รายงานการวิจัย เรื่อง

การพัฒนาอนุพันธ์เชลแล็กเพื่อเป็นสารเคลือบสำหรับการนำส่งยาสู่ลำไส้
Development of shellac derivatives as coating materials for intestinal drug targeting

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กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณสถาบันวิจัยและพัฒนา มหาวิทยาลัยศิลปากร ที่ได้สนับสนุนเงินทุนสำหรับงานวิจัยโครงการนี้ (ประจำปีงบประมาณ 2550) รวมไปถึง สำนักงานกองทุนสนับสนุนการวิจัย สำนักงานคณะกรรมการอุดมศึกษาแห่งชาติ และคณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร ที่ได้สนับสนุนเงินทุนบางส่วน สถานที่ เครื่องมือและอุปกรณ์ต่างๆในการวิจัย

ขอขอบคุณผู้ร่วมวิจัยและผู้ช่วยวิจัยทุกคนที่ได้ช่วยสนับสนุน จนกระทั่งงานวิจัยนี้สำเร็จลุล่วงไปได้ด้วยดี ดังมีรายนามต่อไปนี้

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เซลแล็กเป็นพอลิเมอร์ธรรมชาติที่มีความสามารถในการป้องกันความชื้นและมีความเงางาม จึงถูกประยุกต์ใช้อย่างแพร่หลาย แต่ในปัจจุบันการใช้เซลแล็กเป็นเอนเทอริกพอลิเมอร์ในอุตสาหกรรมยาลดลงอย่างมาก เนื่องจากปัญหาสำคัญสองประการ คือ ปัญหาการละลายในลำไส้เล็ก และปัญหาความคงตัวระหว่างการเก็บรักษา วัตถุประสงค์ของงานวิจัยนี้เพื่อแก้ปัญหาดังกล่าวของเซลแล็กโดยการเตรียมในรูปอนุพันธ์เอสเทอร์ผ่านกระบวนการสังเคราะห์ในภาวะของแข็ง เซลแล็กเอสเทอร์เตรียมด้วยการบดผสมเซลแล็กกับไซคลิกแอนไฮไดรด์ เช่น ซักซินิกแอนไฮไดรด์ ทาลิกแอนไฮไดรด์ และไตรเมลลิติกแอนไฮไดรด์ ร่วมกับการให้ความร้อน ผลการทดลองพบว่าไซคลิกแอนไฮไดรด์โมเลกุลเล็ก (ซักซินิกแอนไฮไดรด์) สามารถเกิดปฏิกิริยากับเซลแล็กได้อย่างมีประสิทธิภาพภายใต้อุณหภูมิที่ต่ำ ในขณะที่ไซคลิกแอนไฮไดรด์ขนาดโมเลกุลที่ใหญ่ขึ้น (ทาลิกแอนไฮไดรด์ และไตรเมลลิติกแอนไฮไดรด์) จำเป็นต้องใช้อุณหภูมิที่สูงขึ้นในการทำปฏิกิริยา ค่าความเป็นกรดของเซลแล็กเอสเทอร์เพิ่มสูงขึ้นเมื่อเพิ่มระยะเวลาในการอบโดยเฉพาะเซลแล็กซักซิเนตและเซลแล็กทาเลต ในขณะที่ปริมาณของแข็งที่ไม่ละลายของเซลแล็กซักซิเนตและเซลแล็กทาเลตมีค่าต่ำกว่า 2% โดยมวล แต่ของแข็งที่ไม่ละลายของเซลแล็กไตรเมลลิเตตมีค่าสูงกว่า 35% โดยมวล ซึ่งแสดงถึงการเกิดเอสเทอริฟิเคชันที่ดีกว่าของซักซินิกแอนไฮไดรด์และทาลิกแอนไฮไดรด์และความล้มเหลวของไตรเมลลิติกแอนไฮไดรด์ในการป้องกันการเสื่อมสภาพของเซลแล็ก การเกิดเซลแล็กเอสเทอร์ถูกยืนยันด้วยเครื่องมือหลายชนิด เช่น เครื่องทดสอบการเลี้ยวเบนรังสีเอกซ์ของผง ดิฟเฟอเรนเชียลสแกนนิงแคลอริมิเตอร์ อินฟราเรดและนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโคปี จากผลการทดลองข้างต้น เซลแล็กซักซิเนตและเซลแล็กทาเลตถูกเลือกมาทดสอบต่อในส่วนคุณสมบัติของฟิล์มและยาเม็ดเพื่อประเมินคุณสมบัติความเป็นเอนเทอริกและความคงตัวต่อ ผลการศึกษาพบว่าฟิล์มเซลแล็กและเซลแล็กเอสเทอร์มีความสามารถในการป้องกันกรดและความชื้นได้ดี ในขณะที่ฟิล์มเซลแล็กเอสเทอร์สามารถละลายได้ดีในพีเอชที่ต่ำลงและมีความยืดหยุ่นที่ดีขึ้นเมื่อเปรียบเทียบกับฟิล์มเซลแล็ก นอกจากนี้ยาเม็ดเคลือบด้วยเซลแล็กซักซิเนตและเซลแล็กทาเลตสามารถละลายในพีเอชของลำไส้เล็กได้รวดเร็วยิ่งขึ้น จากการศึกษาความคงตัว พบว่าเซลแล็กทาเลตมีความคงตัวที่ดีเมื่อเปรียบเทียบกับเซลแล็กอื่น เนื่องจากผลสเตอริกของโครงสร้างอะโรมาติกจากโมเลกุลทาเลตที่สามารถแยกสายโซ่และลดปฏิกิริยาการก่อพอลิเมอร์ภายในโครงสร้างของเซลแล็กได้ กล่าวโดยสรุปคุณสมบัติทางเอนเทอริกและความคงตัวของเซลแล็กสามารถปรับปรุงโดยผ่านกระบวนการที่เป็นมิตรต่อสิ่งแวดล้อมได้

คำสำคัญ: เซลแล็ก / ปฏิกิริยาในสภาวะของแข็ง / ไซคลิกแอนไฮไดรด์ / เอนเทอริกพอลิเมอร์

ABSTRACT

Research Title	Development of shellac derivatives as coating materials for intestinal drug targeting
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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”.

Keyword: Shellac/ Solid-State Reaction/ Cyclic Anhydride/ Enteric Polymer

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List of Abbreviations

$^{\circ}C$	=	degree Celsius
>	=	more than
<	=	less than
%	=	percent
% IS	=	percentage of insoluble solid
% RH	=	percent relatve humidity
% w/w	=	percent weight by weight
δ_C	=	carbon chemical shift
δ_H	=	proton chemical shift
^{1}HNMR	=	proton nuclear magnetic resonance
^{13}C NMR	=	carbon nuclear magnetic resonance
ABS	=	absorbance
AMN	=	ammonium hydroxide
AMP	=	2-amino-2-methyl-1-propanol
AM	=	annealing mixture
ANOVA	=	analysis of variance
AV	=	acid value
CAHs	=	cyclic anhydrides
cm^{-1}	=	inverse centrimeter
cm^{2}	=	square centimeter
cm^{3}	=	cubic centimeter
DMSO	=	dimethyl sulfoxide
DSC	=	differential scanning calorimetry
DT	=	disintegration time
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	=	hours

IC_{50}	=	the half maximal inhibitory concentration
Kp	=	kiloponds
kPa	=	kilopascal
kg	=	kilogram
LD_{50}	=	the median lethal dose
Lot. No.	=	lot number
M	=	molarity
mg	=	milligram
mm	=	millimeter
min	=	minute
mL	=	milliliter
N	=	normality
NaCl	=	sodium chloride
NaOH	=	sodium hydroxide
NaCl	=	sodium chloride
nm	=	nanometer
OD_{550}	=	optical density at 550 mM
PEG	=	polyethylene glycol
PHTA	=	phthalic anhydride
PM	=	physical mixture
PXRD	=	powder X-ray diffraction
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
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
SPSS	=	statistical sackage for the social sciences
s	=	second
T_{50}	=	the 50% drug released
TMTA	=	trimellitic anhydride
USP-NF	=	The United States pharmacopeia and The National Formulary
UV	=	ultraviolet
UV-VIS	=	UV visible
WVP	=	water vapour permeability

Chapter I

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 [1]. 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 [2]. In pharmaceutical industry, shellac has excellent film forming and protective properties. The shellac has been used for sealing, glossing and enteric coating for 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 [3].

Shellac structure comprises mainly of hydroxyl and carboxyl groups [1, 4]. 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 and 6.9, failure of shellac coated tablet 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 [5]. The disintegration time of shellac-coated tablets has been shown to increase dramatically, even when stored at room temperature or elevated temperatures (>70^{O}C) [6, 7]. 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 capsule in simulated intestinal fluid while retained the gastric resistance in 0.1 N hydrochloric acid [8]. 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 [4, 9]. These explained the lower aqueous solubility of shellac. The results demonstrated the solubility of hydrolyzed shellac 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 [4, 10], the stability problem was not yet solved. The polymerization effect still occurred during storage [8]. 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 peak 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 percentage 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 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 percent 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 [11]. 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. [12] 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

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 [13, 14].

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. Most studies of chemical synthesis were achieved with organic solvents; however the toxic organic waste was occurred from the reaction. Solid state reaction is a method based on the idea of green chemistry. 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 chemistry); 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 [15-17]. The research works including nitrones synthesis [18], amorphization [19, 20], methylation [21], amide synthesis [22], and eutectic mixture formation, have been reported [23]. 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.

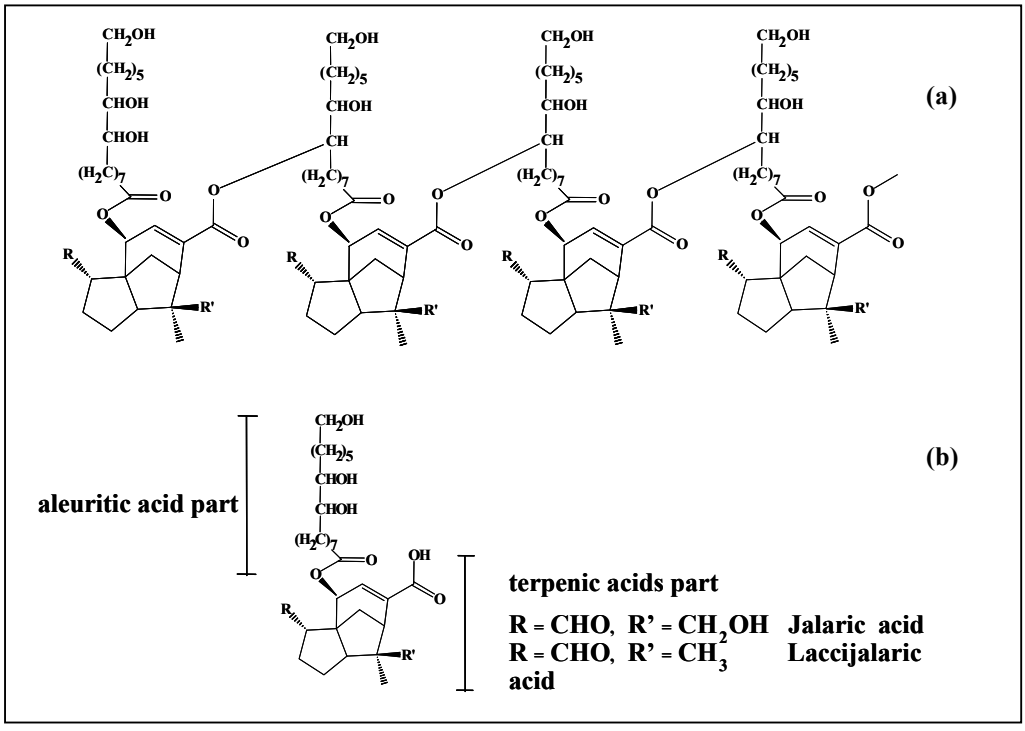

Figure 1 Chemical structure of shellac; polyester (a) and single ester (b)

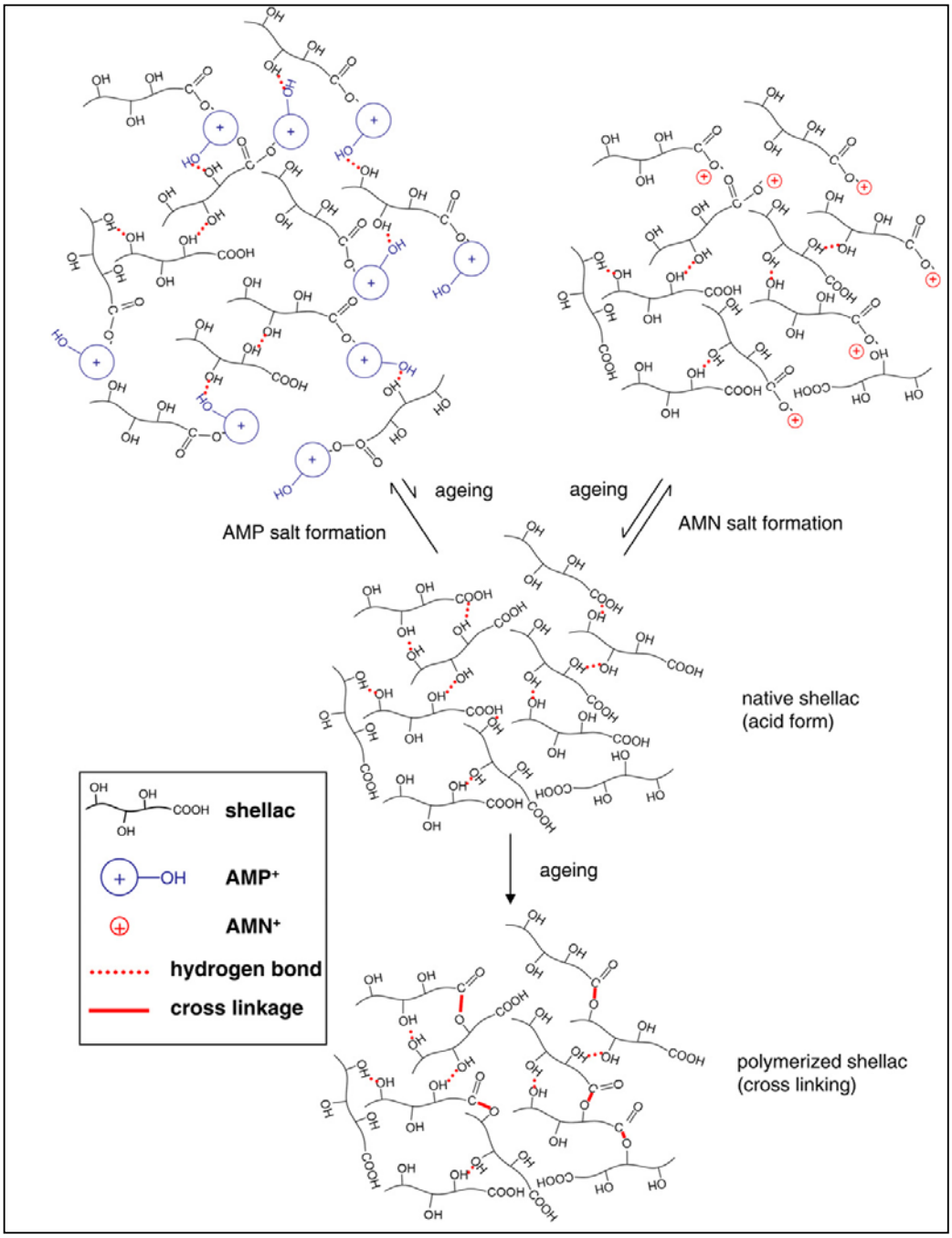

Figure 2 Proposed diagram of salt formation and aging of various forms of shellac

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.

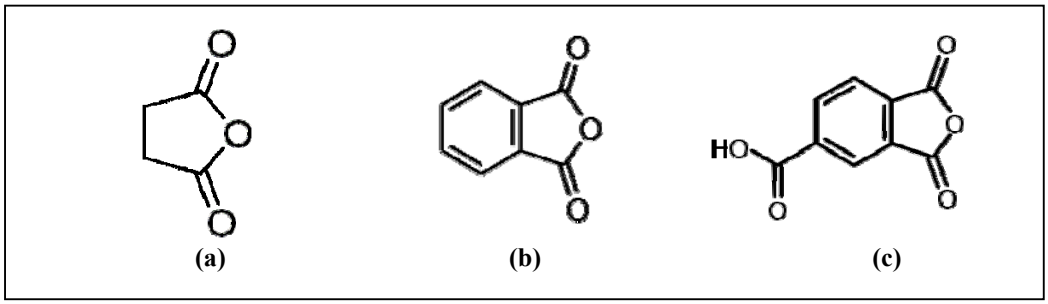

Figure 3 Chemical structure of cyclic anhydrides; succinic anhydride (a), phthalic anhydride (b), and trimellitic anhydride (c)

Chapter II

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. Ethanol 95% grade A
13. Ethyl alcohol absolute (Lot No. J32T04, Mallinckrodt, Malaysia)
14. Calcium chloride anhydrous (Lot No. 7D027277D, Carlo Erba, France)
15. Sodium chloride (Lot No. 0811292, Ajax Pty Ltd, New Zealand)
16. Tri-Sodium phosphate (Lot No. A0121178 008, Merck Darmstadt, Germany)
17. Methanol (Lot No. K36193607 627, Merck Darmstadt, Germany)
18. Acetonitrile (Lot No. I050291 233, Merck Darmstadt, Germany)
19. Methanol-d4 (Lot No. S5033528 939, Merck Darmstadt, Germany)
20. Orthophophoric acid (Lot No. A3B017, Asia Pacific Special Chemical Limited, Australia)
21. Potassium bromide (Lot No. B0351607 905, Merck Darmstadt, Germany)
22. Paracetamol (Lot No. 0135816, Rhodia organique, France)
23. Microcrytalline cellulose (Complecel® M102 D^{+}) (Lot No. 71268, Mingtai chemical, Taiwan)
24. Sodium starch glycolate (Explotab®) (Lot No. E9963, JRS pharma LP, Germany)
25. Lactose monohydrate (Supertab®) (Lot No. FQ010012 T1120, Fonterra, New Zealand)
26. Silicon dioxide (Aerosil® 200) (Lot No. 5042 0110/069, Degussa AG, Germany)
27. Magnesium stearate (Lot No. YW01M0301, Glaxo Wellcome Vidhyasom, Thailand)
28. PEG 400 (Lot No. 1403539, Sigma-Aldrich, Germany)
29. Talcum (Lot No. 000949, Vidhyasom, Thailand)
30. HPMC 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. 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 4). 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.

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 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 performed.

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 $^{\circ}$C for 12 h, and then the percentage of insoluble solid was calculated.

3.2.3 Differential scanning colorimetry (DSC)

The thermogram of shellac sample was determined by differential scanning calorimetry 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 NMRand at 75 MHz for ^{13}C, 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).

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 stabilizes at 25 °C, 75% RH prior to testing.

