react with SHL as compared to SUCA and PHTA.

2. The films of SHL, SHL-SUC and SHL-PHT were prepared in ammonium salt form. From the results of mechanical properties, SHL-SUC demonstrated more percent elongation as compared to SHL-PHT. The aliphatic chain (succinate moiety) might promote more SHL chain movement as compared to rigid aromatic ring (phthalate moiety), resulting in the more flexibility. The increasing hydrophilicity of added carboxylic groups also affected the moisture diffusion through the film; however the increment of WVP coefficient of all SHL-SUC and SHL-PHT film was still in a lower value as compared to other enteric polymers. An importance function of enteric film is gastric protection and to dissolve promptly in small intestine. SHL showed the excellent gastric protection film whereas low solubility at the pH of small intestine which was the major problem of native SHL. The problem was successfully solved by the invention of shellac esters. SHL-SUC and SHL-PHT could increase solubility at intestinal pH, supporting that the increasing carboxylic acid, having

lower pK_a, of attached ester moieties could enhance the solubility in lower pH. Moreover, the percent film dissolved of SHL-SUC and SHL-PHT in SGF was also still low value, indicating the good gastric protection of SHL-SUC and SHL-PHT films.

3. SHL-SUC and SHL-PHT were applied as coating material to deliver the model drug (paracetamol) to the dissolution medium that simulated the gastro-intestinal condition. SHL, SHL-SUC and SHL-PHT coated tablets had excellent gastric resistance. The longest time for 50% drug released of SHL-SUC and SHL-PHT coated tablets in buffer pH 6.8 was within 15 min and 31 min, respectively, while that of SHL coated tablets was more than 2 h. The faster drug release was observed as increasing ester formation and the dissolutions of SHL-SUC AM and SHL-PHT AM coated tablets were not changed as increasing coating level, indicating the good solubility of shellac esters.

4. From the result of cytotoxicity test, the decreasing of IC_{50} of shellac ester was observed, suggesting the higher cell toxicity as compared to SHL. However, the IC_{50} value of all shellac esters was still in high value as compared with other polymers, indicating the low toxicity.

5. The accelerated stability test was investigated at 45 ºC, 75% RH for 6 months. The insoluble solid of SHL film was obviously observed as prolonged storage time, indicating the instability of SHL. After storage, the SHL-SUC film was polymerized after 15 days, as indicated by the increasing of percent insoluble solid, lowering acid value and reducing relative absorbance ratios of the FTIR peaks assigned to C=O stretching of carboxylate and carboxylic acid (ABS_{1556}/ABS_{1716}). In addition, the small PXRD diffraction peak of succinic acid was observed, indicating

the hydrolysis of some succinate moiety from SHL-SUC. For SHL-PHT film, the stability was enhanced as increasing ester formation. Both SHL-SUC film and SHL-PHT film showed better stability as compared to SHL film. Although phthalate moieties were partial hydrolyzed from SHL-PHT (observed from PXRD results), the acid value and the relative absorbance ratios of the FTIR peaks (ABS_{1556}/ABS_{1716}) were slightly declined, indicating that the aging could protect by the sufficient phthalate. For coated tablets, SHL showed lowering percent drug release after storage, while SHL-SUC and SHL-PHT demonstrated better stability and could delay the aging effect of coated tablets.

In summary, the excellent solubility and the delay of aging process of shellac were easily achieved by esterification through solvent free reaction under the concept of “green chemistry”. The finding could give an alternative way, which is simple, safe and efficient, for improving enteric properties and stability of shellac.

BIBLIOGRAPHY

Aiedeh, Khaled, and Mutasem O. Taha. "Synthesis of iron-crosslinked chitosan succinate and iron-crosslinked hydroxamated chitosan succinate and their in vitro evaluation as potential matrix materials for oral theophylline sustained-release beads." European Journal of Pharmaceutical Sciences 13, 2 (May 2001) : 159-168.

AlKhatib, Hatim S. et al. "Modulation of buspirone HCl release from hypromellose matrices using chitosan succinate: Implications for pH-independent release." European Journal of Pharmaceutics and Biopharmaceutics 70, 3 (November 2008) : 804-812.

Allen, Loyd V. et al. Ansel's pharmaceutical dosage forms and drug delivery systems. 8th ed. Philadelphia : Lippincott Williams & Wilkins, 2004.

American Society for Testing and Materials. Annual Book of ASTM Standards. Pennsylvania : ASTM International, 1989.

Asselt, Rob van et al. "New synthetic routes to poly (isothianaphthene) I. Reaction of phthalic anhydride and phthalide with phosphorus pentasulfide." Synthetic Metals 74, 1 (September 1995) : 65-70.

Bechtold, Tomus, and Rita Mussak. Handbook of Natural Colorants. West Sussex : Wiley, 2009.

Belgacem, Mohamed N., and Alessandro Gandini. Monomers, polymers and composites from renewable resources. Amsterdam : Elsevier, 2008.

Berger, Stefan, and Dieter Sicker. Classics in spectroscopy: isolation and structure elucidation of natural products. Germany : WILEY-VCH, 2009.

Bhandari, Praful N., and Rekha S. Singhal. "Studies on the optimisation of preparation of succinate derivatives from corn and amaranth starches." Carbohydrate Polymers 47, 3 (February 2002) : 277-283.

Bley, O. et al. "Protection of moisture-sensitive drugs with aqueous polymer coatings: Importance of coating and curing conditions." International Journal of Pharmaceutics 378, 1-2 (August 2009) : 59-65.

Bruce, L. Diane et al. "Properties of enteric coated sodium valproate pellets." International Journal of Pharmaceutics 264, 1-2 (October 2003) : 85-96.

Brydson, John. Plastics materials. 7th ed. Oxford : Butterworth-Heinemann, 1999.

Bunzli-Trepp, Ursula. Systematic nomenclature of organic, organometallic and coordination chemistry: chemical-abstracts guidelines with IUPAC recommendations and many trivial names. Lausanne. Lausanne : EPFL Press, 2007.