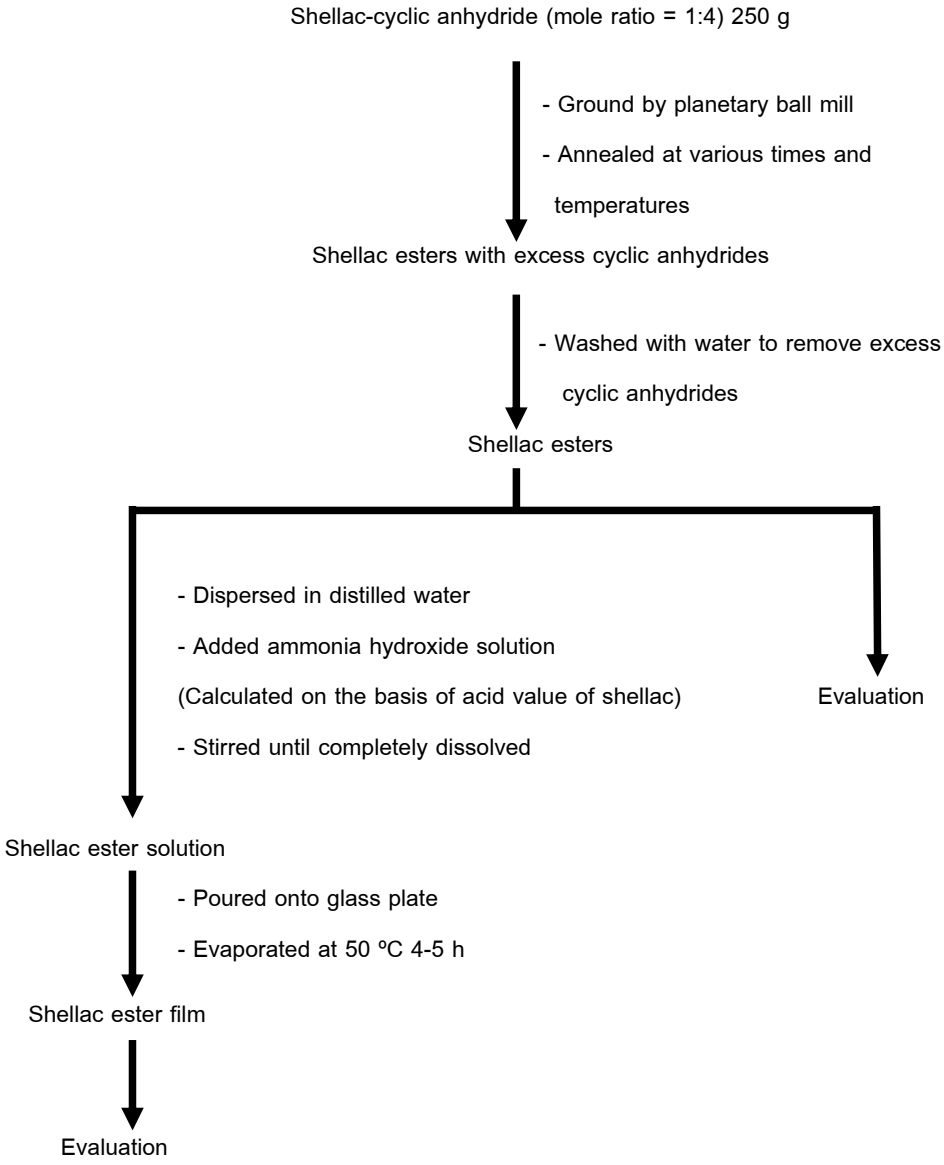

Figure 4 Preparation of shellac esters and their films

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 [24]. 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 (ΔP = 5386.21 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 [4, 11]:

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

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} = $2r\delta$, 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 tablet, paracetamol was selected as the model drug for this study. 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 606	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 (^{o}C)	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 tablet

3.6.1 Weight variation

To study weight variation, 20 tablets of uncoated tablet and coated tablet 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 paracetamol 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^{o}C. The time to achieve disintegration of all 6 coated tablets was recorded.

3.6.5 Dissolution

Dissolution studies of paracetamol coated tablets 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 ± 0.5 °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 6.8 ± 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. The shellac samples were prepared by dissolving in phosphate buffers pH 7.4 at the concentration ranging from 0.001-2 mg/ml. Exposure of celles was strated by replacing of the medium by the shellac solution containing of up to 5 differenct concentrations. After incubation cells with each shellac solution for 24 h, the solution was then removed, and replaced with fresh medium before further incubation for 4 h to stabilize the cells. Finally, the cells were incubated with MTT containing medium (0.1 mg/ml) for 4 h. At the end of incubation period with MTT, the supernatant was removed by inverting and flicking the plate and 100 µl DMSO was added to each well. The trays were then vigorously shaken to dissolve formazan crystals that formed in the living cells and recorded the absorbance value. 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

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

Where $OD_{550,\ sample}$, $OD_{550,\ control}$ and $OD_{550,\ blank}$ 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°C 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 III

RESULTS AND COMMENT

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 [4, 10, 11]. 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 form 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 study of formation of shellac esters. The advantages of this technique were no organic solvent utilization, no organic waste, environmental friendly, easy, and high reactivity [15, 25]. 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 4). 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 5 shows FTIR spectra of SHL-SUC system. In SHL spectrum (Figure 5a), the IR peak at 3400 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 [26]. SUCA (Figure 5b) 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} [27]. The FTIR spectrum of physical mixture of SHL-SUC demonstrated a complete superimposition of the spectra of SHL and SUCA spectra (Figure 5c). 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 5d). 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 5e). As illustrated by star in Figure 5f, the new peaks of SHL-SUC AM were clearly seen 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} was noticed, supporting the succinate formation after grinding and thermal activation.

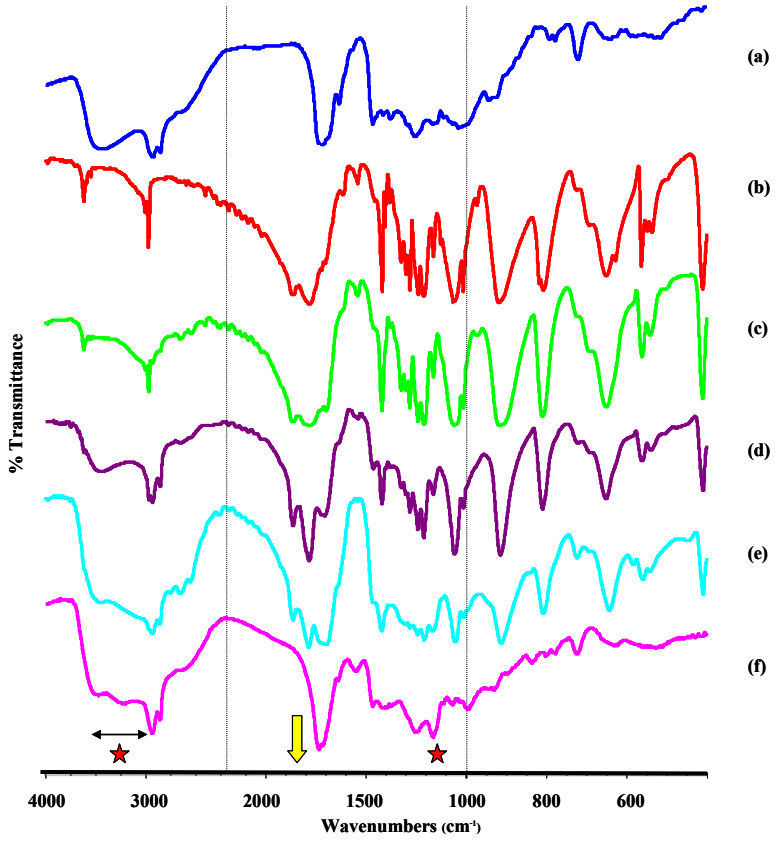

Figure 5 FTIR spectra of SHL-SUC system; SHL (a), SUCA (b), SHL-SUC PM (c), SHL-SUC GM (d), SHL-SUC AM (before washing) (e), and SHL-SUC AM (after washing) (f)

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 6 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 6b). 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 6c 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 6e). Furthermore, the new peaks (as shown by star) were observed and more clearly seen after washing. As compared as SHL and SHL:PHT 1:4 GM, the twin

peak at 1600 cm^{-1} and 1580 cm^{-1} , were due to conjugation of C=O with the aromatic ring from PHTA, were appeared [28]. 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. 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.

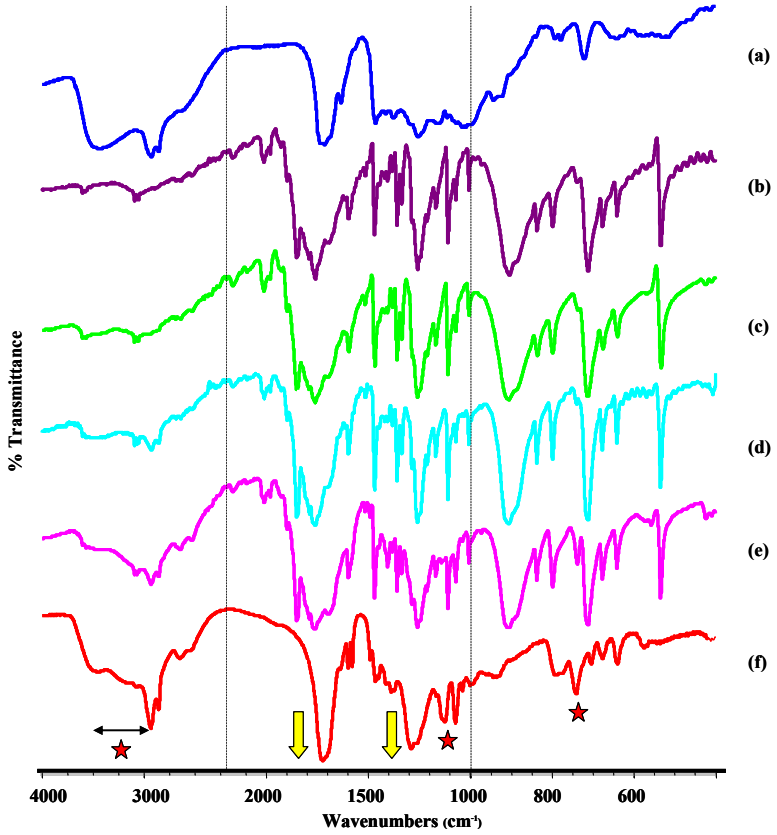

Figure 6 FTIR spectra of SHL-PHT system; SHL (a), PHTA (b), SHL-PHT PM (c), SHL-PHT GM (d), SHL-PHT AM (before washing) (e), and SHL-PHT AM (after washing) (f)

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 system.

As illustrated in figure 7, TMTA demonstrated the characteristic peaks of carbonyl stretching at 1864, 1778 and 1720 cm^{-1} (Figure 7b). 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 7c and 10d). SHL-TMT AM (before washing) did not clearly show the new peak (Figure 7e). After removing excess TMTA (Figure 7f), 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 may occurred. However, the intensity of new peaks was very low peaks, the data demonstrated that the less esterification of SHL-TMT system might be occurred as compared to SHL-SUC and SHL-PHT system 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.

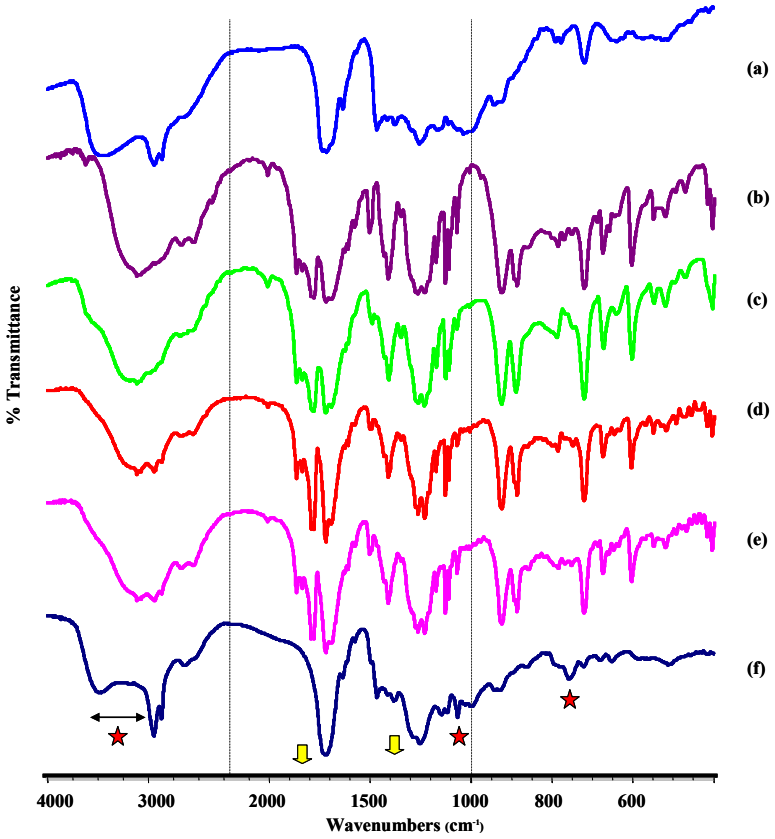

Figure 7 FTIR spectra of SHL-TMT system; SHL (a), TMTA (b), SHL-TMT PM (c), SHL-TMT GM (d), SHL-TMT AM (before washing) (e), and SHL-TMT AM (after washing) (f)

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 5). 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 [29-31]. Figure 8 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 [32, 33], cellulose [34, 35] and starch [36]. 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 [5]. The percent insoluble solid of SHL-SUC AM during annealing process is illustrated in Figure 8 (line bar), 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.

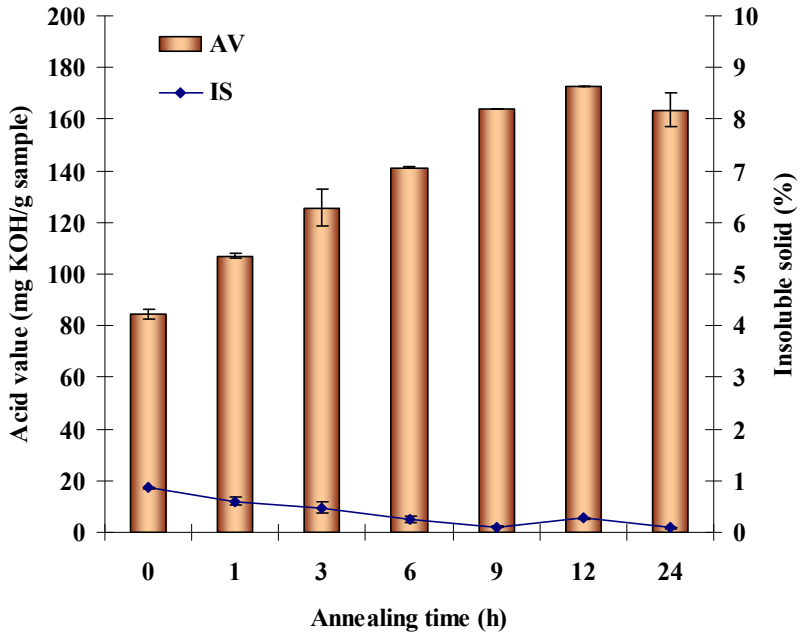

Figure 8 Acid value and insoluble solid of SHL-SUC AM after annealing at 60 ^{O}C for various times

2.1.1.2 Fourier transformed infrared (FTIR) spectroscopy

Figure 9 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 10 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 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 11.

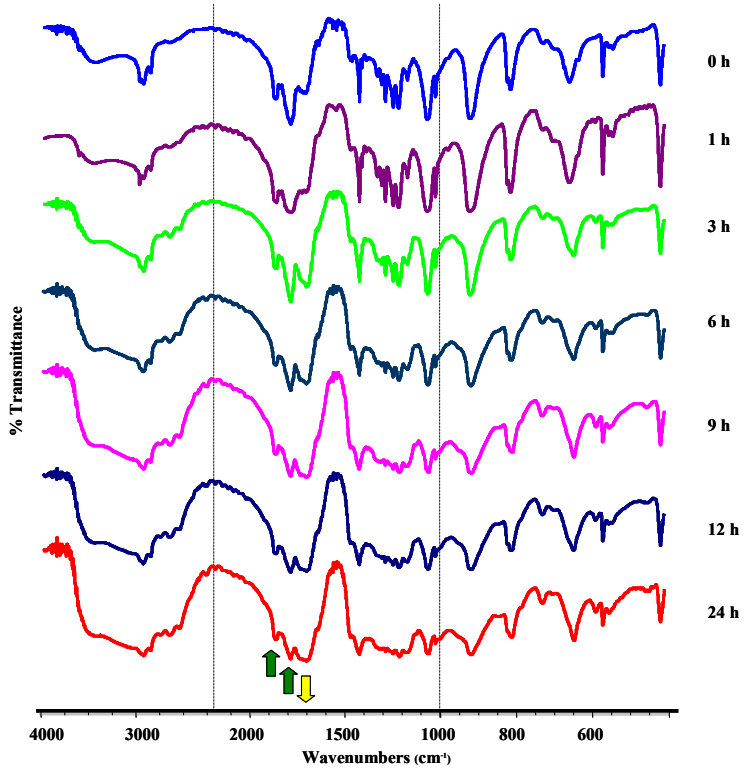

Figure 9 FTIR spectra of SHL-SUC AM after annealing at 60 ºC for various times (before washing)

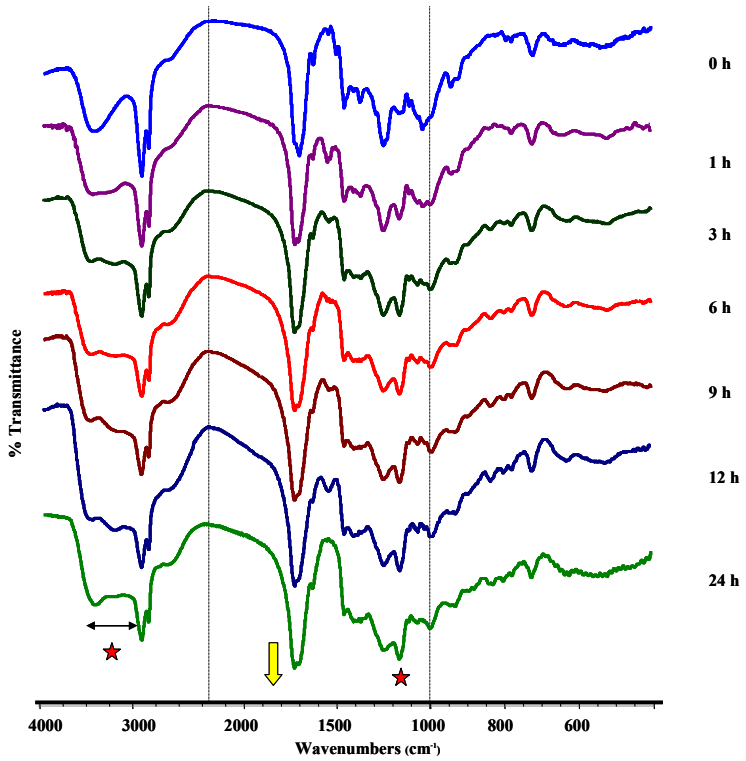

Figure 10 FTIR spectra of SHL-SUC AM after annealing at 60 ºC for various times (after washing)

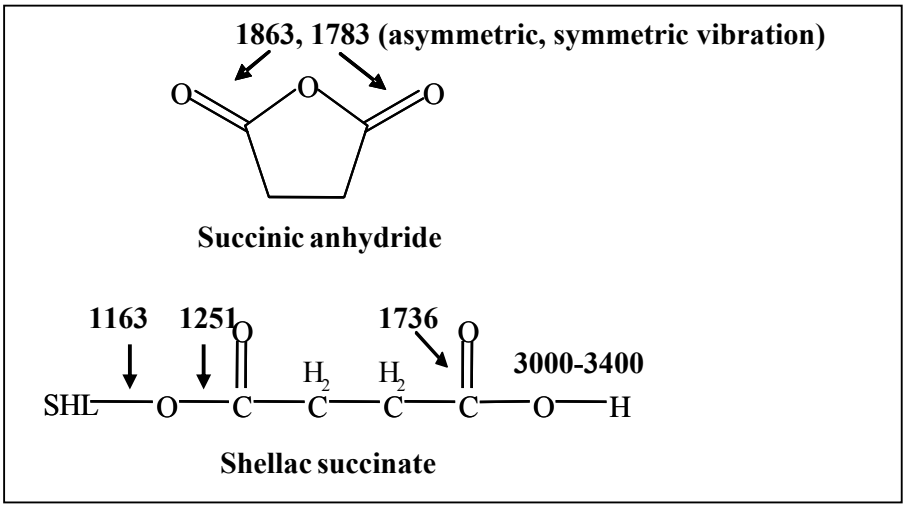

Figure 11 FTIR peak assignment of succinic anhydride and shellac succinate

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 12 shows DSC curves of SHL-SUC AM after annealing at 60 °C for various times (before washing). SHL-SUC AM 0h 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 9).

The DSC curves of SHL and the washed SHL-SUC AM samples are shown in Figure 13. 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.

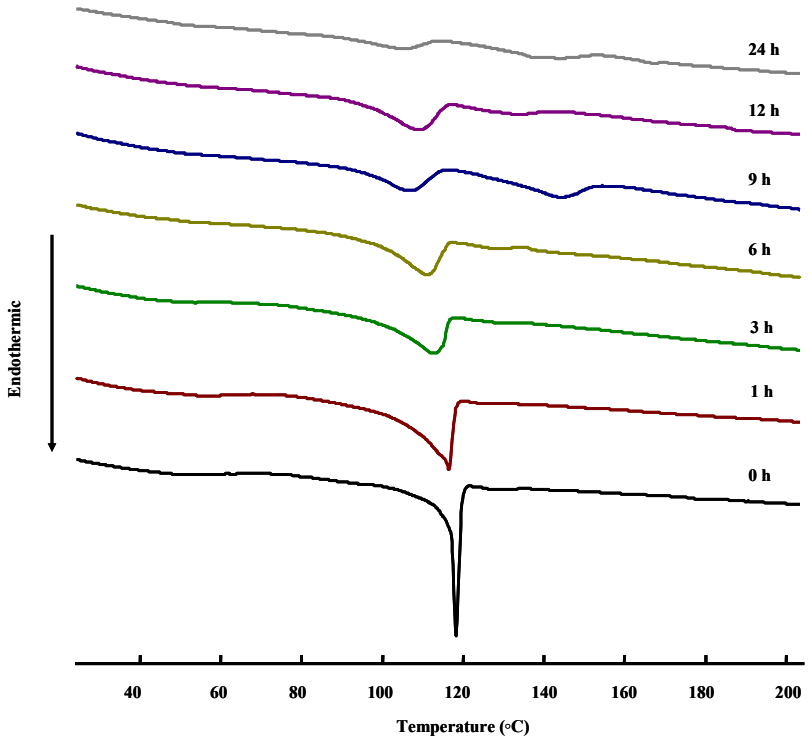

Figure 12 DSC curves of SHL-SUC AM after annealing at 60 °C for various times (before washing)

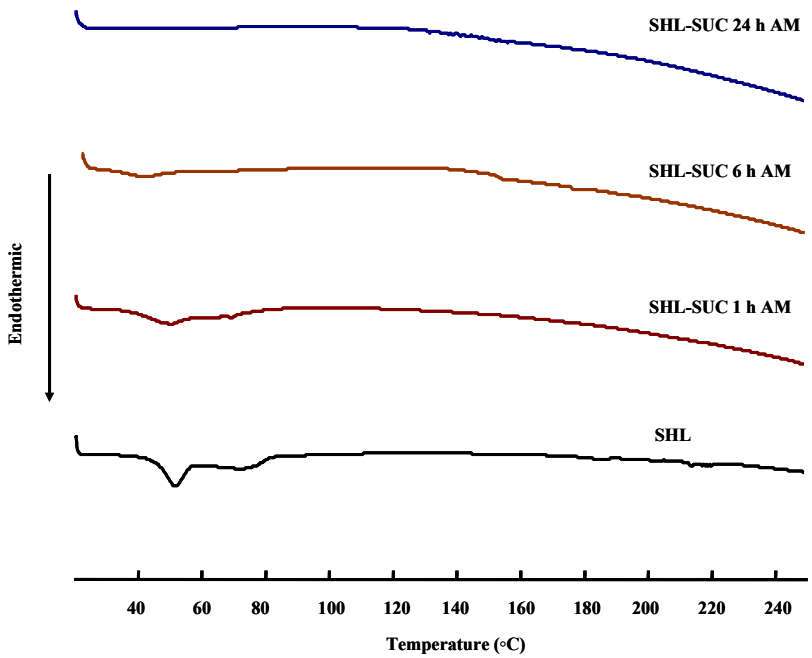

Figure 13 DSC curves of SHL, SHL-SUC AM (after washing)

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 13 shows the PXRD patterns of SHL and SHL-SUC system (before washing). A halo pattern of SHL was clearly observed because of its amorphous phase (Figure 14a). The PXRD patterns of SHL-SUC PM (Figure 14) and SHL-SUC GM (Figure 14d) were the superimposition of PXRD patterns of SHL and SUCA in which the intensity of diffraction peaks of SHL-SUC GM was lower. With increase of annealing time, the intensity of diffraction peaks of SUCA gradually decreased (Figure 14e-14g), 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 15. 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.

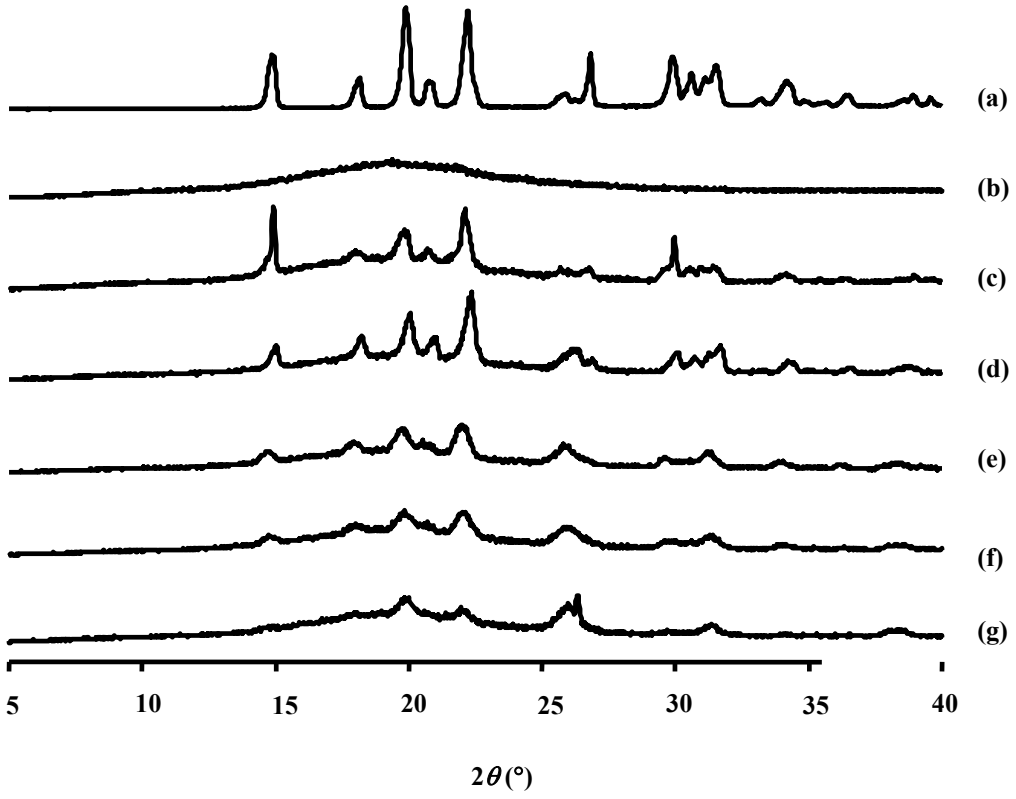

Figure 14 Powder X-ray diffraction pattern of SHL-SUC system before washing: SUCA (a), SHL (b), SHL-SUC PM (c), SHL-SUC GM (d), SHL-SUC 1 h AM (e), SHL-SUC 6 h AM (f) and SHL-SUC 24 h AM (g)

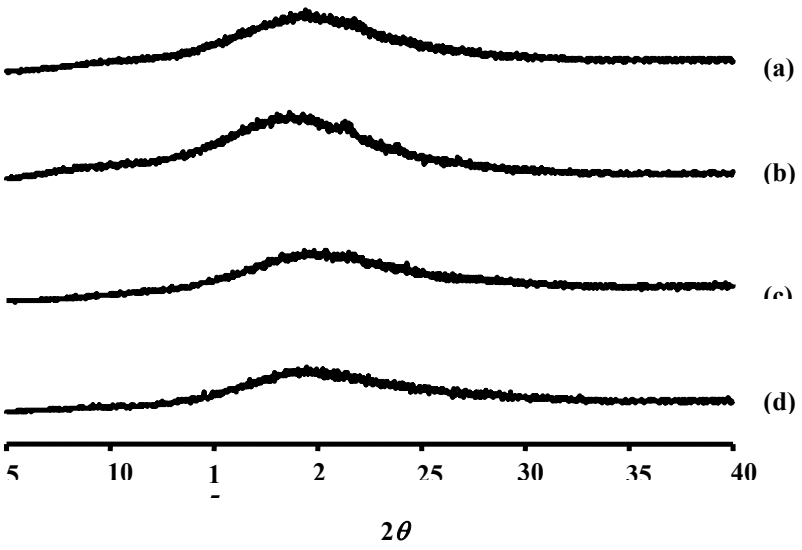

Figure 15 Powder X-ray diffraction pattern of SHL-SUC system after washing: SHL (a), SHL-SUC 1 h AM (b), SHL-SUC 6 h AM (c) and SHL-SUC 24 h AM (d)

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 [37, 38].

To confirm the esterification between SHL and SUCA, the ^{1}H NMR spectrum of SHL-SUC 24 h AM (Figure 16c) was recorded and compared with SHL (Figure 16a). 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 [39]. The finding was also supported by ^{13}C NMR (Figure 17), 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.

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.

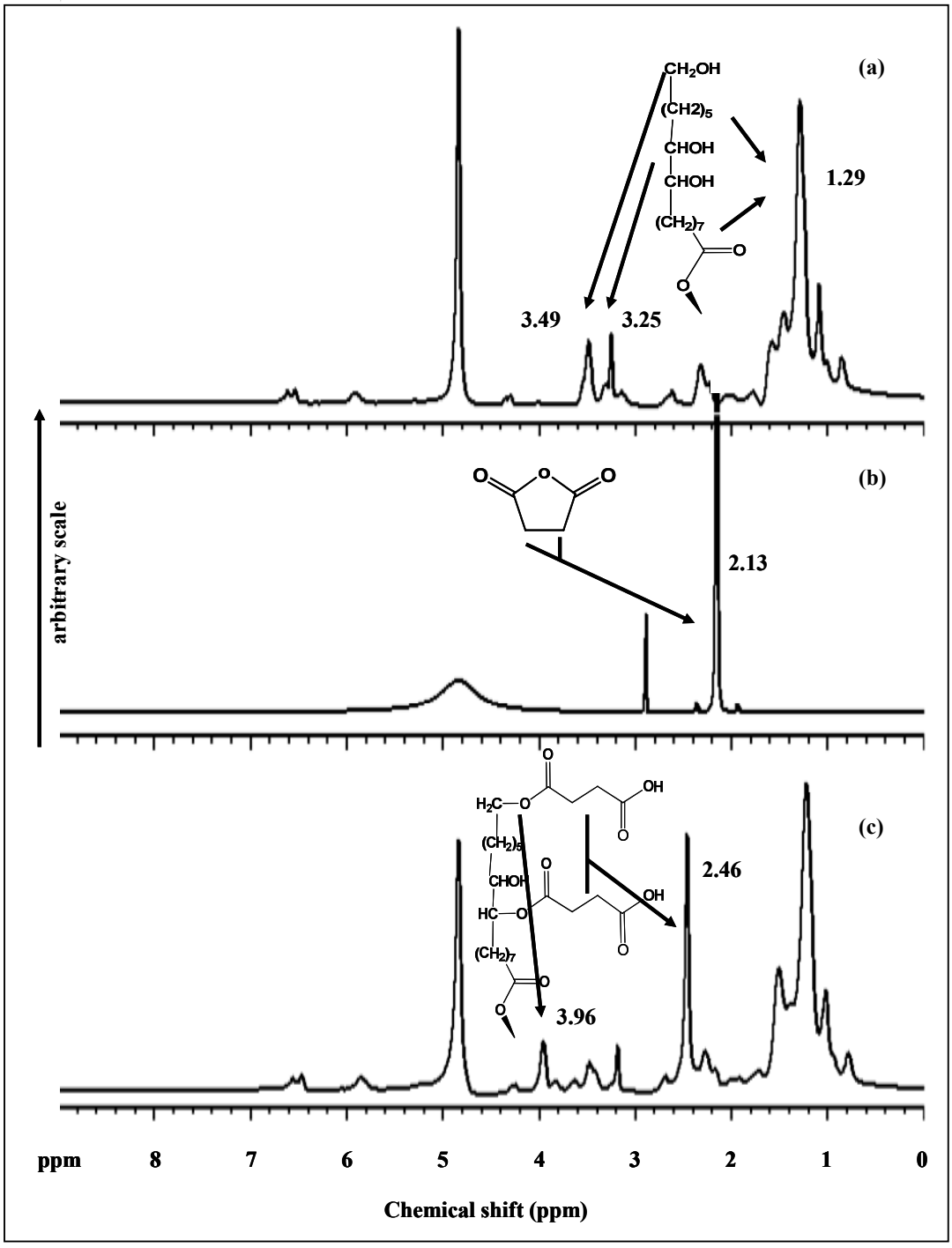

Figure 16 ^{1}H NMR of SHL (a), SUCA (b) and SHL-SUC 24 h AM (c)

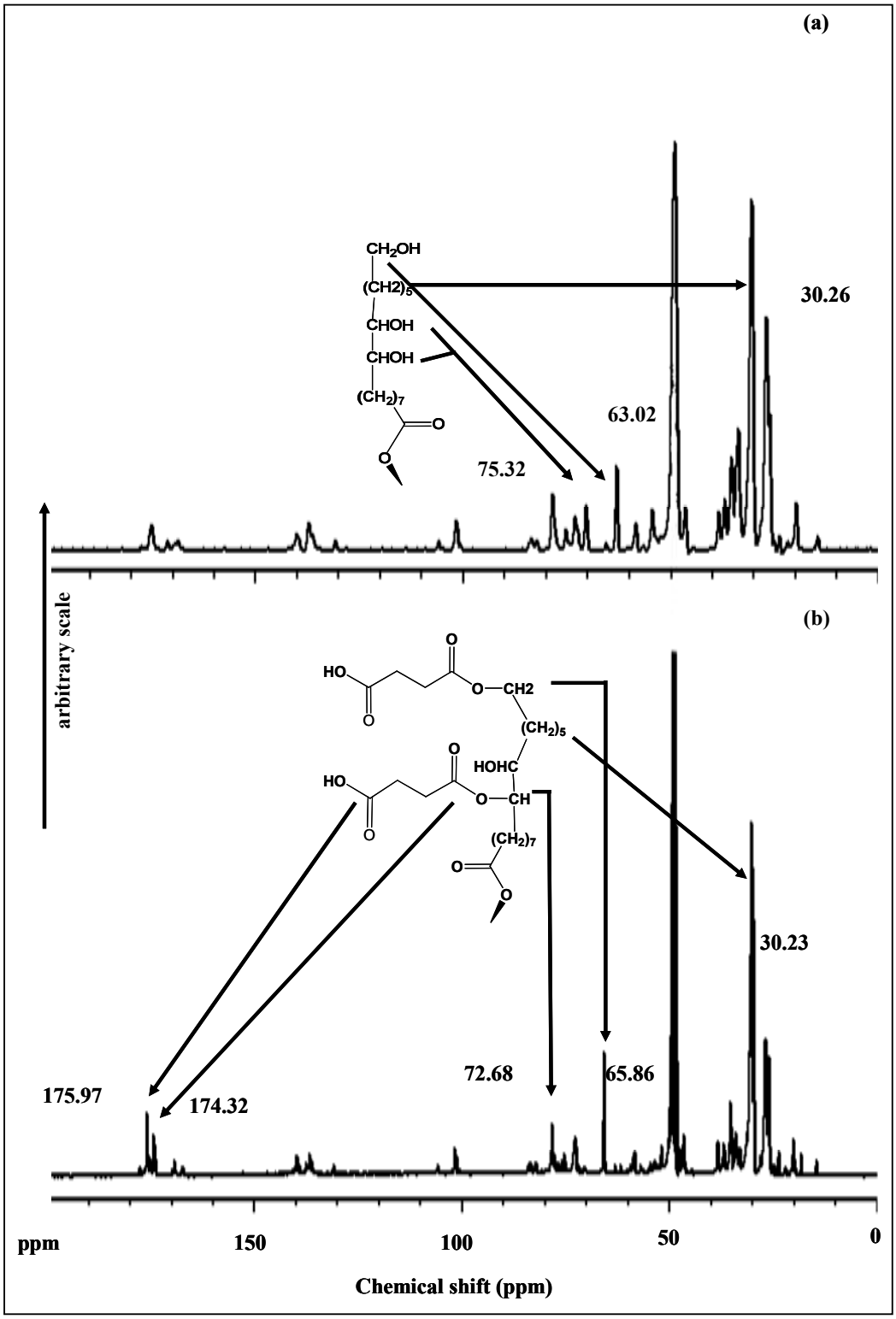

Figure 17 ^{13}C NMR of SHL (a) and SHL-SUC 24 h AM (b)

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 native shellac. 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 [40, 41], several approaches including adding organic acid [8], plasticizer [40, 42] and hydrophilic polymer [43], 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 18. The modulus at puncture of SHL showed the highest value about 243 kPa. The modulus was gradually decreased by prolonging annealing time (Figure 18a). 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 18b). 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 [44]. In addition, several natural polymers were investigated e.g. chitosan [45], oat starch [46], gelatin [47]. 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 [44, 48]. 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.

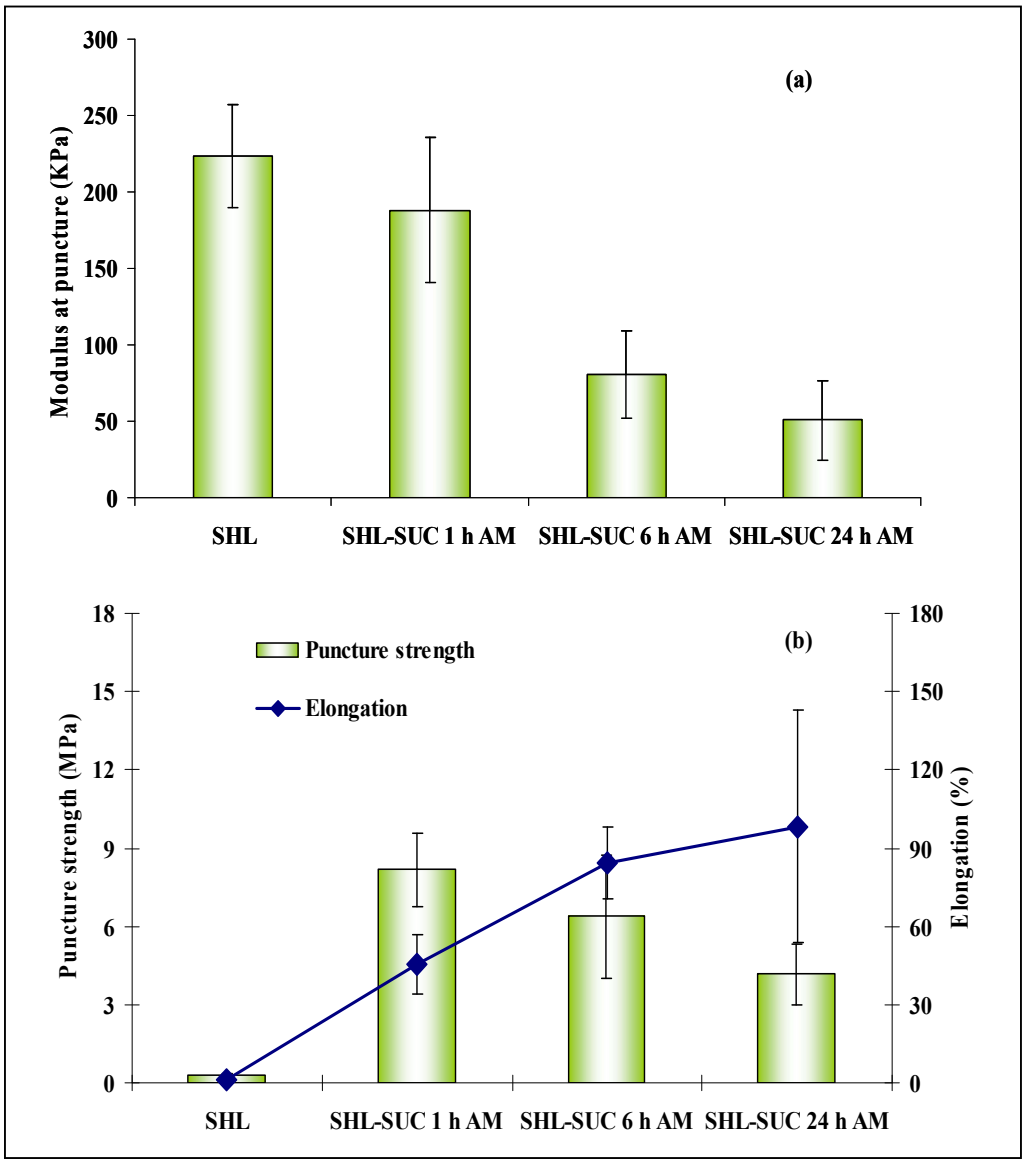

Figure 18 Mechanical properties of film prepared from SHL-SUC: modulus at puncture (a), puncture strength and percentage of elongation (b)

2.1.2.3 Water vapor permeability

Shellac is one of the effective coating barriers against the passage of water vapor [49] 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 19 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 coefficient of all SHL-SUC films were still in a low value as compared to several other enteric polymers such as Eudragit® [50], cellulose acetate phthalate [51].

The more hydrophilicity of carboxylic groups from succinate moieties might promote the diffusion of water vapor through the film. The results were agreed well 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 [52]. The adding of hydrophilic plasticizer could increased water penetration of Eudragit® [53], ethyl cellulose [54] and some protein [44].