Byrn, Stephen R., Wei Xu, and Ann W. Newman. "Chemical reactivity in solid-state pharmaceuticals: formulation implications." Advanced Drug Delivery Reviews 48, 1 (May 2001) : 115-136.

Cao, Na, Xinmin Yang, and Yuhua Fu. "Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films." Food Hydrocolloids 23, 3 (May 2009) : 729-735.

Colacinoa, Evelina et al. "Solvent-free synthesis of nitrones in a ball-mill." Tetrahedron 64, 23 (June 2008) : 5569-5576.

Clark, James H., and Duncan J. Macquarrie. Handbook of green chemistry and technology. Oxford : Wiley-Blackwell, 2002.

Davis, M. et al. "Comparison and evaluation of enteric polymer properties in aqueous solutions." International Journal of Pharmaceutics 28, 2-3 (February 1986) : 157-166.

Der Marderosian, Ara Harold, and John A. Beutler. The review of natural products. 2nd ed. St. Louis : Fact and Comparison, 2002.

Doble, Mukesh, and Anil K. Green chemistry and engineering. London : Elsevier, 2007.

Eastaugh, Nicholus et al. Pigment Compendium: A Dictionary and Optical Microscopy of Historic Pigments. Burlington : Butterworth-Heinemann, 2008.

Francis, Frederick J. Wiley Encyclopedia of food science and technology. New York : John Wiley & Sons, 2000.

Frohoff-Hulsmann, Maria A., Bernhard C. Lippold, and James W. McGinity. "Aqueous ethyl cellulose dispersion containing plasticizers of different water solubility and hydroxypropyl methyl-cellulose as coating material for diffusion pellets II: properties of sprayed films." European Journal of Pharmaceutics and Biopharmaceutics 48, 1 (July 1999) : 67-75.

Fukui, Eiji, Nobuteru Miyamura, and Masao Kobayashi. "An in vitro investigation of the suitability of press-coated tablets with hydroxypropylmethylcellulose acetate succinate (HPMCAS) and hydrophobic additives in the outer shell for colon targeting." Journal of Controlled Release 70, 1-2 (January 2001): 97-107.

Fukuoka, Eihei et al. "Solid-State Reaction between Sulfacetamide and Phthalic Anhydride by Grinding." Chemical and Pharmaceutical Bulletin 46, 6 (February 1994) : 1342-1344.

Gad, Shayne C. Pharmaceutical manufacturing handbook: production and processes. New Jersey : Wiley-Interscience, 2008.

Gad, Shayne C. Preclinical Development Handbook: ADME and Biopharmaceutical Properties. New Jersey : John Wiley & Sons, 2008.

Galdeano, Melícia Cíntia et al. "Effects of plasticizers on the properties of oat starch films." Materials Science and Engineering: C 29, 2 (March 2009) : 532-538.

Gardner, Henry A., and George G. Sward. Paint testing manual: physical and chemical examination of paints, varnishes, lacquers, and colors. 13th ed. Philadelphia : American Society for Testing and Materials, 1972.

Ghatak, K. L. Techniques and Methods in Biology. New Delhi : PHI Learning Pvt. Ltd., 2011.

Ghosh, Premamoy. Adhesive and Coating Technology. New Delhi : McGraw-Hill Education, 2008.

Graaf, Robin A. D. In vivo NMR spectroscopy: principles and techniques. 2nd ed. West Sussex : John Wiley & Sons, 2007.

Gubbins, P. O., and K. E. Bertch. "Drug absorption in gastrointestinal disease and surgery. Clinical pharmacokinetic and therapeutic implications." Clinical Pharmacokinetics 21, 6 (December 1991) : 431-47.

Hogan, John, Graham Cole, and Michael E. Aulton. Pharmaceutical Coating Technology. London : Taylor & Francis, 2002.

Holzgrabe, Ulrike, Iwona Wawer, and Bernd Diehl. NMR spectroscopy in drug development and analysis. Weinheim : Wiley-VCH, 1999.

Horton, R. E., F. G. M. Ross, and G. H. Darling. "Determination of the Emptying-time of the Stomach by Use of Enteric-coated Barium Granules." British Medical Journal 5449, 1 (June 1965) : 1537-1539.

Ibekwe, Valentine C., et al. "A comparative in vitro assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery." International Journal of Pharmaceutics 308, 1-2 (February 2006) : 52-60.

Jabde, Pradip V. Text Book Of Applied Zoology. New Delhi : Discovery Publishing House, 2005.

Jacobsen, Neil E. NMR spectroscopy explained: simplified theory, applications and examples for organic chemistry and structural biology. New Jersey : Wiley-Interscience, 2007.

Jones, David. Pharmaceutical Applications of Polymers for Drug Delivery. Shrewbury : Rapra Technology, 2004.

Kappe, C. Oliver, Doris Dallinger, and S. Shaun Murphree. Practical microwave synthesis for organic chemists: strategies, instruments, and protocols. Darmstadt : Wiley-VCH, 2009.

Kararli, Tugrul T., Carol F. Kirchhoff, and James E. Truelovel. "Ionic strength dependence of dissolution for Eudragit S-100 coated pellets." Pharmaceutical Research 12, 11 (November 1995) : 1813-1816.

Karsa, D. R. et al. Chemical aspects of drug delivery systems. Cambridge : Royal Society of Chemistry Information Services, 1996.

Kaupp, G., J. Schmeyers, and J. Boy. "Waste-free solid-state syntheses with quantitative yield." Chemosphere 43, 1 (April 2001) : 55-61.

Kidwai, Mazaahir. "Dry media reactions." Pure and Applied Chemistry 73, 1 (2001) : 147-151.

Kim, James H., Herman J. Gibb, and Anneatte Iannucci. Cyclic acid anhydrides: human health aspects. Stuttgart : World Health Organization, 2009.

Kim, Seok Hoon and Joon Ho Kim. "Preparation and characterization of high molecular weight poly(trimethylene terephthalate) by solid-state polymerization." Fibers and Polymers 11, 2 (February, 2010): 170-176.