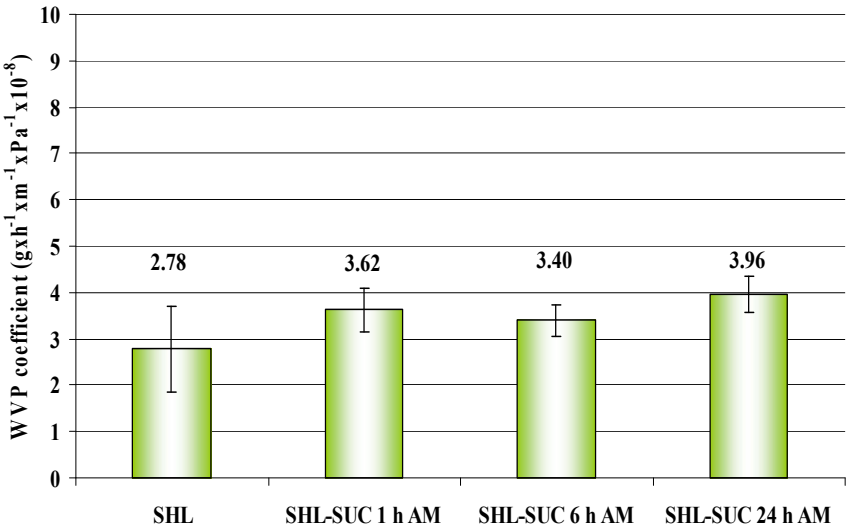

Figure 19 WVP coefficient of SHL and SHL-SUC AM system

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 profile 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 percentage

dissolved of films prepared from SHL-SUC in SGF was slightly higher as compared to SHL. The percentage dissolved exhibited low value (less than 5%), suggesting the gastric resistance of SHL-SUC was still in an acceptable range (Figure 20).

Figure 21 shows the percentage dissolved of all shellac film after soaking in buffer solution at various pH values. The results demonstrated that the percentage 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 22). 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 [33, 55-57].

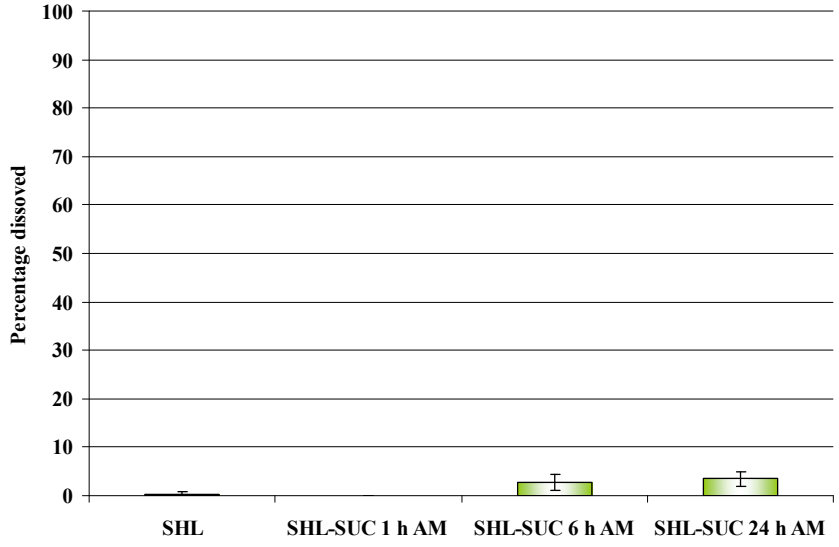

Figure 20 Percentage dissolved of SHL and SHL-SUC in SGF

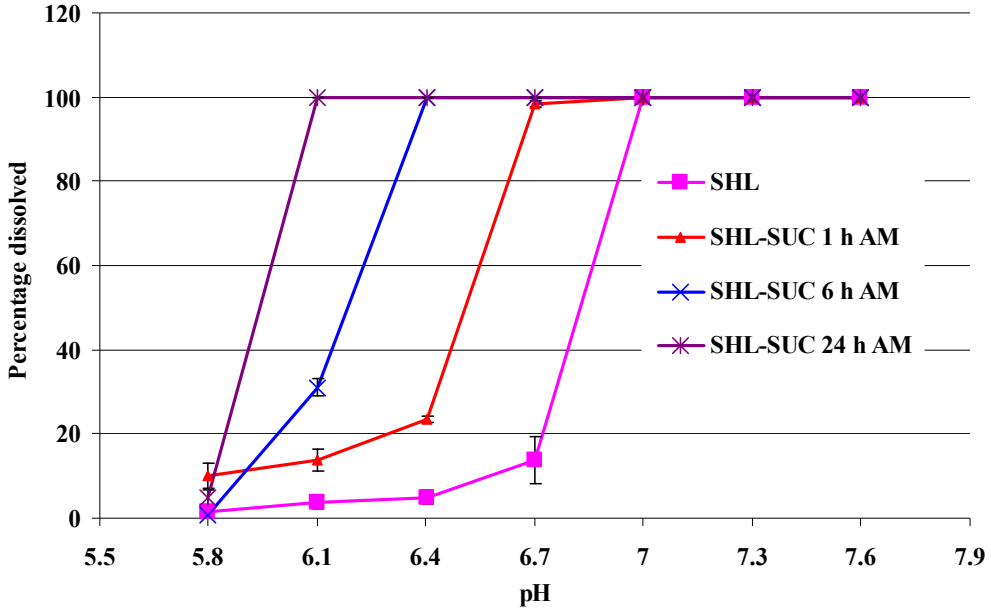

Figure 21 pH solubility profile of SHL and SHL-SUC

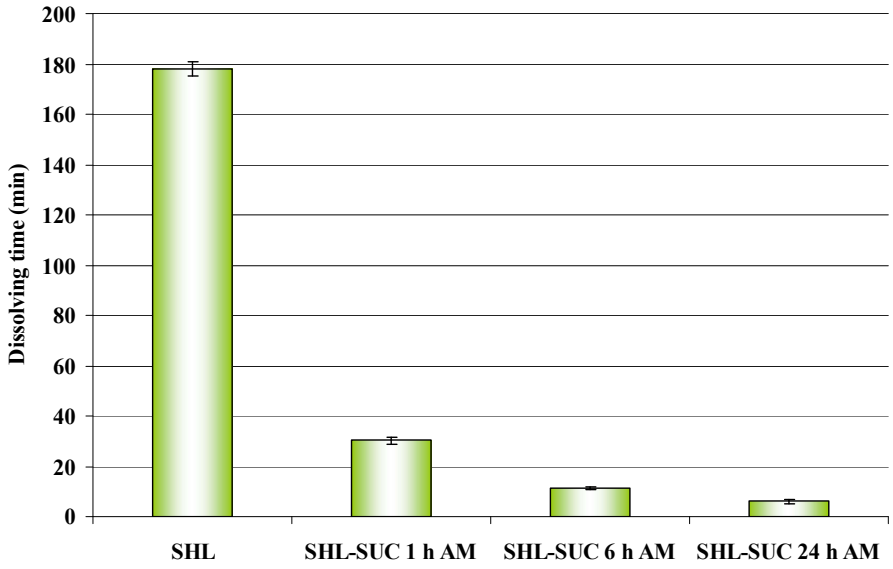

Figure 22 Dissolving time of SHL and SHL-SUC in buffer pH 7.0

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 6. 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.

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 23 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.

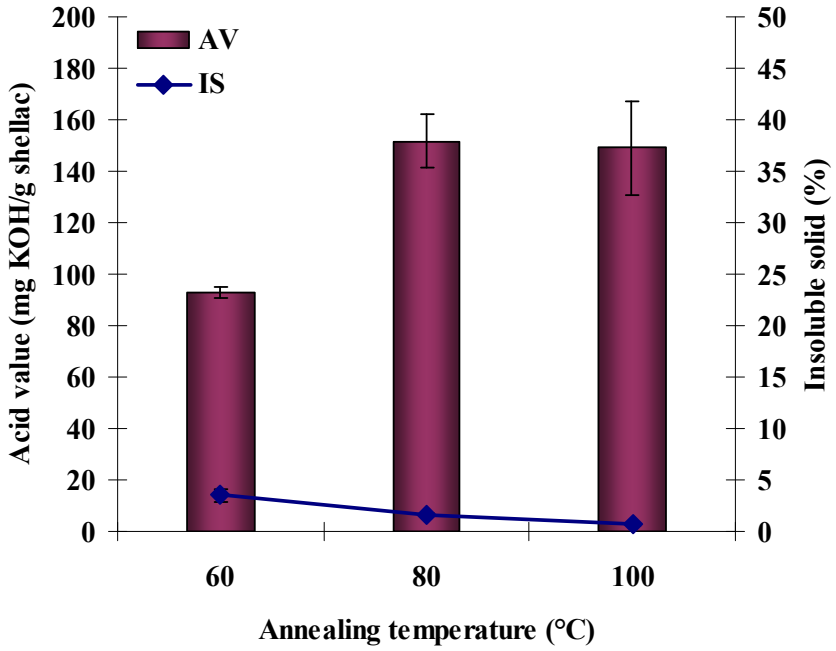

Figure 23 Effect of annealing temperature on acid value and insoluble solid of SHL-PHT AM (after washing)

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 24 illustrates the effect of annealing time on acid value (bar chart) and percent alcohol insoluble (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 to 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.

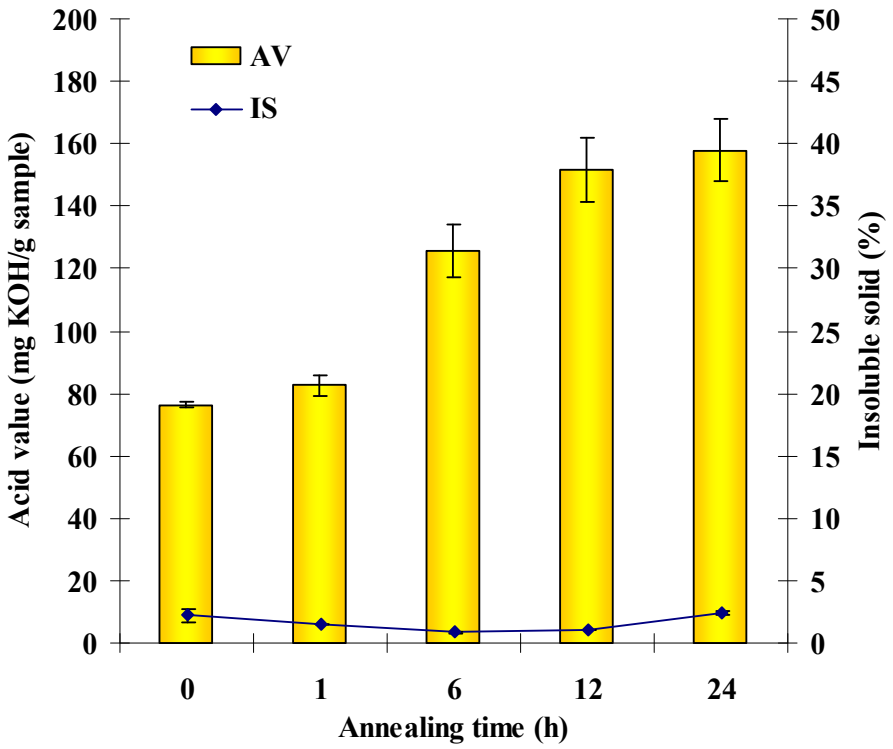

Figure 24 Acid value and insoluble solid of SHL-PHT AM after annealing at 80 ºC for various times

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 24). 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 various times are demonstrated in Figure 25. 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 26). In addition, the different O-H stretching band in the range of 3000-3400 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 27 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 53 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 25), PHTA should be utilized for esterification during thermal activation process.

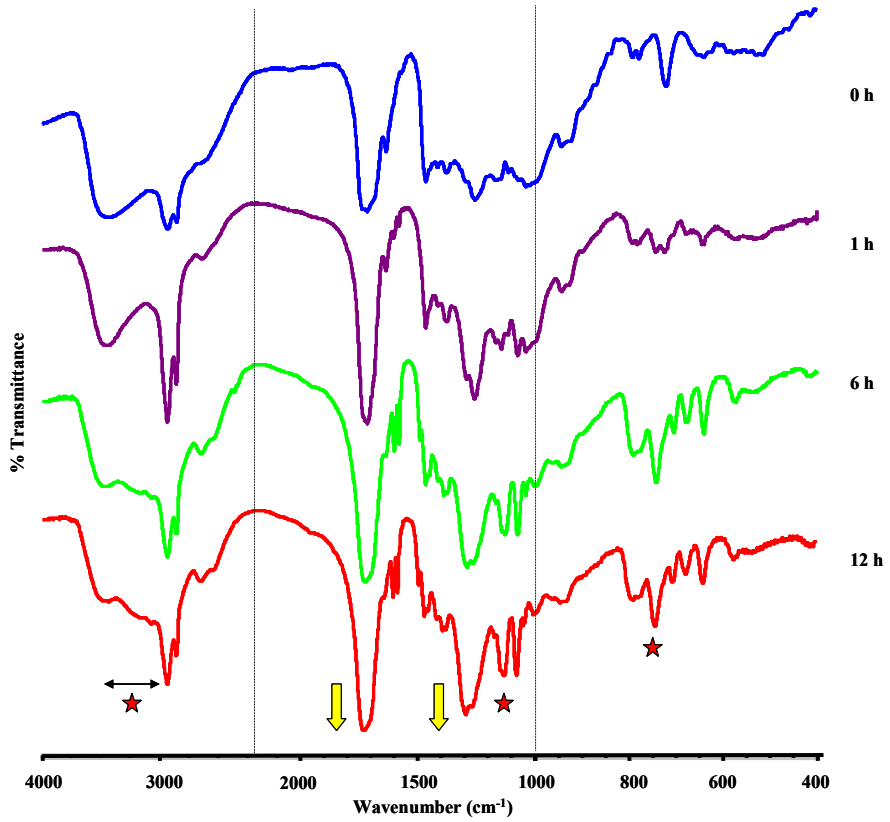

Figure 25 FTIR spectra of SHL-PHT AM after annealing at 80 ºC for various times (after washing)

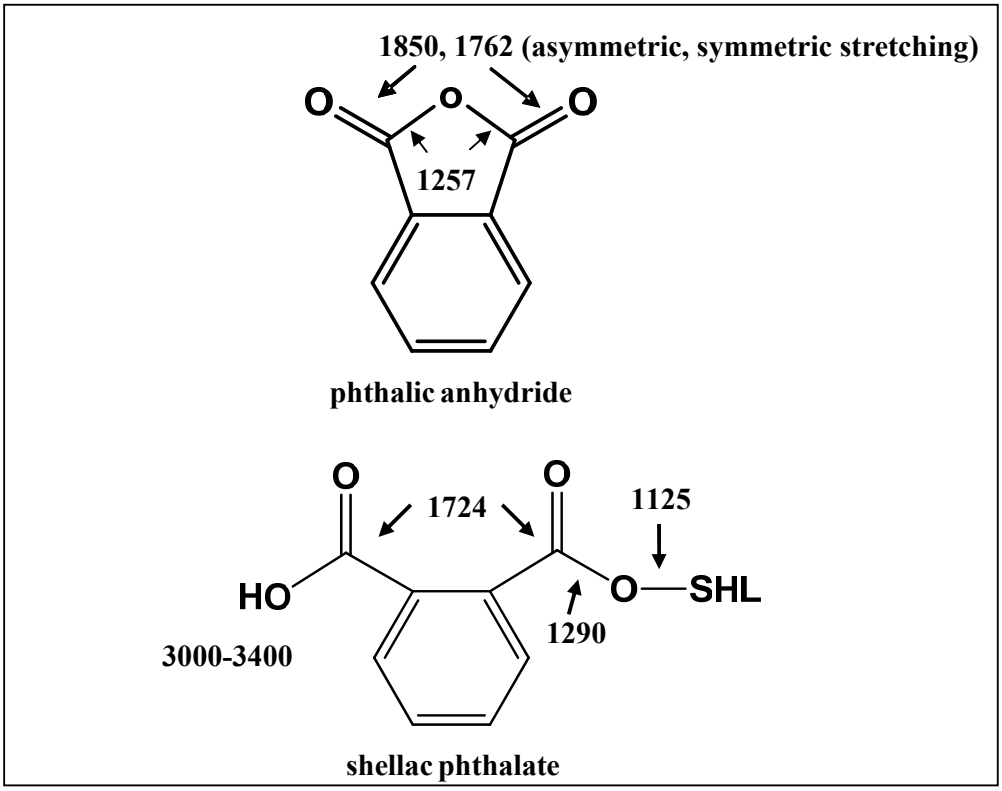

Figure 26 FTIR peak assignment of phthalic anhydride and shellac phthalate

The DSC curves of SHL-PHT AM washed samples are displayed in Figure 28. SHL showed an endothermic peak at 51 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.

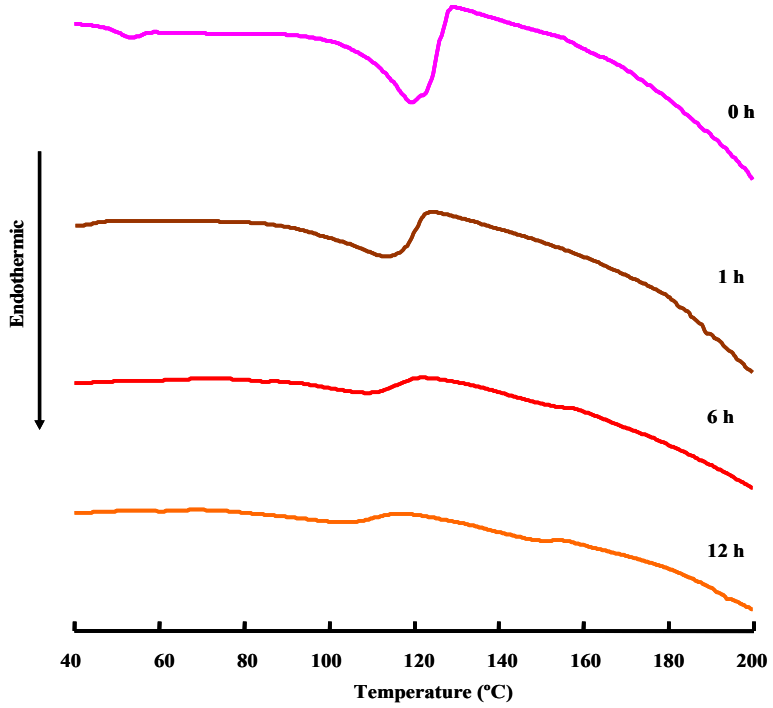

Figure 27 DSC curves of SHL-PHT AM after annealing at 80 °C for various times (before washing)

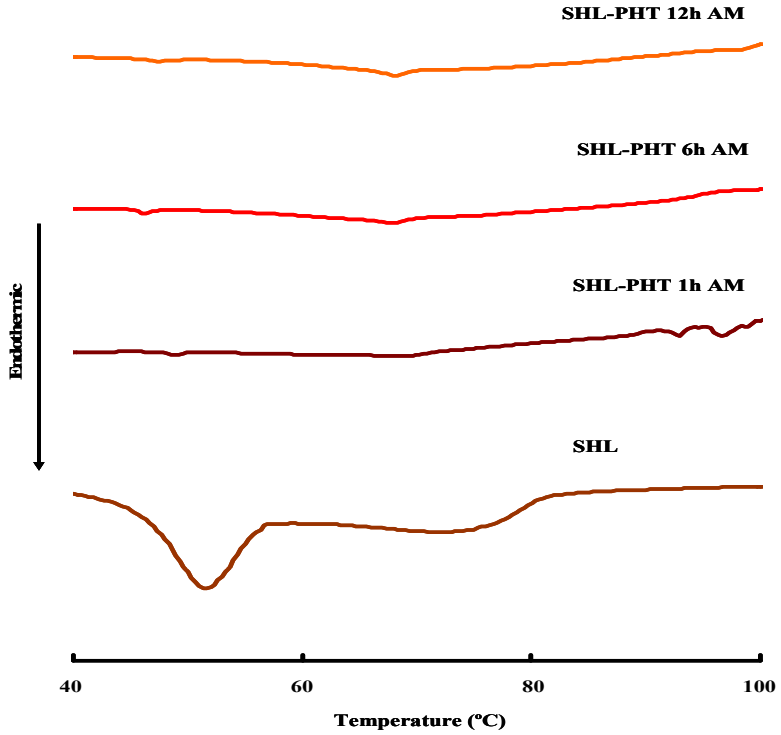

Figure 28 DSC curves of SHL and SHL-PHT AM after washing.