Kokubo, H., et al. "Development of cellulose derivatives as novel enteric coating agents soluble at pH 3.5-4.5 and higher." Chemical and Pharmaceutical Bulletin 45, 8 (August 1997) : 1350-1353.

Krause, K. P., and R. H. Muller. "Production of aqueous shellac dispersions by high pressure homogenisation." International Journal of Pharmaceutics 223, 1-2 (July 2001) : 89-92.

Lai, Xiaolin et al. "Evaluation of poly(styrene-alt-maleic anhydride)–ethanol as enteric coating material." International Journal of Pharmaceutics 352, 1-2 (March 2008) : 66-73.

Lalena, John N., and David A. Cleary. Inorganic materials synthesis and fabrication. 2nd ed. New Jersey : Wiley-Interscience, 2008.

Lancaster, Mike. Green Chemistry: an introductory text. Cambride : Royal Society Of Chemistry, 2010.

Lew, Kristi. Chemical Reactions. New York : Chelsea House Publishers, 2008.

Li, Xin-Ming, Guo-Wei Le, and Yong-Hui Shi. "Microwave-assisted solid-phase oligosaccharides synthesis reaction and scavenging activity of synthetic product to free radical." Carbohydrate Polymers 64, 2 (May 2006) : 274-281.

Liu, Fang et al. "A novel concept in enteric coating: A double-coating system providing rapid drug release in the proximal small intestine." Journal of Controlled Release 133, 2 (January 2009) : 119-124.

Mallick, Subrata et al. "Formation of physically stable amorphous phase of ibuprofen by solid state milling with kaolin." European Journal of Pharmaceutics and Biopharmaceutics 68, 2 (February 2008) : 346-351.

Mallick, Subrata et al. "Physicochemical characterization of interaction of ibuprofen by solid-state milling with aluminum hydroxide." Drug Development and Industrial Pharmacy 34, 7 (July 2008) : 726-734.

Manee Luangtana-Anan et al. "Effect of salts and plasticizers on stability of shellac film." Journal of Agricultural and Food Chemistry 55, 3 (February 2007) : 687-692.

Marṫnez-Gonzlez, Ilona, and Leopoldo Villafuerte-Robles. "Influence of enteric citric acid on the release profile of 4-aminopyridine from HPMC matrix tablets." International Journal of Pharmaceutics 251, 1-2 (January 2003) : 183-193.

Matlack, Albert S. Introduction to green chemistry. 2nd ed. New York : CRC Press, 2010.

Meehan, Elizabeth. "Characterisation of hydroxypropylmethylcellulose phthalate (HPMCP) by GPC using a modified organic solvent." Analytica Chimica Acta 557, 1-2 (January 2006) : 2-6.

Montazerozohori, Morteza et al. "Fast and Highly Efficient Solid State Oxidation of Thiols." Molecules 12, 3 (March 2007) : 694-702.

Morris, Christopher G. Academic Press dictionary of science and technology. London : Academic Press, 1992.

Nesbitt, R. U., F.W. Goodhart, and R.H. Gordon. "Evaluation of polyvinyl acetate phthalate as an enteric coating material." International Journal of Pharmaceutics 26, 3 (October 1985) : 215-226.

Nielsen, S. Suzanne. Food Analysis. 4th ed. New York : Springer, 2010.

Ohashi, T., K. Tokuno, and Y. Otoda. "Organic Sulfur Compounds. XV. : Organic Solid-State Methylations Using Sulfonium Salt." Journal of the Pharmaceutical Society of Japan 108, 8 (1988) : 733-739.

Omari, Derar M., et al. "Lactic acid-induced modifications in films of Eudragit RL and RS aqueous dispersions." International Journal of Pharmaceutics 274, 1-2 (April 2004) : 85-96.

Ortega-Aviles, M., et al. "Spectroscopic investigation of a `Virgin of Sorrows' canvas painting: A multi-method approach." Analytica Chimica Acta 550, 1-2 (September 2005) : 164-172.

Nantarat Pearnchob, and Roland Bodmeier. "Dry polymer powder coating and comparison with conventional liquid-based coatings for Eudragit (R) RS, ethylcellulose and shellac." European Journal of Pharmaceutics and Biopharmaceutics 56, 3 (November 2003): 363-369.

Nantarat Pearnchob, Andrei Dashevsky, and Roland Bodmeier. "Improvement in the disintegration of shellac-coated soft gelatin capsules in simulated intestinal fluid." Journal of Controlled Release 94, 2-3 (February 2004) : 313-321.

Papaspyrides, Constantine D. and Stamatina N. Vouyiouka. Solid state polymerization. New Jersey : John Wiley and Sons, 2009.

Pharmaceutical Society of Great Britain. Pharmaceutical journal: A weekly record of pharmacy and allied sciences. London : J. Churchill, 1901.

Qussi, B., and W. G. Suess. "Investigation of the effect of various shellac coating compositions containing different water-soluble polymers on in vitro drug release." Drug Development and Industrial Pharmacy 31, 1 (January 2005) : 99-108.

Qussi, B., and W. G. Suess. "The influence of different plasticizers and polymers on the mechanical and thermal properties, porosity and drug permeability of free shellac films." Drug Development and Industrial Pharmacy 32, 4 (April 2006) : 403-412.

Rao, P. Rama, and Prakash. V. Diwan. "Permeability studies of cellulose acetate free films for transdermal use: Influence of plasticizers." Pharmaceutica Acta Helvetiae 72, 1 (February 1997) : 47-51.

Rathbone, Michael. J., Jonathan Hadgraft, and Michael S. Roberts. Modified-release drug delivery technology. New York : Informa Health Care, 2003.

Ren, Lili et al. "Influence of surface esterification with alkenyl succinic anhydrides on mechanical properties of corn starch films." Carbohydrate Polymers 82, 3 (October 2010) : 1010-1013.

Rowe, Raymond C., Paul J. Sheskey, and Siân C. Owen. Handbook of pharmaceutical excipients. 5th ed. Michigan : Pharmaceutical Press, 2006.

Rudnik, Ewa et al. "Thermal properties of starch succinates." Thermochimica Acta 427, 1-2 (March 2005) : 163-166.

Rungsinee Sothornvit, and John M. Krochta. "Plasticizer effect on mechanical properties of [beta]-lactoglobulin films." Journal of Food Engineering 50, 3 (November 2001) : 149-155.

Safari, Javad et al. "A rapid and efficient method for synthesis of new 3-arylideneisobenzofuran-1(3H)-one derivatives catalyzed by acetic anhydride under solvent-free and microwave conditions." Journal of Molecular Catalysis A: Chemical 270, 1-2 (June 2007) : 236-240.

Sakata, Yukoh et al. "Effects of solid-state reaction between paracetamol and cloperastine hydrochloride on the pharmaceutical properties of their preparations." International Journal of Pharmaceutics 335, 1-2 (April 2007) : 12-19.

Sauer, Dorothea et al. "Influence of polymeric subcoats on the drug release properties of tablets powder-coated with pre-plasticized Eudragit L 100-55." International Journal of Pharmaceutics 367, 1-2 (February 2009) : 20-28.

Schoenwald, Ronald D. Pharmacokinetic principles of dosing adjustments: understanding the basics. London : CRC Press, 2001.

Schreier, Hans. Drug targeting technology: physical, chemical, biological methods. New York: Marcel Dekker, 2001.

Sharma, S. K., S. K. Shukla, and D. N. Vaid. "Shellac-Structure, Characteristics & Modification." Defence Science Journal 33, 3 (July 1983) : 261-271.

Shigemasa, Yoshihiro et al. "Chemical modification of chitin and chitosan 1: preparation of partially deacetylated chitin derivatives via a ring-opening reaction with cyclic acid anhydrides in lithium chloride/N,N-dimethylacetamide." Carbohydrate Polymers 39, 3 (July 1999) : 237-243.

Silverstein, Robert Milton, Francis X. Webster, and David J. Kiemle. Spectrometric identification of organic compounds. 7th ed. New York : John Wiley & Sons, 2005.

Singh, Rajendra. Elements of Entomology. Meerut : Rastogi Publications, 2006.

Smith, Michael. Organic Chemistry: An Acid-Base Approach. Florida : Taylor and Francis, 2010.

Sontaya Limmatvapirat et al. "Modification of physicochemical and mechanical properties of shellac by partial hydrolysis." International Journal of Pharmaceutics 278, 1 (June 2004) : 41-49.

Sontaya Limmatvapirat et al. "Enhanced enteric properties and stability of shellac films through composite salts formation." European Journal of Pharmaceutics and Biopharmaceutics 67, 3 (November 2007) : 690-698.

Sontaya Limmatvapirat et al. "Effect of alkali treatment on properties of native shellac and stability of hydrolyzed shellac." Pharmaceutical Development and Technology 10, 1 (2005) : 41-46.

Srinivasa, P. C., M. N. Ramesh, and R. N. Tharanathan. "Effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films." Food Hydrocolloids 21, 7 (October 2007) : 1113-1122.

Toda, Fumio. Organic solid state reactions. London : Springer, 2002.

Tarcha, Peter. J. Polymers for Controlled Drug Delivery. London : CRC Press, 1999.

Thomas, John M. "Organic reactions in the solid state: Accident and design." Pure and Applied Chemistry, no. 51 (1979) : 1065-1082.

Tundo, Pietro et al. "Synthetic pathways and processes in green chemistry. Introductory overview." Pure and applied chemistry 72, 7 (2000) : 1207-1228.

United States Pharmacopeial Convention. The United States pharmacopeia: National formulary (USP30-NF25). Rockville : United States Pharmacopeial Convention, 2007.

Velayutham, Tamil S. et al. "Synthesis and characterization of polyurethane coatings derived from polyols synthesized with glycerol, phthalic anhydride and oleic acid." Progress in Organic Coatings 66, 4 (December 2009) : 367-371.

Wang, Li Kuan et al. "Microwave assisted solid state reaction synthesis of methionine complexes of iron (II)." Food Chemistry 106, 1 (2008) : 315-323.

Washington, Neena, Clive Washington, and Clive Wilson. Physiological Pharmaceutics: Barriers to Drug Absorption. 2nd ed. London : CRC Press, 2001.

West, Anthony. R. Solid state chemistry and its applications. New York : Wiley, 1991.

Wolny, Philip. Chemical Reactions. New York : The Rosen Pub Group, 2011.

Wruble, J. S. "Enteric coating." American Journal of Pharmacy, no. 102 (1930) : 318.

Wu, C. and J. W. McGinity. "Influence of relative humidity on the mechanical and drug release properties of theophylline pellets coated with an acrylic polymer containing methylparaben as a non-traditional plasticizer." European Journal of Pharmaceutics and Biopharmaceutics 50, 2 (September 2000) : 277-284.

Zhao, Bo, Can Hui Lu, and Mei Liang. "Solvent-free esterification of poly(vinyl alcohol) and maleic anhydride through mechanochemical reaction." Chinese Chemical Letters 18, 11 (November 2007) : 1353-1356.

APPENDIX

Acid value

Acid value (AV) of each sample was calculated using the following formula.

$$AV = V_{NaOH}\ N_{NaOH}\ \frac{56.11}{W_{sample}}$$

where

AV is acid value (mg KOH/g sample),

V_{NaOH} is the amount of NaOH consumed at the equivalent point (ml),

N_{NaOH} is The molarity concentration of NaOH (M),

56.11 is the molecular weight of KOH, and

W_{sample} is the amount of NaOH (g)

Example

1.6782 g of SHL utilized 25.14 mL of 0.0968 M NaOH to neutralize the acid.