2.2.1.3 Powder X-ray diffraction

PXRD patterns of SHL and SHL-SUC system (before washing) are shown in Figure 29. SHL demonstrated a very broad peak (halo pattern) (Figure 29a). The PXRD patterns of SHL-PHT PM (Figure 29c) and SHL-PHT GM (Figure 29d) were the combination patterns of SHL and PHTA. The PXRD pattern of PHTA was decreased as increased annealing time (Figure 29e-29g), 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 30).

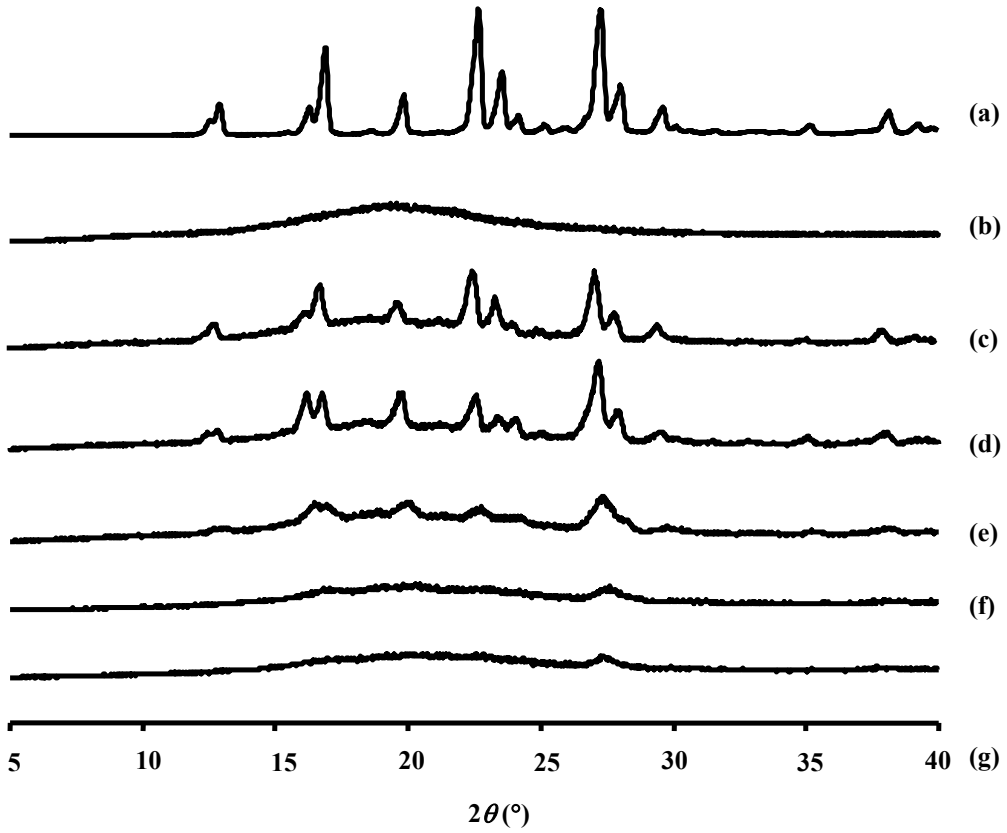

Figure 29 Powder X-ray diffraction pattern of SHL-PHT system before washing: PHTA (a), SHL (b), SHL-PHT PM (c), SHL-PHT GM (d), SHL-PHT 1 h AM (e), SHL-PHT 6 h AM (f) and SHL-PHT 12 h AM (g)

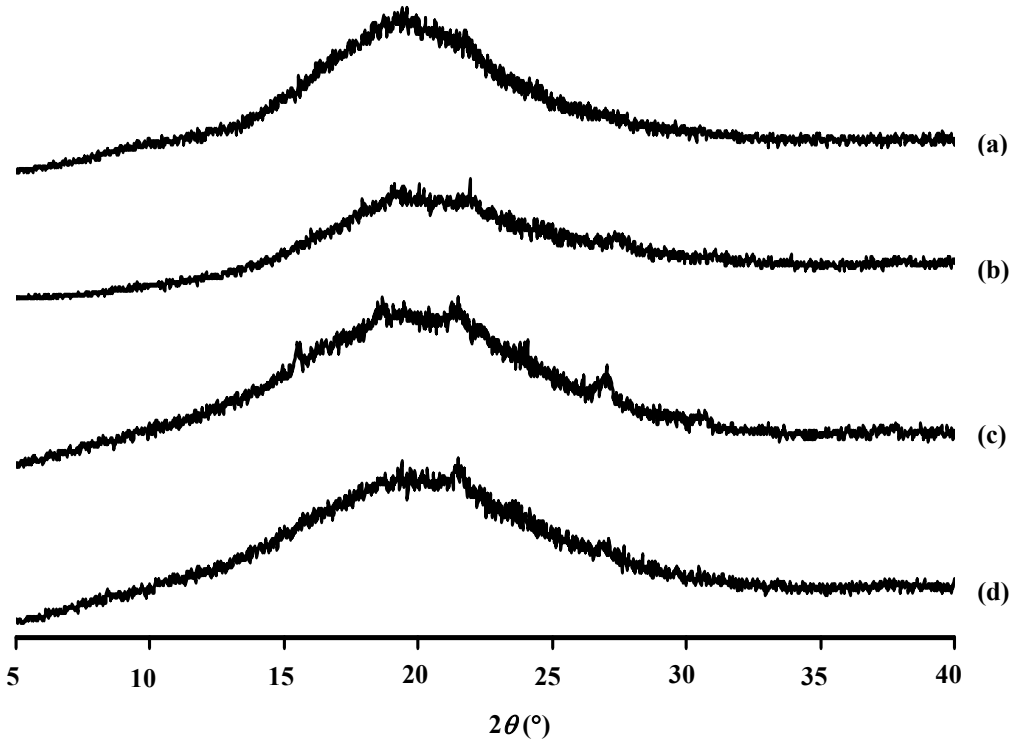

Figure 30 Powder X-ray diffraction pattern of SHL-PHT system after washing: SHL (a), SHL-PHT 1 h AM (b), SHL-PHT 6 h AM (c) and SHL-PHT 12 h AM (d)

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 were comparatively evaluated with SHL as shown in Figure 31 (^{1}H NMR spectra) and Figure 32 (^{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 31a). 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 31c). 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 32b). The data confirmed shellac phthalate formation.

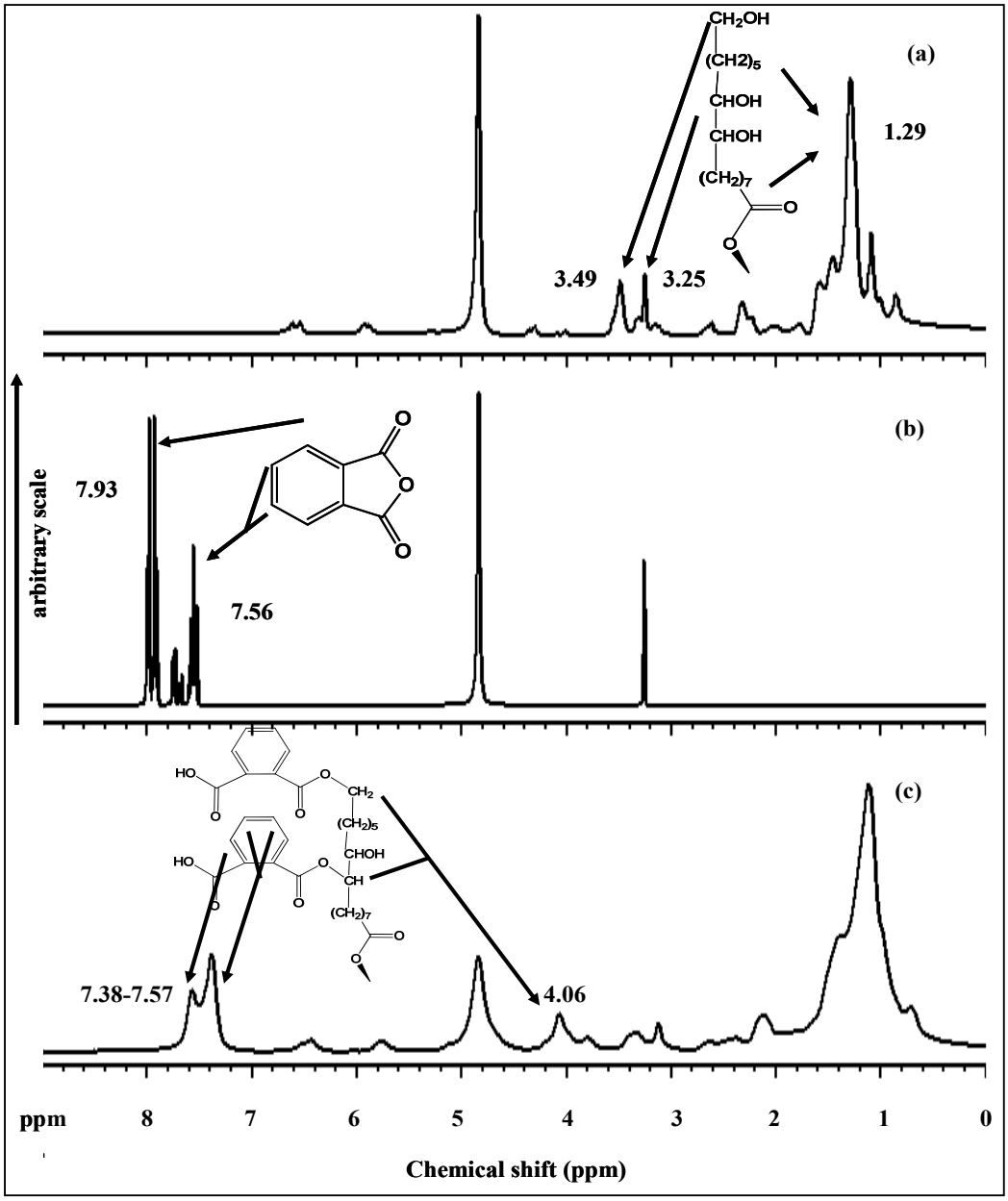

Figure 31 ^{1}H NMR of SHL (a), PHTA (b) and SHL-PHT 12 h AM (c)

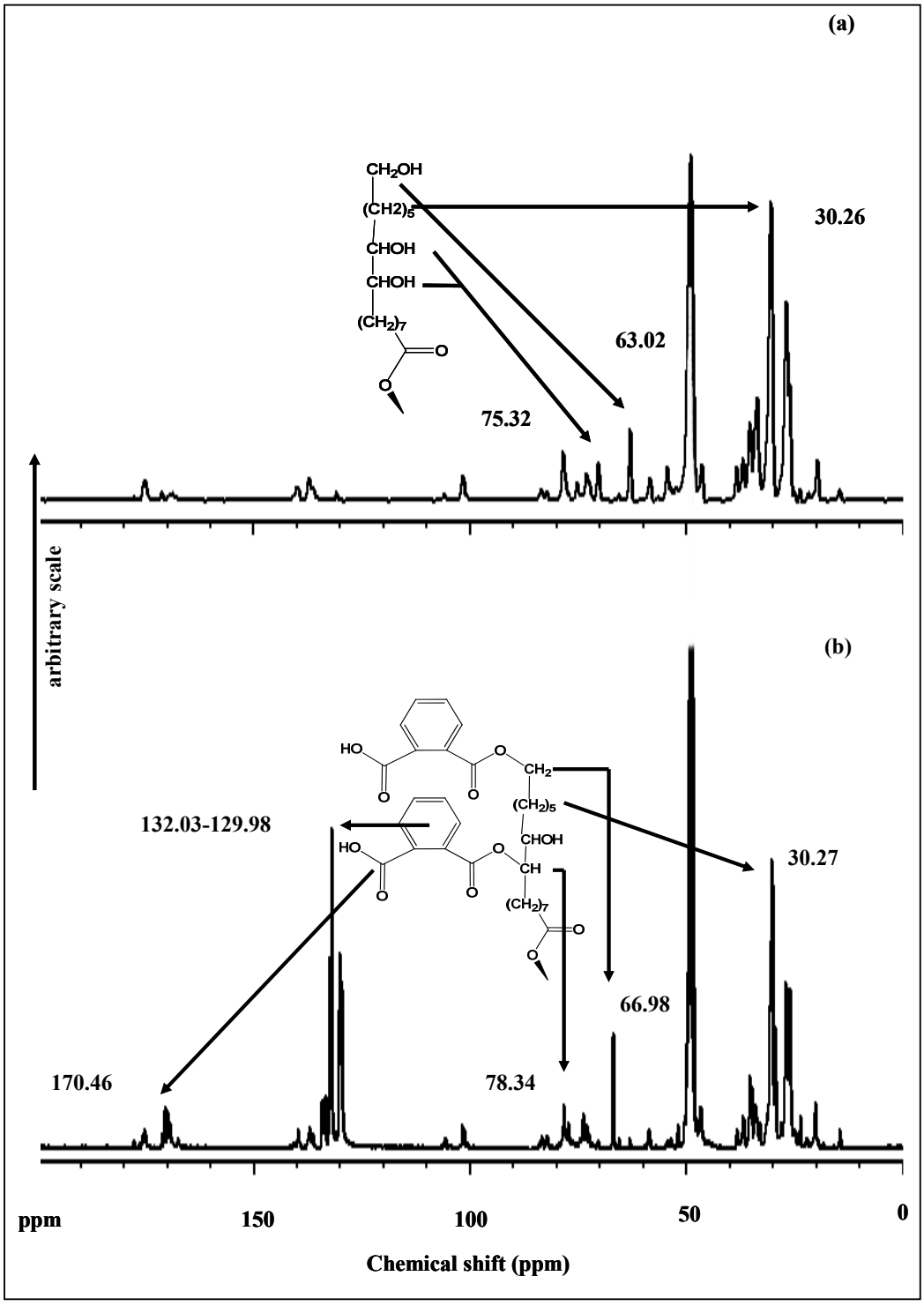

Figure 32 ^{13}C NMR of SHL (a) and SHL-PHT 12 h AM (b)

2.2.2 Characterization of shellac phthalate film

SHL and SHL-PHT AM were prepared by the same method as described in SHL-SUC system 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 33. 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 33a). 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 decrease 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, resulted 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 33. 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. Additinaly, the WVP coefficient of either SHL-SUC or SHL-PHT was still in low level as compared to the reported values (1×10^{-7} to 3×10^{-5} g h^{-1} m^{-1} Pa^{-1}) of several enteric polymers, including cellulose acetate phthalate (CAP), hydroxypropyl methylcellulose phthalate (HPMCP, HP-55), hydroxypropyl methylcellulose acetate succinate (HPMCAS, AS-LG), and methacrylic acid-ethyl acrylate copolymer (Eudragit L30D) [58-61].

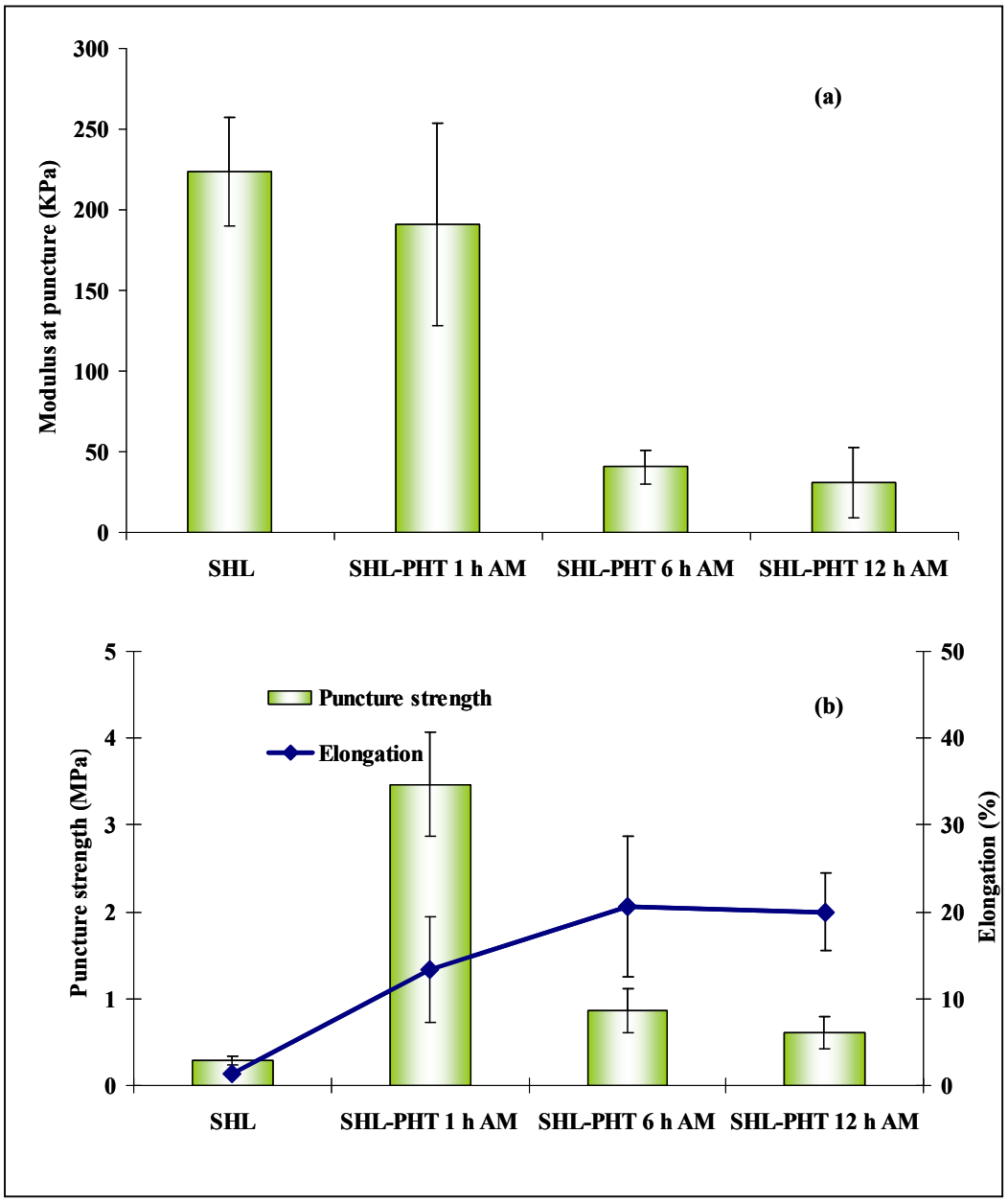

Figure 33 Mechanical properties of SHL-PHT AM system: modulus at puncture (a), puncture strength and percentage of elongation (b)

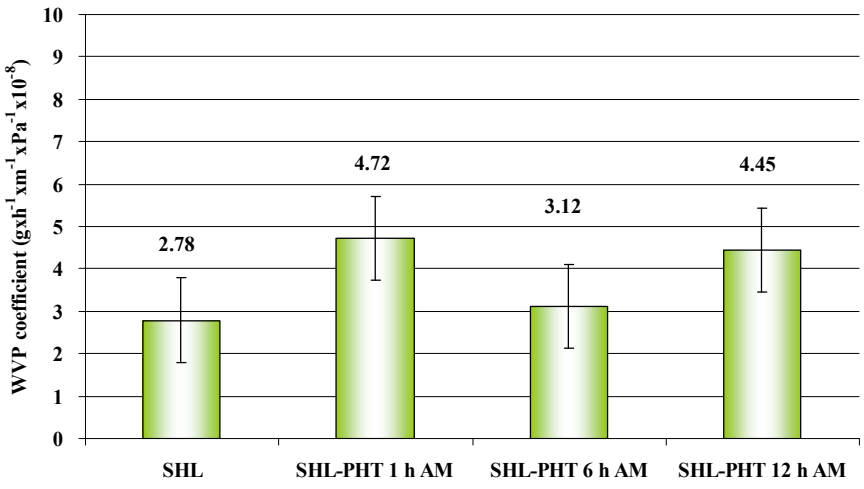

Figure 34 WVP coefficient of SHL and SHL-PHT AM system

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 [62]. Although shellac has a good protection in gastric fluid, however shellac still has a limited solubility in intestinal fluid [4, 11, 63-65], thus SHL-PHT had to test for gastric resistance and solubility at the pH of intestine.

Figure 35 demonstrates percentage 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 36 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 37 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 [33, 55-57].