$$AV_{SHL} = 25.14 \times 0.0968 \times \frac{56.11}{1.6782}$$

$$AV_{SHL} = 81.36$$

The acid value of SHL was 81.36 mg KOH/g sample

Table 18 The mechanical properties of SHL, SHL-SUC and SHL-PHT films

Sample	Puncture strength (MPa)	Elongation (%)	Modulus at puncture (kPa)
SHL	0.28±0.04	1.24±0.42	223.47±33.79
SHL-SUC 1 h AM	8.17±1.42	45.38±11.39	188.04±47.40
SHL-SUC 6 h AM	6.38±2.37	84.60±13.75	80.61±28.45
SHL-SUC 24 h AM	4.17±1.14	98.22±44.64	50.75±25.88
SHL-PHT 1 h AM	0.63±0.07	1.35±0.27	366.84±49.78
SHL-PHT 6 h AM	0.54±0.08	53.58±18.46	8.73±1.48
SHL-PHT 12 h AM	0.71±0.02	47.22±6.49	21.62±9.92

Table 19 The percentage of drug released of SHL coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.37	0.09	0.18	0.21	0.14
	30	0.09	0.06	0.10	0.08	0.02
	60	0.09	0.05	0.08	0.07	0.02
	90	0.08	0.05	0.08	0.07	0.02
	120	0.05	0.04	0.06	0.05	0.01
(buffer pH 6.80)	5	0.00	0.00	0.00	0.00	0.00
	10	0.05	0.05	0.02	0.04	0.02
	15	0.34	0.27	0.20	0.27	0.07
	20	1.08	0.93	0.74	0.92	0.17
	30	2.64	2.42	2.25	2.44	0.20
	45	74.45	28.34	12.66	38.48	32.12
	60	86.12	94.12	94.94	91.73	4.87
	90	93.56	97.49	100.09	97.05	3.29
	120	94.01	99.53	103.75	99.10	4.89
	150	96.22	101.86	105.94	101.34	4.88
	180	98.71	104.46	105.07	102.74	3.51
	210	99.00	106.18	105.79	103.66	4.04
	240	106.39	106.33	105.36	106.03	0.58
	270	105.53	109.45	106.07	107.02	2.13
	300	106.39	109.03	106.22	107.21	1.58
	330	106.96	107.62	105.65	106.74	1.00
	360	103.98	105.38	104.11	104.49	0.78

Table 20 The percentage of drug released of SHL-SUC 1 h AM coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.34	0.45	0.37	0.38	0.06
	30	0.12	0.31	0.20	0.21	0.09
	60	0.14	0.30	0.20	0.22	0.08
	90	0.14	0.30	0.35	0.26	0.11
	120	0.22	0.33	0.37	0.31	0.08
(buffer pH 6.80)	5	1.30	1.12	1.08	1.16	0.12
	10	78.72	63.04	65.31	69.03	8.47
	15	96.46	78.84	78.27	84.52	10.34
	20	98.40	82.28	82.02	87.57	9.39
	30	97.36	86.30	84.86	89.50	6.84
	45	100.03	91.04	86.64	92.57	6.83
	60	100.76	91.48	88.41	93.55	6.43
	90	102.82	94.12	91.35	96.09	5.98
	120	103.69	95.43	92.95	97.36	5.62
	150	104.12	97.46	95.43	99.00	4.55
	180	104.99	96.88	96.15	99.34	4.91
	210	103.84	96.60	98.16	99.53	3.81
	240	104.98	98.88	98.02	100.63	3.80
	270	105.13	98.45	99.87	101.15	3.52
	300	105.27	99.02	100.72	101.67	3.23
	330	105.13	99.30	100.58	101.67	3.06
	360	105.13	99.02	100.30	101.48	3.22

Table 21 The percentage of drug released of SHL-SUC 6 h AM coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.11	0.15	0.16	0.14	0.03
	30	0.10	0.12	0.11	0.11	0.01
	60	0.07	0.11	0.10	0.09	0.02
	90	0.10	0.31	0.09	0.17	0.13
	120	0.09	0.14	0.09	0.10	0.03
(buffer pH 6.80)	5	1.67	1.73	1.45	1.62	0.15
	10	80.85	51.15	57.87	63.29	15.57
	15	101.80	68.06	77.88	82.58	17.35
	20	99.37	75.79	84.72	86.63	11.90
	30	99.97	80.77	89.10	89.95	9.63
	45	101.33	84.67	91.66	92.55	8.37
	60	99.53	88.10	92.71	93.45	5.75
	90	101.77	91.22	97.02	96.67	5.28
	120	103.99	94.62	98.95	99.18	4.69
	150	101.92	96.23	102.78	100.31	3.56
	180	106.33	98.72	104.54	103.19	3.98
	210	104.28	106.43	105.41	105.37	1.07
	240	104.57	105.99	105.56	105.37	0.73
	270	103.56	106.86	106.42	105.61	1.79
	300	105.00	106.71	107.14	106.28	1.13
	330	105.00	105.57	107.85	106.14	1.51
	360	105.28	106.71	108.99	106.99	1.87

Table 22 The percentage of drug released of SHL-SUC 24 h AM coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.11	0.06	0.02	0.06	0.04
	30	0.07	0.01	0.02	0.04	0.03
	60	0.10	0.04	0.04	0.06	0.03
	90	0.06	0.02	0.06	0.05	0.02
	120	0.04	0.05	0.04	0.04	0.01
(buffer pH 6.80)	5	3.77	4.68	33.28	13.91	16.78
	10	77.65	87.06	67.16	77.29	9.95
	15	91.58	96.20	78.34	88.71	9.27
	20	93.24	95.75	81.95	90.31	7.35
	30	95.79	96.65	84.64	92.36	6.70
	45	95.49	95.30	85.39	92.06	5.78
	60	94.01	96.80	86.57	92.46	5.29
	90	96.08	97.39	88.19	93.89	4.97
	120	94.61	100.79	92.73	96.04	4.22
	150	96.65	98.00	94.92	96.52	1.54
	180	95.49	97.70	94.92	96.04	1.47
	210	96.65	97.41	94.20	96.09	1.68
	240	97.51	99.87	96.21	97.86	1.86
	270	96.65	101.31	97.92	98.63	2.41
	300	97.65	100.60	98.49	98.91	1.52
	330	99.06	99.32	100.04	99.47	0.51
	360	100.47	101.30	98.36	100.04	1.52

Table 23 The percentage of drug released of SHL-PHT 1 h AM coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.15	0.41	0.59	0.38	0.22
	30	0.15	0.41	0.62	0.39	0.24
	60	0.18	0.45	0.63	0.42	0.22
	90	0.24	0.44	0.59	0.42	0.18
	120	0.35	0.48	0.64	0.49	0.15
(buffer pH 6.80)	5	0.01	0.01	0.89	0.30	0.51
	10	0.28	0.68	0.89	0.61	0.31
	15	0.41	0.88	0.97	0.75	0.30
	20	1.03	1.37	1.55	1.32	0.26
	30	19.59	75.49	36.25	43.78	28.70
	45	92.76	97.18	87.87	92.60	4.66
	60	96.42	98.37	95.89	96.90	1.31
	90	95.64	97.45	97.09	96.73	0.96
	120	97.97	97.98	98.01	97.99	0.02
	150	96.43	97.98	97.75	97.38	0.84
	180	97.84	99.01	98.93	98.59	0.66
	240	98.35	104.17	99.19	100.57	3.15
	300	97.71	100.20	96.86	98.26	1.73
	360	98.47	99.43	99.05	98.98	0.48

Table 24 The percentage of drug released of SHL-PHT 6 h AM coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.31	0.15	0.07	0.18	0.13
	30	0.24	0.10	0.09	0.14	0.08
	60	0.34	0.13	0.15	0.21	0.12
	90	0.56	0.16	0.19	0.30	0.22
	120	0.65	0.24	0.28	0.39	0.23
(buffer pH 6.80)	5	3.23	1.26	1.45	1.98	1.09
	10	37.04	3.43	3.83	14.77	19.29
	15	73.81	18.62	35.48	42.64	28.28
	20	83.15	59.87	73.67	72.23	11.71
	30	95.54	95.71	97.16	96.14	0.89
	45	96.69	99.57	98.21	98.16	1.44
	60	98.99	100.21	99.65	99.62	0.61
	90	99.12	99.58	99.65	99.45	0.29
	120	99.12	99.45	99.78	99.45	0.33
	150	101.38	100.46	99.52	100.45	0.93
	180	100.38	99.58	101.05	100.34	0.74
	240	101.50	100.45	100.67	100.87	0.55
	300	100.77	100.82	100.67	100.75	0.07
	360	101.74	101.18	99.56	100.83	1.13

Table 25 The percentage of drug released of SHL-PHT 12 h AM coated tablets (initial)

Time (min)		Tab 1	Tab 2	Tab 3	Average	SD
(0.1N HCl)	15	0.12	0.20	0.19	0.17	0.05
	30	0.10	0.17	0.15	0.14	0.04
	60	0.15	0.45	0.22	0.27	0.16
	90	0.21	0.34	0.29	0.28	0.07
	120	0.26	0.39	0.37	0.34	0.07
(buffer pH 6.80)	5	1.84	1.84	2.27	1.98	0.25
	10	7.24	4.83	7.29	6.45	1.41
	15	56.61	22.19	65.36	48.05	22.82
	20	79.38	71.67	97.77	82.94	13.41
	30	91.04	97.00	99.05	95.69	4.16
	45	93.65	98.82	98.34	96.94	2.86
	60	95.46	97.84	100.87	98.06	2.71
	90	96.24	97.84	97.93	97.34	0.95
	120	97.01	97.29	98.21	97.50	0.63
	150	96.62	100.74	96.83	98.06	2.32
	180	98.78	99.23	99.44	99.15	0.34
	240	98.66	100.87	101.36	100.29	1.44
	300	98.53	100.87	100.54	99.98	1.27
	360	101.78	100.06	99.05	100.30	1.38

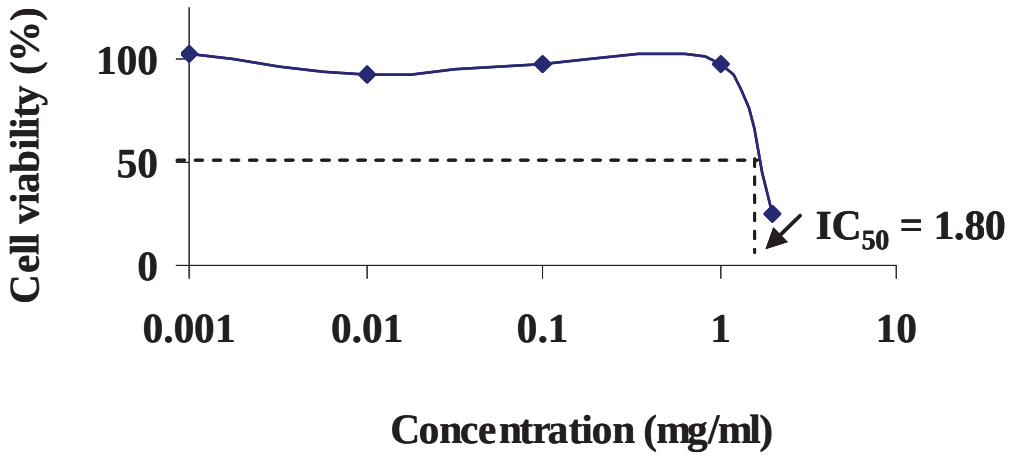


Figure 91 The effect of SHL on cytotoxicity incubated with Caco-2 cells

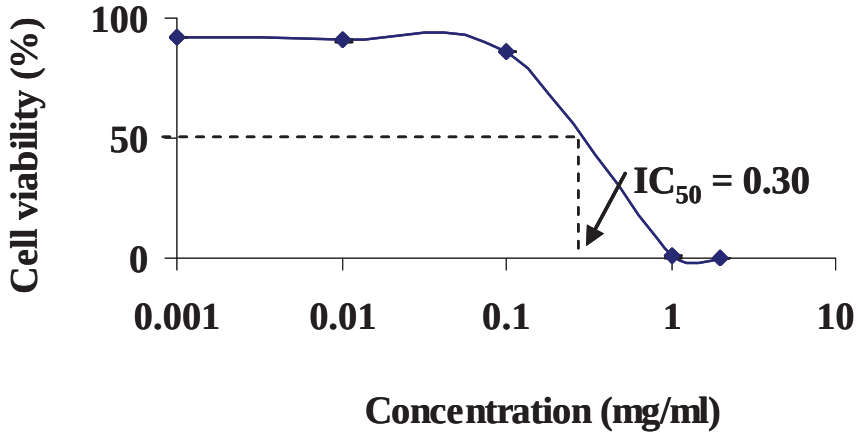


Figure 92 The effect of SHL-SUC 24 h AM on cytotoxicity incubated with Caco-2 cells