Respective pH threshold of common enteric polymers, such as cellulose derivatives, vinyls and acrylate co-palymer, was in the range of 4.8-7.0. While lowest pH thresholds of SHL-SUC and SHL-PHT system were 6.1 and 6.4, respectively. The results suggested that the dissolved pH of modified SHL could improve and properly used for delivering drug to small intestine, as well as the other commercial enteric polymers.

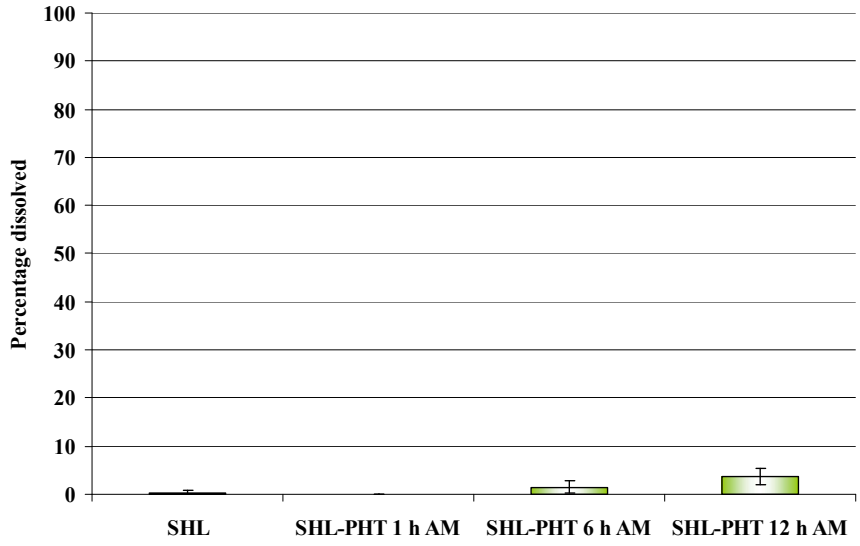

Figure 35 Percentage dissolved of SHL and SHL-PHT AM in SGF

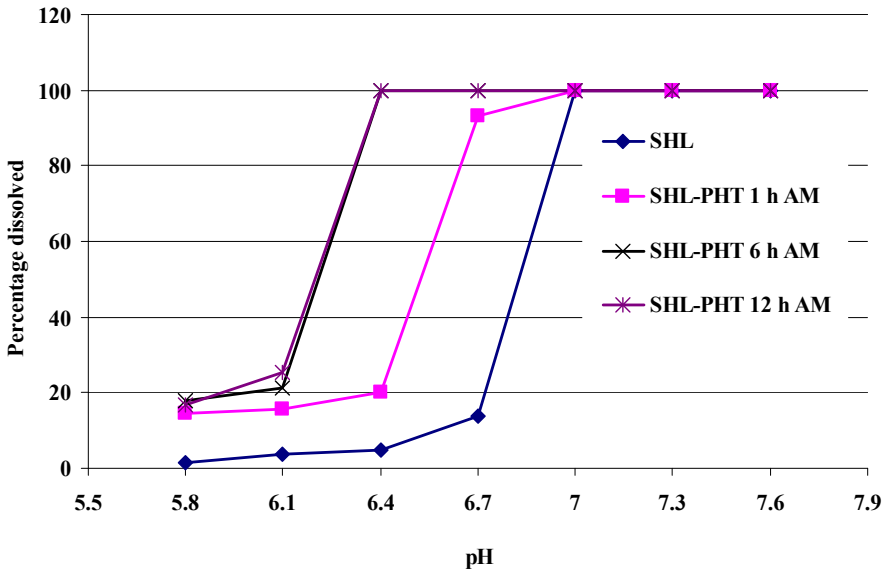

Figure 36 pH solubility profiles of SHL and SHL-PHT AM

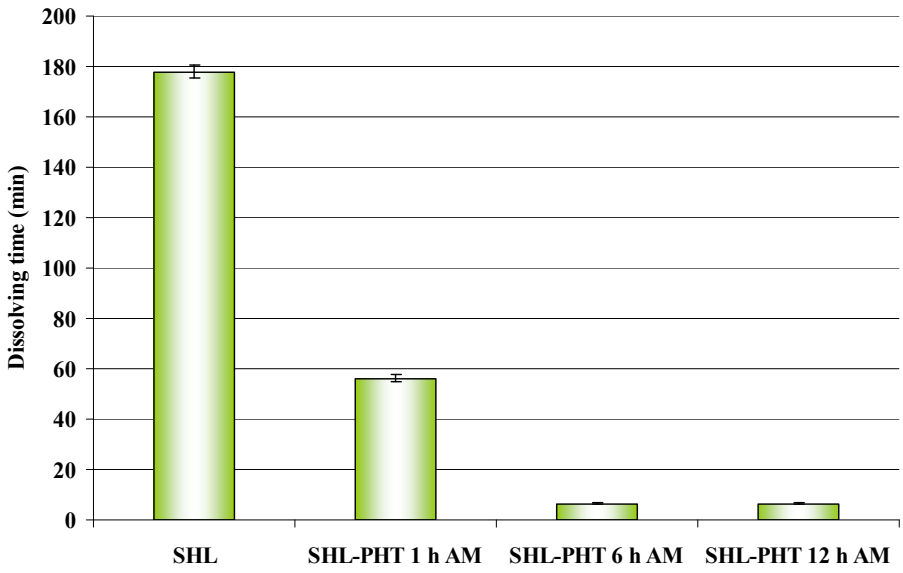

Figure 37 Dissolving time of SHL and SHL-PHT AM in buffer pH 7.0

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 system.

2.3.1 Acid value and percentage of insoluble solid

Figure 38 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. The acid value of SHL-TMT after annealing at 100 $^{\circ}$C (100 $^{\circ}$C AM) was approximately 1.5 times as compared to that of SHL. However, SHL-TMT 100 $^{\circ}$C AM showed lower acid value as compared to SHL-PHT. Since acid value represent 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.

In order to investigate the stability of SHL-TMT during heat treatment, the percentage of insoluble solid was monitored. As illustrated in Figure 38 (line bar), 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.

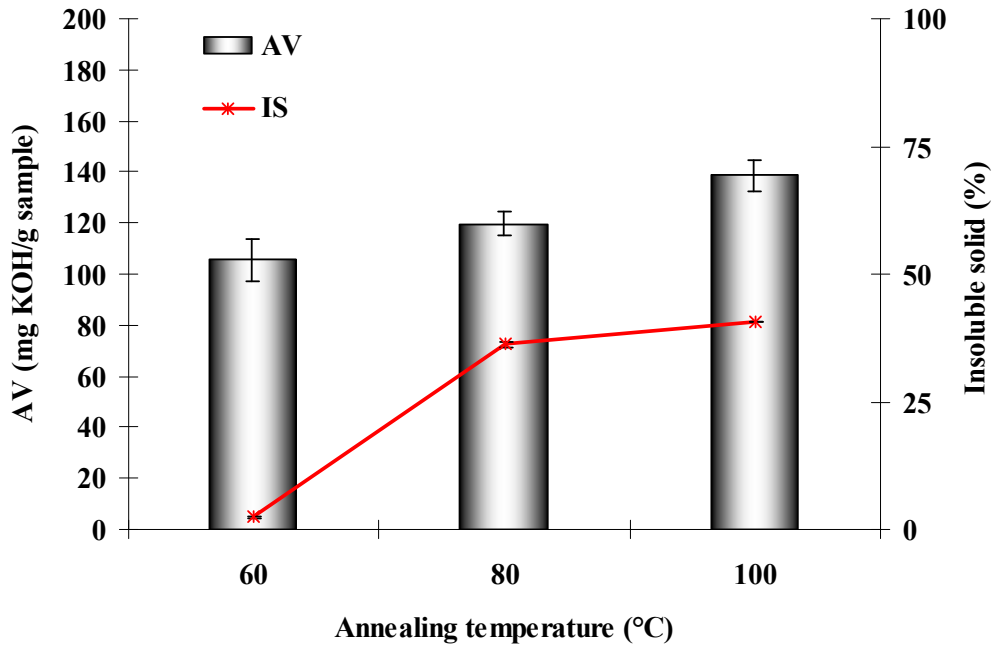

Figure 38 Acid value and insoluble solid of SHL-TMT after annealing at various temperatures for 12 h

2.3.2 FTIR spectroscopy

As illustrated in Figure 39, 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 system, especially at low annealing temperature. The results were agreed well with that of AV increment, supporting the lower degree of esterification by TMTA.

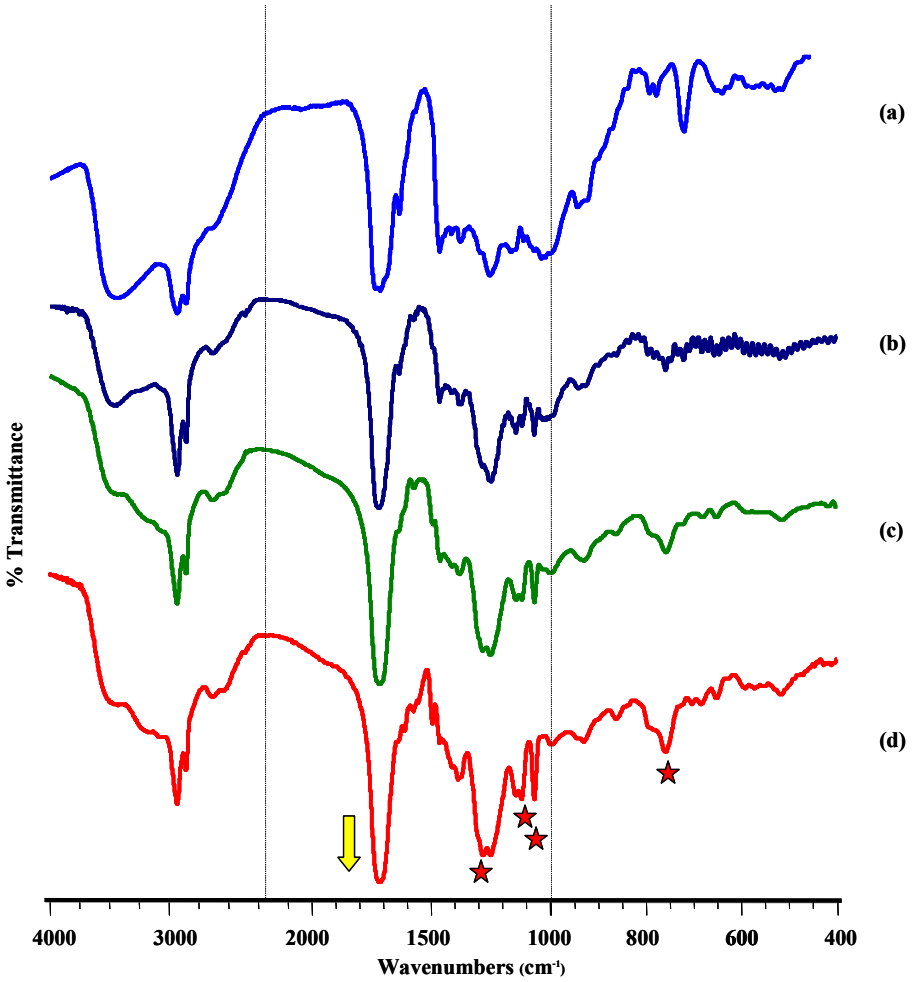

Figure 39 FTIR spectra of SHL-TMT AM system; SHL (a), SHL-TMT 60°C AM (b), SHL-TMT 80°C AM (c) and SHL-TMT 100°C AM (d)

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 showed different thermogram as compared to SHL (Figure 40). 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.

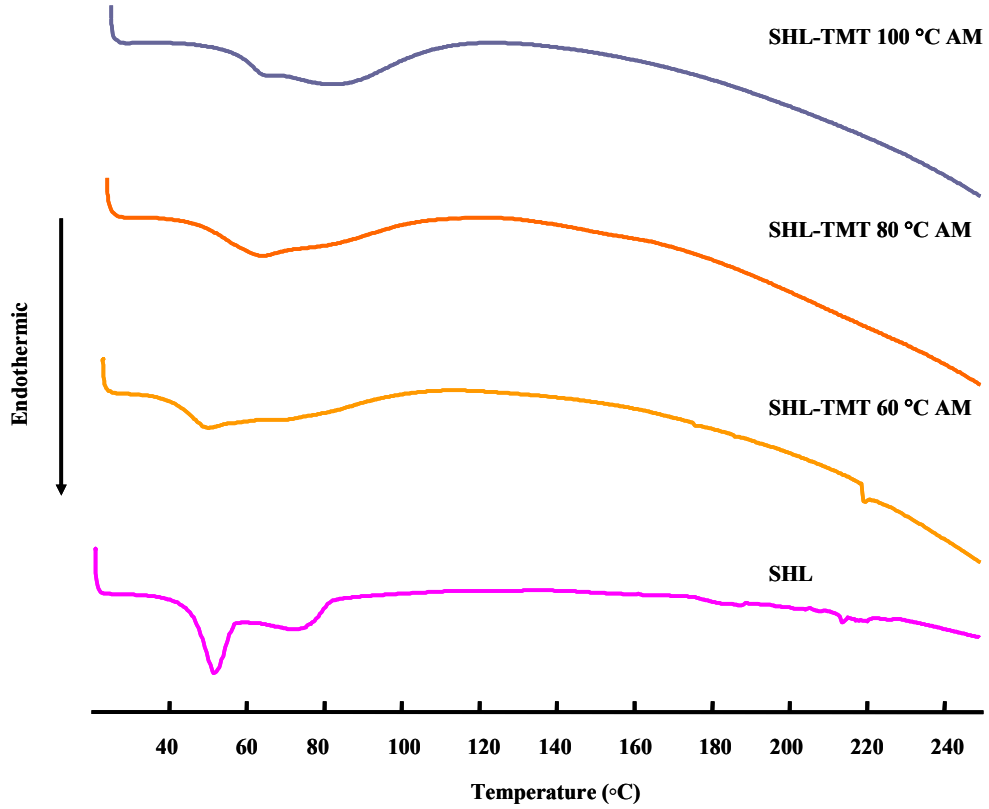

Figure 40 DSC curves of SHL, SHL-TMT AM (after washing)

2.3.4 Powder X-ray diffraction

Figure 41 demonstrates the PXRD patterns of SHL and SHL-TMT (before washing). An amorphous phase of SHL showed a halo pattern (Figure 41a). The PXRD patterns of SHL-TMT PM (Figure 41c) and SHL-TMT GM (Figure 41d) were the superimposed pattern of SHL and TMTA in which the intensity of diffraction peaks of SHL-TMT GM was a lower. As raised the temperature, the intensity of diffraction peaks of TMTA gradually declined (Figure 41e-41g), 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 system, 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 42. The halo pattern of all SHL-TMT AM was observed, suggesting the amorphous state of SHL-TMT.

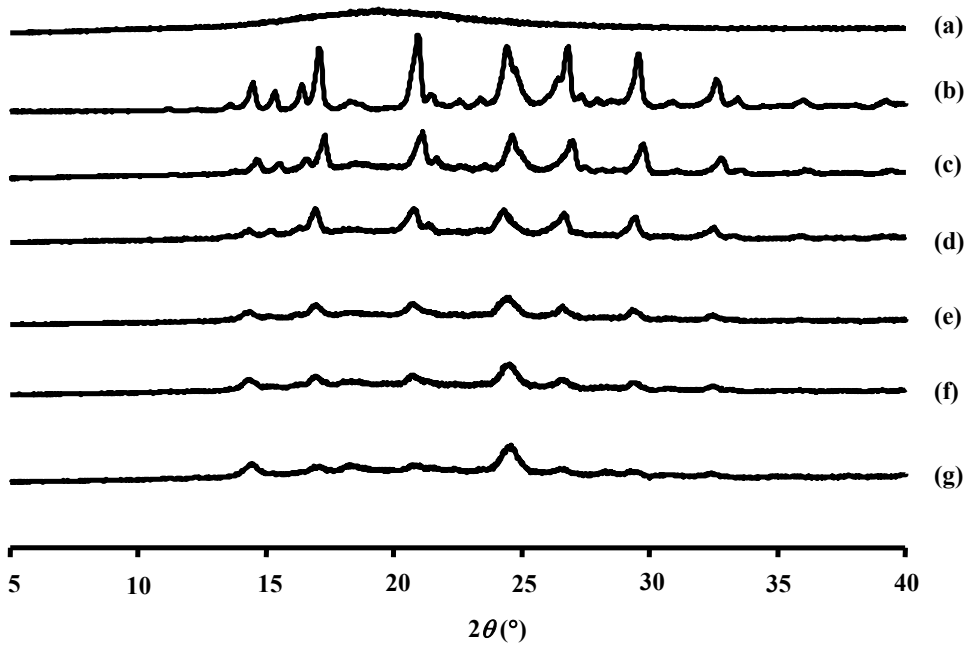

Figure 41 Powder X-ray diffraction pattern of SHL-TMT system before washing: SHL (a), TMTA (b), SHL-TMT PM (c), SHL-TMT GM (d), SHL-TMT 60°C AM (e), SHL-TMT 80°C AM (f) and SHL-TMT 100°C AM (g)

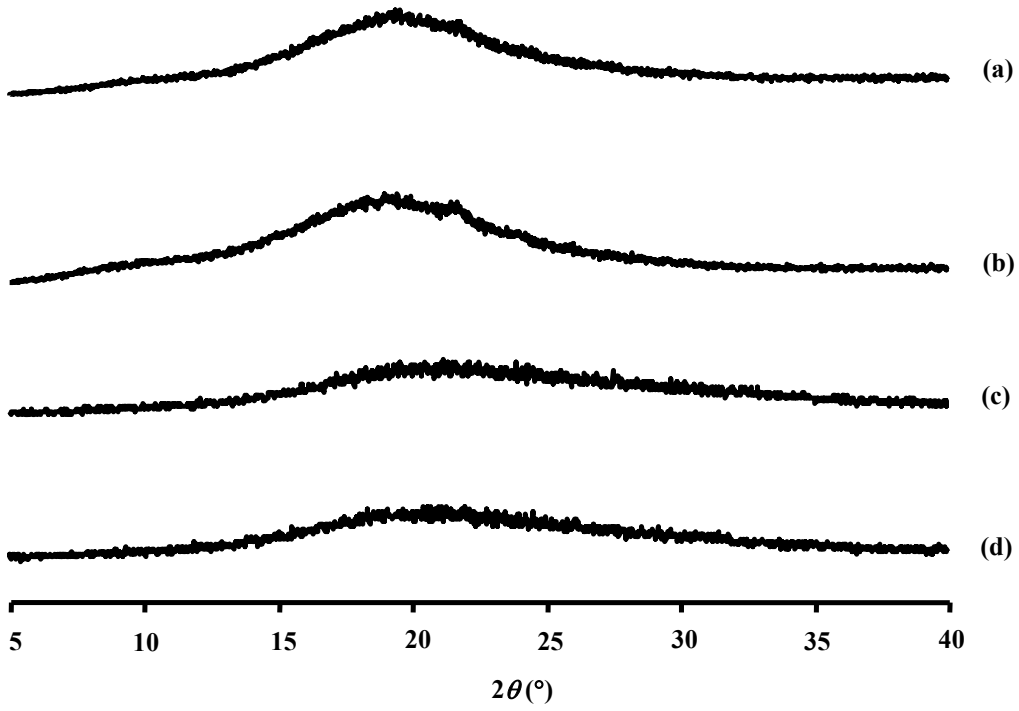

Figure 42 Powder X-ray diffraction pattern of SHL-TMT system after washing: SHL (a), SHL-TMT 60°C AM (b), SHL-TMT 80°C AM (c) and SHL-TMT 100°C AM (d)

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 C AM were comparatively analyzed with those of SHL. Figure 43 illustrated the ^{1}H NMR spectrum of SHL-TMT system. The relative resonance of methylene group (-CH_2-OH) and methine group (-CH-OH) of SHL-TMT (Figure 43c) 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 44). The presence of peaks assigned as aromatic carbon on the spectrum of SHL-TMT (Figure 44b) 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 compare with SHL-SUC and SHL-PHT, the intensity of all new signals and the reduction of characteristic resonance of SHL were much lower. 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 lower 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.