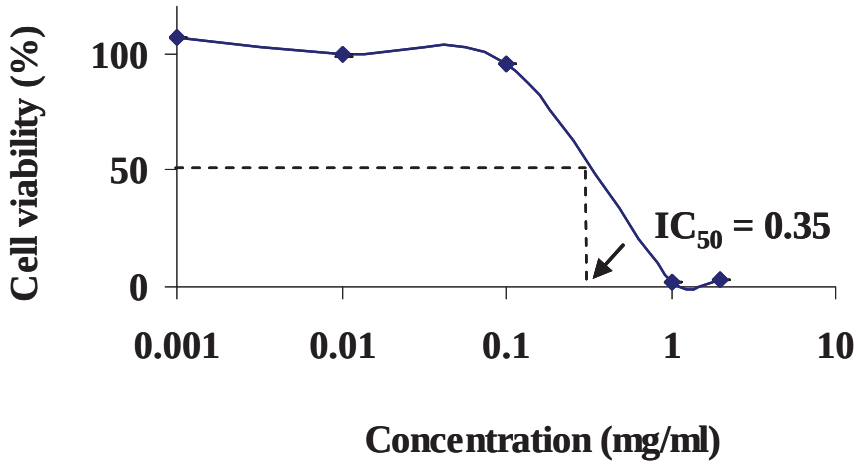


Figure 93 The effect of SHL-PHT 12 h AM on cytotoxicity incubated with Caco-2 cells

Table 26 List of abbreviations

Symbol	Definition
^{o}C	degree Celsius
>	more than
<	less than
%	percent
%IS	percentage od insoluble solid
%w/w	percent weight by weight
^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon nuclear magnetic resonance
AMN	ammonium hydroxide

Table 26 List of abbreviations (continue)

Symbol	Definition
AMP	2-amino-2-methyl-1-propanol
AM	annealing mixture
ANOVA	analysis of variance
AV	acid value
CAHs	cyclic anhydrides
cm^2	square centimeter
cm^3	cubic centimeter
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
FTIR	fourier transform infrared spectroscopy
g	gram
GI	gastro intestinal
GM	ground mixture
HCl	hydrochloric acid
HPLC	high pressure liquid chromatography
HPMC	hydroxypropyl methylcellulose
h	hour
IC_{50}	the half maximal inhibitory concentration
kp	kiloponds
kPa	kiloPascal
kg	kilogram

Table 26 List of abbreviations (continue)

Symbol	Definition
LD_{50}	the median lethal dose
Lot. No.	lot number
mg	milligram
min	minute
mL	milliliter
N	normality
NaCl	sodium chloride
NaOH	sodium hydroxide
NaCl	sodium chloride
nm	nanometer
PEG	polyethylene glycol
PHTA	phthalic anhydride
PM	physical mixture
p	p-value
pH	The negative logarithm of the hydrogen ion concentration
pK_a	The negative logarithm of the dissociation constants
ppm	parts per million
qs. to	add to
R^2	coefficient of determination

Table 26 List of abbreviations (continue)

Symbol	Definition
rpm	revolution per minute
SD	standard deviation
SGF	simulated gastric fluid
SHL	shellac
SHL-PHT	shellac phthalate
SHL-SUC	shellac succinate
SHL-TMT	shellac trimellitate
s	second
T_{50}	the 50% drug released
TMTA	trimellitic anhydride
UV	ultraviolet
UV-VIS	UV visible

BIOGRAPHY

Name Danuch Panchapornpon, Mr.

Address 15/39 Rajamankha Nai Rd., Phra Pathom Chedi, Muang, Nakhon Pathom 73000

Workplace

2004-2006 Pharmacy Department, Mettapracharak Watraikhing Hospital, Nakhonpathom, Thailand

Institution Attended

1999-2004 Silpakorn University: Bachelor of Pharmacy (Honors)

2006 Education in Silpakorn University: Doctor of Philosophy (Pharmaceutical Technology)

Award

2007 Poster Presentation Award from 4th Thailand Pharmacy Congress, Bangkok, Thailand

2008 Poster Presentation Award from Silpakorn University Research Fair 2, Bangkok, Thailand

2009 Travel Grant from 17th Symposium on Microencapsulation, Nagoya, Japan

Refereed journals

1. Limmatvapirat S, Panchapornpon D, Limmatvapirat C, Nunthanid J, Luangtana-anan M, Puttipipatkhachorn S. Formation of shellac succinate having improved enteric film properties through dry media reaction, European Journal of Pharmaceutics and Biopharmaceutics, 2008; 70(1): 335-344.

2. Panchapornpon D, Nunthanid J, Luangtana-Anan M, Limmatvapirat C, Puttipipatkhachorn S, Limmatvapirat S. Investigation of modified shellac as a potential material for enhanced enteric drug delivery of xenobiotic agents. Drug Metabolism Reviews 2009, 41: 183.

3. Panchapornpon D, Limmatvapirat C, Luangtana-anan M, Nunthanid J, Sriamornsak P, Limmatvapirat S. Fabrication of thermally stabilized shellac through solid state reaction with phthalic anhydride, Material letters 2011, 65 : 1241-1244

4. Panchapornpon D, Limmatvapirat C, Nunthanid J, Luangtana-anan M, Sriamornsak P, Puttipipatkhachorn S, Limmatvapirat S. Development of shellac from source available in Thailand as an alternative polymer for postharvest treatment, Thai Journal of Agricultural Science. (*In Press*)

Conference proceedings

1. Limmatvapirat, S. Limmatvapirat, C. Panchapornpon, D. Rangnim, V. Nuntanid, J. Luangtana-anan, M. and Yamamoto, K. Modification of shellac by composite salt formation, Proceeding of the 21st Congress of Federation of Asian Pharmaceutical Associations, Yokohama, Japan, 2006, 18-21 November.

2. Limmatvapirat S, Limmatvapirat C, Panchapornpon D, Nunthanid J, Luangtana-Anan, M, Sittisombat C, Sriamornsak P, Puttipipatkhachorn S. Development of shellac from source available in Thailand as a potential polymer for enteric drug delivery. การประชุมนักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส สกว. 16-18 ตุลาคม 2551. เพชรบุรี

3. Panchapornpon D, Jongjherdsad N, Nunthanid J, Luangtana-anan M, Limmatvapirat C, Puttipipatkhachorn S, Limmatvapirat S., Solid state formation of shellac phthalate having improved enteric property and stability., 22nd Federation of Asian Pharmaceutical Associations Congress (FAPA 2008) 7-10 November 2008. Singapore.
4. Panchapornpon D, Limmatvapirat C, Nuntanid J, Luangtana-anan M, Limmatvapirat S. Synthesis of Shellac Phthalate through Environmental Friendly Reaction. The 2th Silpakorn University Research Fairs (SURF), 18-19 December 2009, Silpakorn University, Bangkok, Thailand.
5. Panchapornpon D, Puttipipatkhachorn S, Nunthanid J, Luangtana-anan M, Limmatvapirat C, Limmatvapirat S. Effect of Molecular Structure of Cyclic Anhydrides on Solid State Formation and Enteric Properties of Shellac Esters. 17th International Symposium on Microencapsulation, Nagoya, Japan, 29 September- 1 October 2009.
6. Sangnim T, Panjapornpon D, Limmatvapirat C, and Limmatvapirat S. Study of factors affecting solid state formation of shellac phthalate. The 3rd Silpakorn University Research Fairs (SURF), 28-29 January 2010, Silpakorn University, Nakhon Pathom, Thailand.
7. Panchapornpon D, Limmatvapirat C, Nuntanid J, Luangtananan M, and Limmatvapirat S. Effect of molecular structure modification on solubility and stability of shellac. The 4th Silpakorn University Research Fairs (SURF), 19-21 January 2011, Silpakorn University, Nakhon Pathom, Thailand.

Conference abstract

1. Panchapornpon, D., Nuntanid, J., Luangtana-anan, M., Sittisombat, C., Puttipipatkhachorn, S., Limmatvapirat, C. and Limmatvapirat, S. Solid-state formation of shellac succinate through mechanical and thermal activation. การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว, Phetchaburi, October 12-14, 2006.
2. Panchapornpon, D., Nuntanid, J., Luangtana-anan, M., Sittisombat, C., Puttipipatkhachorn, S., Limmatvapirat, C. and Limmatvapirat, S. Solid-state formation of shellac polycarboxylate esters through mechanical and thermal activation. RGJ- Ph.D. Congress VIII, Chonburi, April 20-22, 2007.
3. Panchapornpon, D., Nuntanid, J., Luangtana-anan, M., Sittisombat, C., Puttipipatkhachorn, S., Limmatvapirat, C. and Limmatvapirat, S. Formation of shellac polycarboxylate esters through solid-state reaction. The 1st Silpakorn University Research Fair, Nakhon Pathom, November 22, 2007.
4. Panchapornpon, D. Puttipipatkhachorn, S. Nuntanid, J. Luangtana-anan, M. Limmatvapirat, C. and Limmatvapirat, S. Formation of shellac succinate having improved enteric properties through dry media reaction.4th Thailand Pharmacy Congress, Bangkok, December 6-7, 2007. (The most outstanding poster presentation award)
5. Limmatvapirat, S. Limmatvapirat, C. Panchapornpon, D. Nuntanid, J. Luangtana-anan, M. Sittisombat, C. Sriamornsak, P. Puttipipatkhachorn, S. Evaluation of Shellac as a Matrix Forming Material for Sustained Drug Release.การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว ครั้งที่ 8, Phetchaburi, October 16-18, 2008.

6. Panchapornpon D, Nunthanid J, Luangtana-Anan M, Limmatvapirat C, Puttipipatkhachorn S, Limmatvapirat S. Investigation of modified shellac as a potential material for enhanced enteric drug delivery of xenobiotic agents. 3rd Asian Pacific ISSX meeting, The Imperial Queen's Park Hotel, Bangkok, Thailand, May 10-12, 2009.

7. Limmatvapirat, S. Panchapornpon, D. Limmatvapirat, C. Nuntanid, J. Luangtana-anan, M. Sriamornsak, P. Puttipipatkhachorn, S. Effect of Molecular Conformation of Cyclic Anhydrides on Solid State Formation of Shellac Esters and Corresponding Physicochemical Properties.การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว ครั้งที่ 10, Phetchaburi, October 14-16, 2010.

8. Panchapornpon D, Limmatvapirat C, Nuntanid J, Luangtana-anan M, Sriamornsak P, Puttipipatkhachorn S, and Limmatvapirat S. Development of Shellac from Source Available in Thailand as an Alternative Polymer for Postharvest Treatment. International Conference on Agriculture and Agro-Industry (ICAAI2010) Food, Health and Trade, 19-20 November 2010, Mae Fah Luang University, Chiang Rai, Thailand.

9. Panchapornpon D, Limmatvapirat C, Luangtana-anan M, Sriamornsak P, Puttipipatkhachorn S, and Limmatvapirat S. Stability Assessment for Enteric Properties of Shellac Phthalate in Film and Coated tablets. 2nd International Symposium Frontiers in Polymer Science, 29-31 May 2011, Centre de Congrès, Lyon, France

10. Chongcherdsak N, Suriyaampor P, Laksananukul V, Keawchuthamani A, Panchapornpon D, Limmatvapirat C, and Limmatvapirat S. Fabrication of Shellac as Matrix Forming Agent for Floating Drug Delivery System. 2nd

International Symposium Frontiers in Polymer Science, 29-31 May 2011, Centre de Congrès, Lyon, France