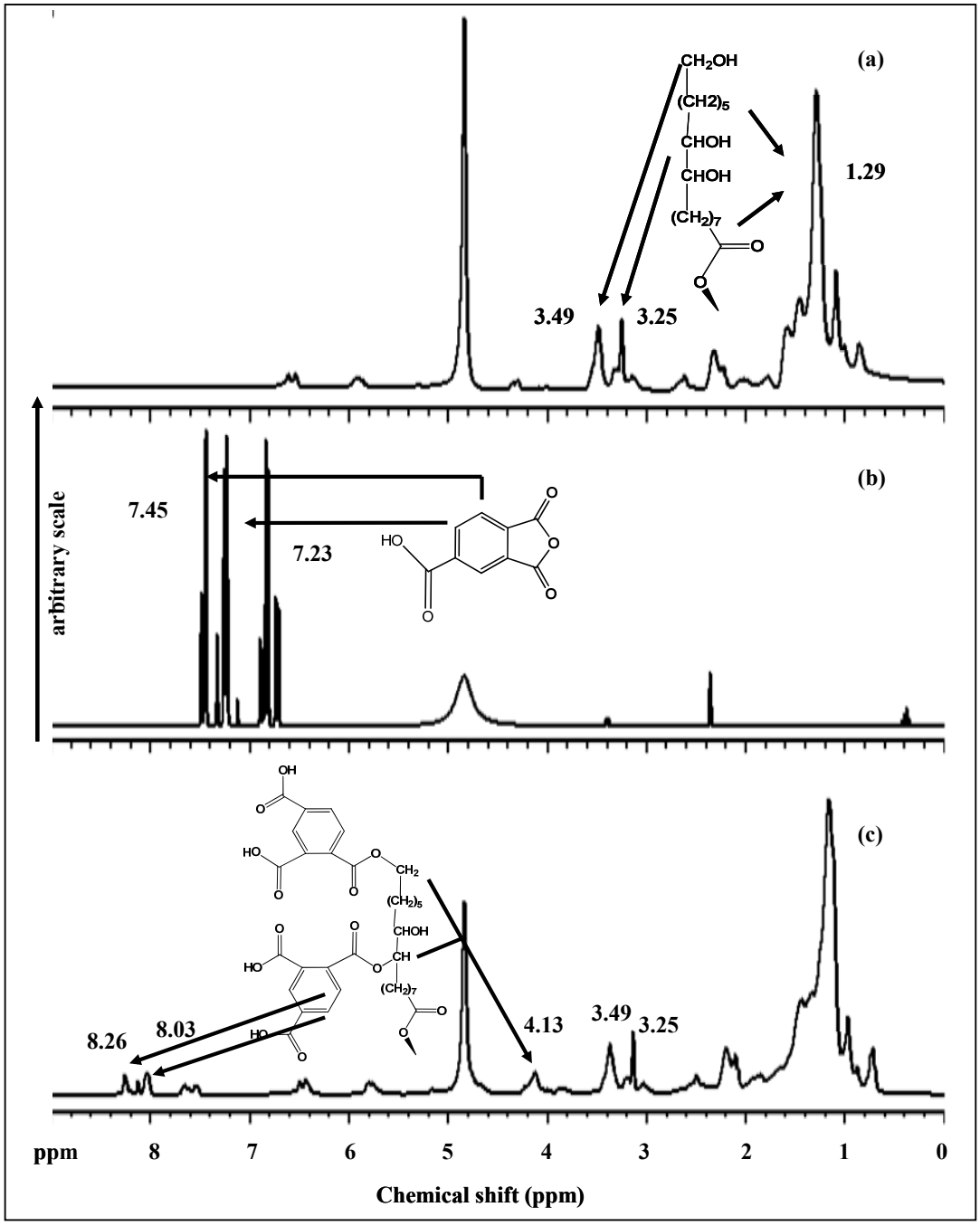

Figure 43 ^{1}H NMR of SHL (a), TMTA (b) and SHL-TMT 80^{o}C AM (c)

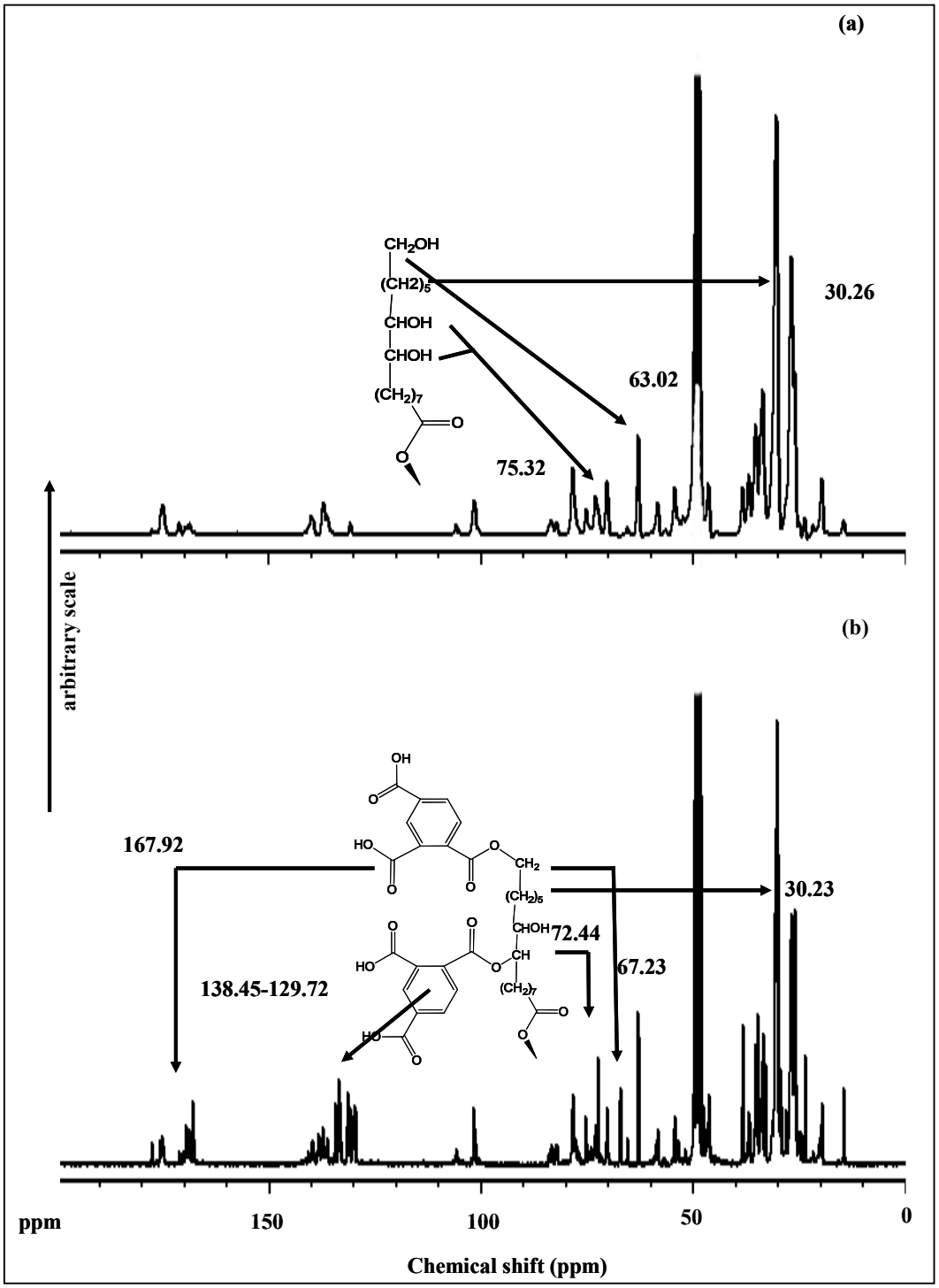

Figure 44 ^{13}C NMR of SHL (a) and SHL-TMT 80^{o}C AM (b)

3. Characterizations of coated tablet

In order to study enteric properties of shellac ester coated tablet, the SHL and shellac esters coated tablet 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 tablet

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.

Tablet properties of SHL and SHL-SUC AM coated tablet at different coating levels such as weight, thickness, hardness, tablet disintegration and dissolution profile were comparatively evaluated as shown in Table 1-4. As increasing coating level, the change of tablet properties was observed, especially the disintegration time of coated tablet.

Table 1 demonstrates tablet properties of shellac coated tablet at various coating levels. Weight and thickness of coated tablet gradually increased, while hardness was insignificant changed as increasing coating level. For tablet disintegration, uncoated tablet and coated tablet 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 tablet 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 1 Tablet properties of SHL coated tablet 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	0.9987±0.0057	8.868±0.085	14.4±2.4	< 1.0	-
1	1.0057±0.0093	8.858±0.070	13.9±2.5	< 1.0	-
2	1.0155±0.0086	8.925±0.053	13.8±1.0	> 2 h*	19.2±18.0
3	1.0261±0.0080	8.968±0.048	14.4±1.4	> 2 h**	43.5±2.1
4	1.0321±0.0084	8.969±0.074	15.5±1.0	> 2 h	41.0±0.8
5	1.0440±0.0112	9.030±0.059	17.0±0.7	> 2 h	58.6±9.3
6	1.0469±0.0086	9.034±0.058	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 tablet properties of SHL-SUC 1 h AM, SHL-SUC 6 h AM and SHL-SUC 24 h AM coated tablet are shown in table 2-4, respectively. The results were similar to that observed in SHL coated tablet. The coating level directly affected the tablet properties of all SHL-SUC AM coated tablet. SHL-SUC 1 h AM coated tablet could be protected from acid condition at the coating level of 4 mg/cm^2 which was the same as SHL coated tablet. However, SHL-SUC 1 h AM coated tablet showed significant rapid disintegration in buffer pH 6.8, as compared to SHL coated tablet (p<0.01). For SHL-SUC 6 h AM and SHL-SUC 24 h AM, the picking of coated tablet 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 20-22). 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 2 Tablet properties of SHL-SUC 1 h AM coated tablet 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	0.9987±0.0057	8.868±0.085	14.4±2.4	< 1.0	-
1	1.0010±0.0120	8.793±0.131	10.0±2.0	34.0±18.0	-
2	1.0132±0.0081	8.862±0.055	9.0±1.0	> 2 h*	3.3±0.7
3	1.0197±0.0085	8.889±0.059	9.3±0.6	> 2 h**	6.0±2.8
4	1.0286±0.0084	8.931±0.048	10.6±0.8	> 2 h	7.4±2.3
5	1.0387±0.0124	8.977±0.082	13.9±2.0	> 2 h	9.7±0.1
6	1.0414±0.0093	8.987±0.111	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 3 Tablet properties of SHL-SUC 6 h AM coated tablet 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	0.9987±0.0057	8.868±0.085	14.4±2.4	< 1.0	-
1	1.0009±0.0118	8.848±0.045	10.6±4.4	< 1.0	-
2	1.0123±0.0080	8.911±0.040	10.0±1.3	5.7±0.9	-
3	1.0168±0.0090	8.941±0.058	9.3±1.0	31.9±4.9	-
4	1.0264±0.0092	8.972±0.051	13.3±1.8	> 2 h*	2.4±0.7
5	1.0315±0.0069	8.961±0.057	14.8±2.2	> 2 h**	4.2±0.2
6	1.0417±0.0085	9.050±0.062	15.9±1.5	> 2 h***	4.5±0.3
7	1.0450±0.0086	9.070±0.052	16.3±1.7	> 2 h****	4.7±1.4
8	1.0544±0.0076	9.098±0.055	14.2±2.0	> 2 h	6.5±0.3
9	1.0579±0.0098	9.160±0.049	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 4 Tablet properties of SHL-SUC 24 h AM coated tablet 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	0.9987±0.0057	8.868±0.085	14.4±2.4	< 1.0	-
1	1.0034±0.0056	8.819±0.068	16.6±1.5	< 1.0	-
2	1.0067±0.0101	8.830±0.071	14.5±1.4	< 1.0	-
3	1.0150±0.0072	8.915±0.050	14.6±0.8	1.1±0.3	-
4	1.0237±0.0139	8.966±0.084	15.0±0.8	15.1±14.7	-
5	1.0298±0.0095	8.996±0.093	15.3±1.9	42.2±40.6	-
6	1.0343±0.0080	9.019±0.052	16.4±1.8	> 2 h*	2.2±0.8
7	1.0474±0.0096	9.056±0.059	17.4±0.9	> 2 h**	1.3±0.3
8	1.0496±0.0078	9.097±0.056	18.4±0.4	> 2 h	2.4±0.9
9	1.0562±0.0069	9.093±0.075	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 tablet 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 tablet at various coating levels was also evaluated. Figure 45 demonstrates the dissolution profile of SHL coated tablet. SHL coated tablet 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 1). 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 46-48, all SHL-SUC AM coated tablet could inhibit drug dissolved in gastric condition for 2 h, similar to SHL coated tablet. 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 tablet 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 tablet is shown in Figure 49. T_{50} of SHL coated tablet 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 tablet 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 21), confirming the enhanced enteric properties of SHL by succinate formation.

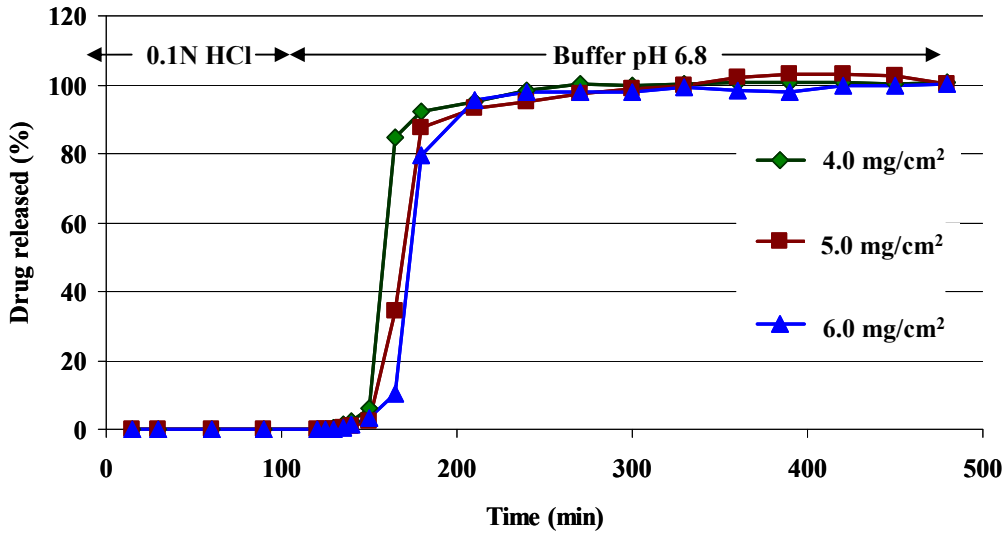

Figure 45 Dissolution profile of shellac coated tablet

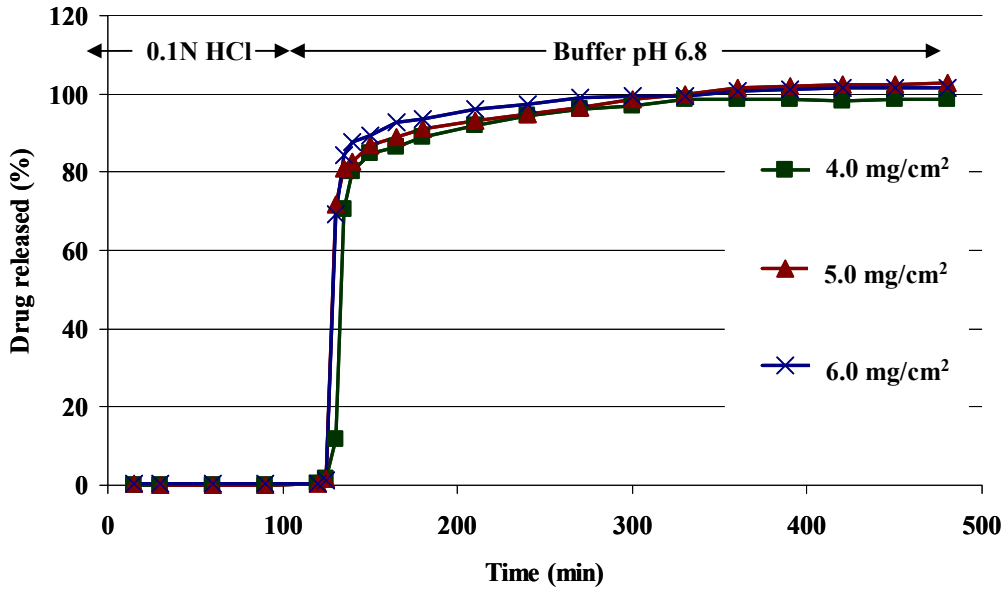

Figure 46 Dissolution profile of SHL-SUC 1 h AM coated tablet

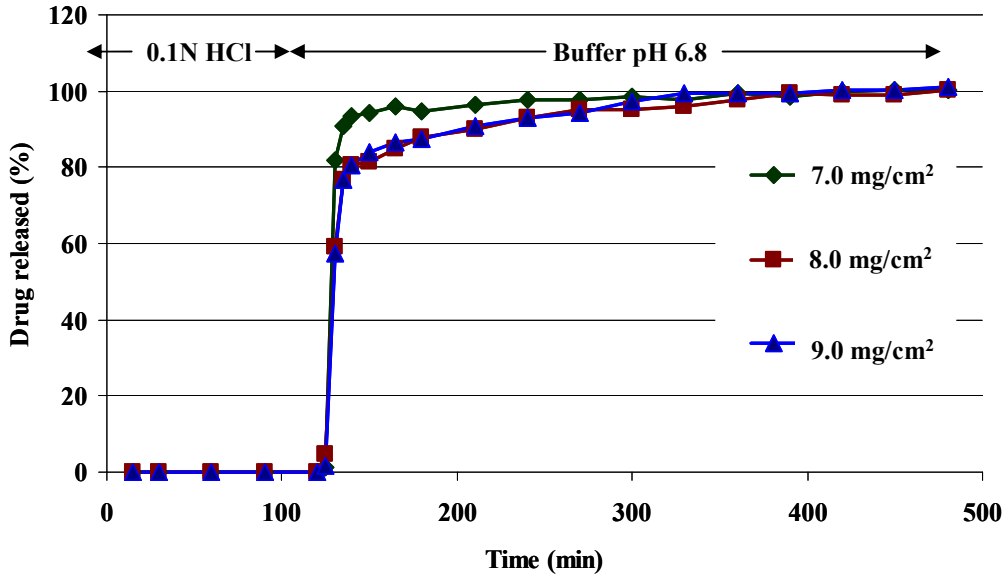

Figure 47 Dissolution profile of SHL-SUC 6 h AM coated tablet

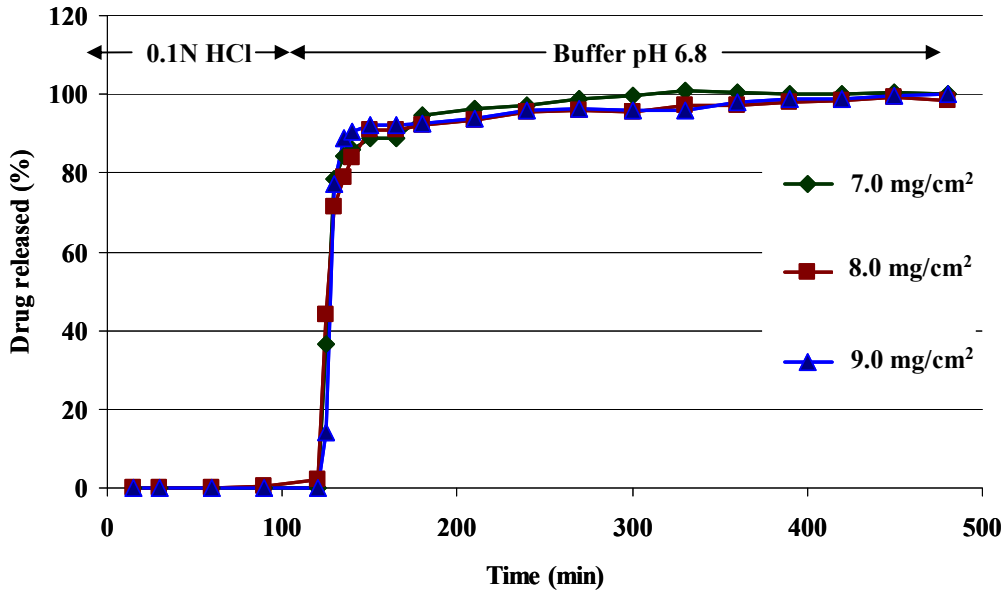

Figure 48 Dissolution profile of SHL-SUC 24 h AM coated tablet

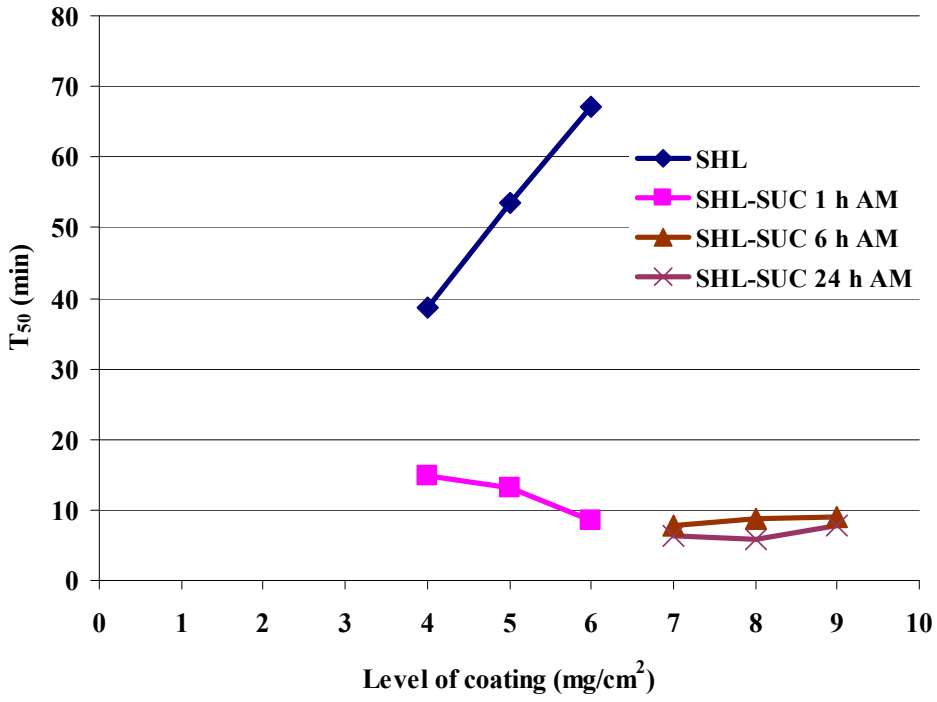

Figure 49 Time for 50% drug released (T_{50}) of SHL and SHL-SUC AM coated tablet with various coating levels

3.2 Characterization of SHL-PHT AM coated tablet

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 tablet 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 system.

Table 5 demonstrates properties of SHL coated tablet at various coating levels. Weight and thickness of coated tablet increased progressively, while hardness was not significantly changed as increasing of coating level. For tablet disintegration, uncoated tablet and coated tablet 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 tablet 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 tablet in the small intestine condition.

The tablet properties of SHL-PHT coated tablet are demonstrated in Table 6-8. The effect of coating level on tablet hardness, weight and thickness was similar to that of SHL coated tablet. 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 tablet was occurred during coating process, so the coating level was increased to obtain sufficient gastric resistance. However, SHL-PHT AM coated tablet still demonstrated fast disintegration in buffer solution even at the high coating level. As compared to SHL coated tablet, SHL-PHT AM coated tablet 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 tablet was less than 15 min at all of coating level (Table 7-8).

Table 5 Tablet properties of SHL coated tablet 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	1.0060±0.0111	8.645±0.116	37.2±3.8	< 1.0	-
1	1.0117±0.0411	8.597±0.141	38.3±3.9	44.5±3.6	-
2	1.0254±0.0368	8.644±0.280	39.1±4.1	> 2h*	23.0±9.9
3	1.0290±0.0394	8.650±0.137	37.5±7.9	> 2h*	59.6±30.7
4	1.0373±0.0409	8.667±0.139	38.4±3.4	> 2h*	> 3h
5	1.0429±0.0436	8.692±0.089	39.4±1.7	> 2h*	> 3h
6	1.0492±0.0421	8.717±0.174	38.9±4.0	> 2h*	> 3h
7	1.0596±0.0269	8.786±0.102	39.3±4.8	> 2h	> 3h

* swell in 0.1N HCl

Table 6 Tablet properties of SHL-PHT 1 h AM coated tablet 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	1.0060±0.0111	8.645±0.116	37.2±3.8	< 1.0	-
1	1.0225±0.0259	8.607±0.133	37.3±3.6	12.7±2.3	-
2	1.0285±0.0314	8.628±0.062	37.5±4.8	69.3±9.1	-
3	1.0345±0.0349	8.715±0.134	38.4±3.6	84.0±23.9	-
4	1.0470±0.0317	8.735±0.094	38.6±3.1	> 2h*	7.5±0.6
5	1.0540±0.0262	8.757±0.067	39.1±2.6	> 2h	14.8±5.3
6	1.0600±0.0454	8.774±0.226	38.4±4.9	> 2h	25.2±12.5
7	1.0680±0.0378	8.822±0.169	37.8±4.8	> 2h	71.9±11.1
8	1.0760±0.0241	8.839±0.105	39.7±6.0	> 2h	91.2±4.5

* 2 of 6 tablets disintegrated in 89.5±30.41 min

Table 7 Tablet properties of SHL-PHT 6 h AM coated tablet 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	1.0060±0.0111	8.645±0.116	37.2±3.8	< 1.0	-
1	1.0140±0.0329	8.673±0.103	37.4±4.9	6.08±0.98	-
2	1.0150±0.0202	8.683±0.061	38.6±3.2	13.60±1.92	-
3	1.0230±0.0350	8.710±0.129	38.3±4.2	23.61±0.77	-
4	1.0310±0.0328	8.730±0.102	37.3±2.2	70.95±13.90	-
5	1.0400±0.0401	8.735±0.075	39.8±2.6	100.33±7.51	-
6	1.0550±0.0287	8.768±0.063	37.5±3.7	> 2h*	5.55±1.27
7	1.0590±0.0094	8.803±0.028	38.6±1.3	> 2h**	4.43±0.97
8	1.0630±0.0257	8.818±0.192	37.8±1.0	> 2h***	4.35±0.42
9	1.0720±0.0142	8.823±0.110	41.4±2.7	> 2h****	4.63±0.45
10	1.0845±0.317	8.830±0.128	36.8±2.3	> 2h	6.96±1.58
11	1.0885±0.0139	8.863±0.108	39.9±3.3	> 2h	9.51±1.53
12	1.0930±0.0338	8.870±0.026	39.4±2.9	> 2h	9.69±3.80
13	1.1090±0.0192	8.888±0.117	39.8±3.0	> 2h	10.39±1.79
14	1.1140±0.0217	8.918±0.326	38.6±3.8	> 2h	10.64±1.84
15	1.1170±0.0365	9.015±0.039	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 8 Tablet properties of SHL-PHT 12 h AM coated tablet 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	1.0060±0.0111	8.645±0.116	37.2±3.8	< 1.0	-
1	0.990±0.0433	8.635±0.131	40.2±1.9	8.33±1.55	-
2	1.0160±0.0338	8.638±0.182	39.1±2.1	10.44±1.54	-
3	1.0270±0.0255	8.660±0.088	37.5±2.9	34.41±5.81	-
4	1.0340±0.0347	8.675±0.093	40.0±2.2	37.66±5.36	-
5	1.0350±0.0429	8.700±0.253	37.3±3.0	48.59±5.79	-
6	1.0460±0.0228	8.710±0.144	37.6±3.4	> 2h*	5.02±1.08
7	1.0530±0.0201	8.728±0.108	37.8±4.1	> 2h**	5.78±0.38
8	1.0580±0.0250	8.753±0.071	38.7±5.2	> 2h**	6.73±0.48
9	1.0640±0.0331	8.755±0.040	38.4±5.4	> 2h**	7.86±1.54
10	1.0770±0.0372	8.780±0.197	39.9±3.0	> 2h**	13.15±3.31
11	1.0820±0.0273	8.795±0.103	38.8±3.5	> 2h	13.27±0.43
12	1.0860±0.0096	8.843±0.085	40.4±2.0	> 2h	13.57±0.38
13	1.0940±0.0290	8.875±0.116	39.2±1.8	> 2h	13.84±2.48
14	1.1040±0.0306	8.893±0.034	39.7±2.4	> 2h	14.42±1.63
15	1.1110±0.0322	8.918±0.071	40.4±2.7	> 2h	14.85±2.23

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

Figure 50 shows the dissolution profile of SHL coated tablet at various coating levels. SHL enteric coated tablet 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 51-53. 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 tablet 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 51). 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 52-53). Moreover, as compared to SHL coating, SHL-PHT AM coating showed the better enteric properties and solubility in the small intestine.

Figure 54 illustrates the time for 50% drug released (T_{50}) of shellac and shellac phthalate coated tablet. As shellac has low solubility in the pH of small intestine (Banker and Agyilirah 1999: 39-66), thus the increment of coating level caused the significant increasing T_{50} of SHL coated tablet (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.

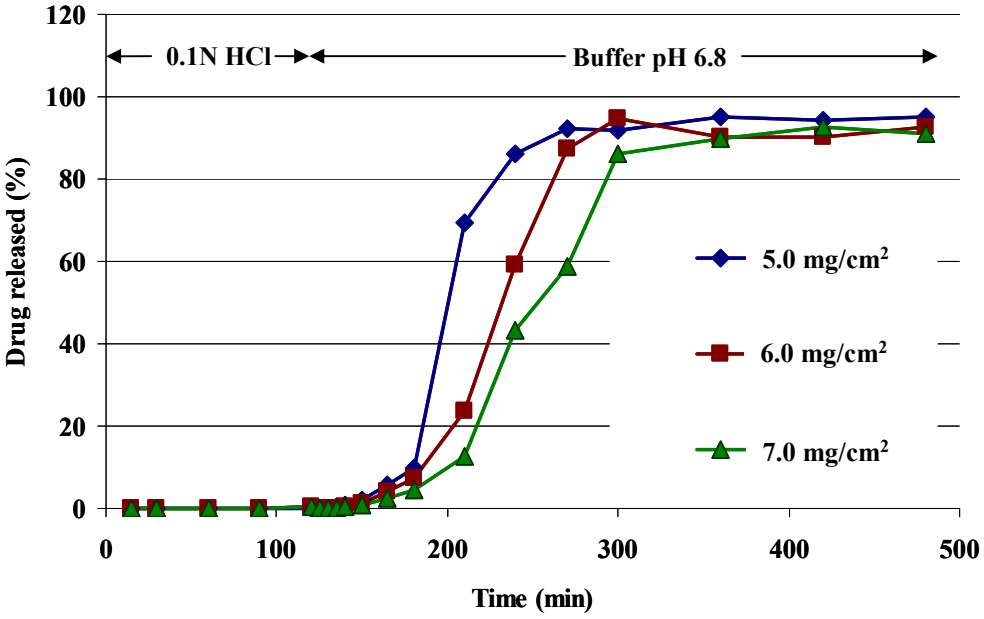

Figure 50 Dissolution profile of SHL coated tablet

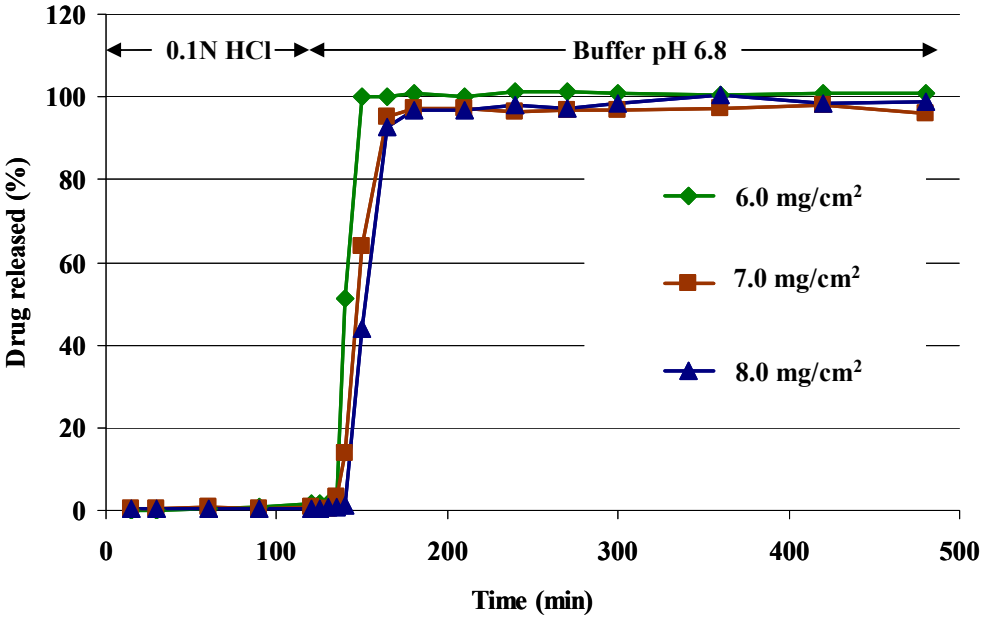

Figure 51 Dissolution profile of SHL-PHT 1 h AM coated tablet

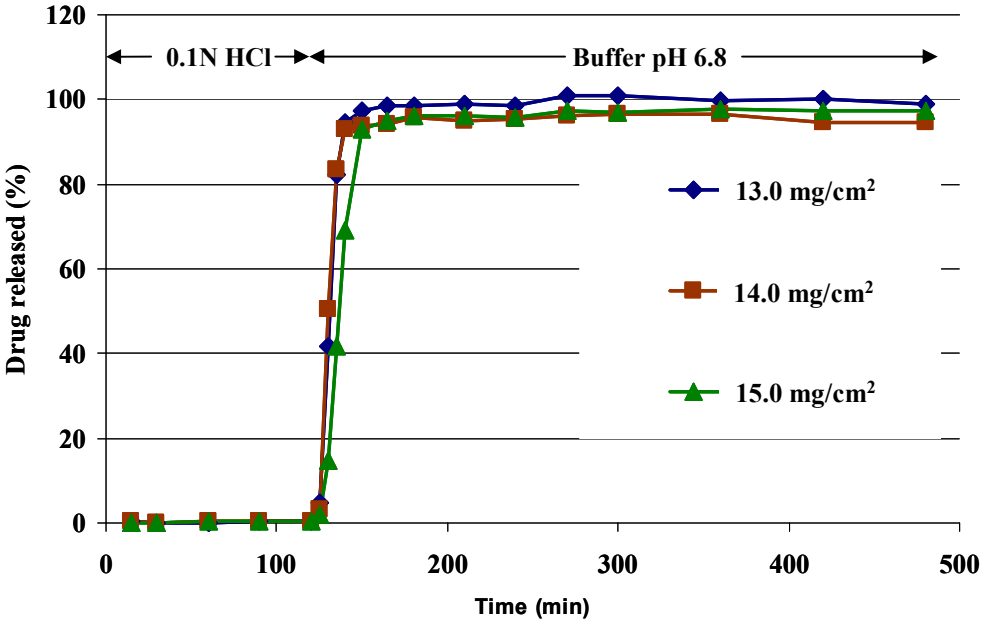

Figure 52 Dissolution profile of SHL-PHT 6 h AM coated tablet

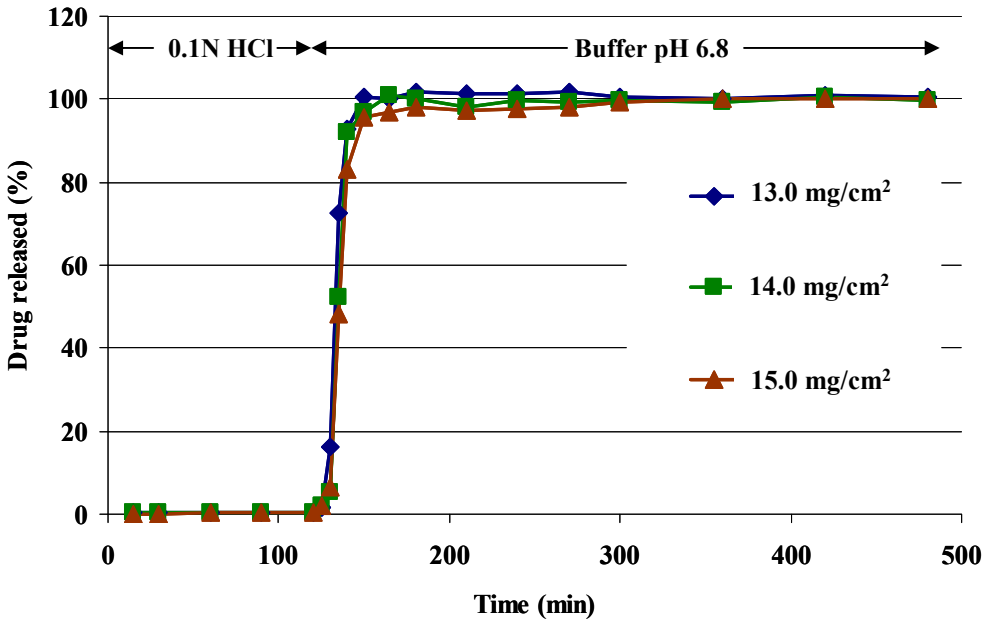

Figure 53 Dissolution profile of SHL-PHT 12 h AM coated tablet

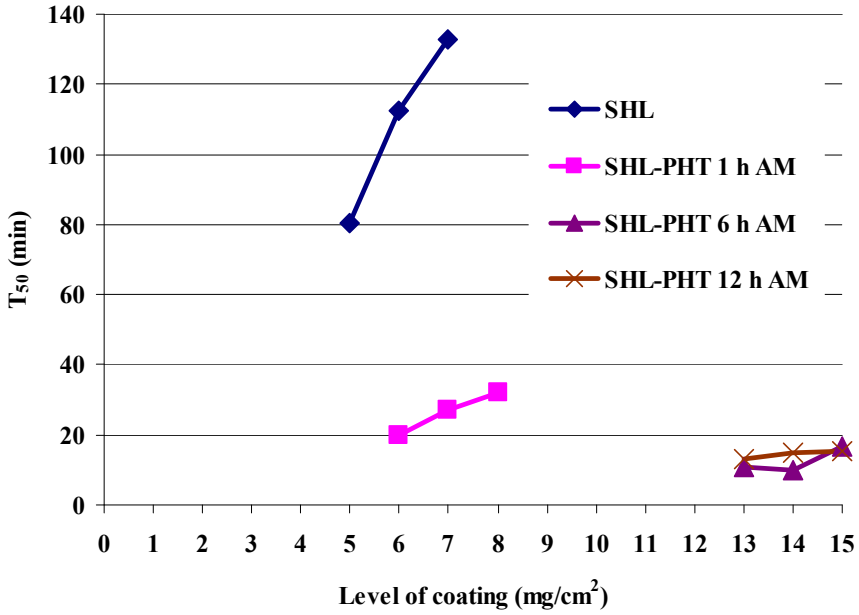

Figure 54 Time for 50% drug released (T_{50}) from SHL and SHL-PHT AM coated tablet with various coating levels

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 88-90 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 (Limmatvapirat et al. 2007: 690-698). Figure 55 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 56. 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 (Limmatvapirat et al. 2005: 41-6).

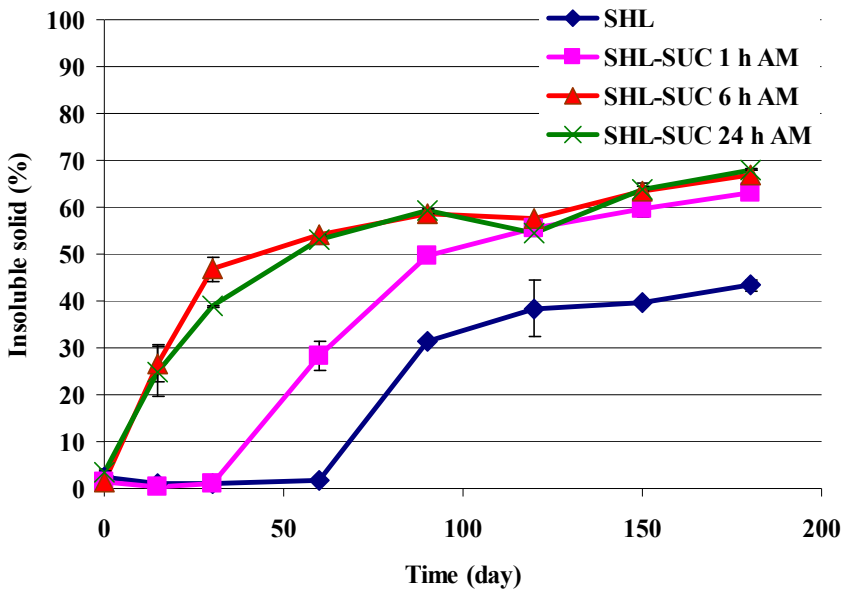

Figure 55 Change of percent insoluble solid of SHL and SHL-SUC films after storage at 40°C, 75% RH for 6 months

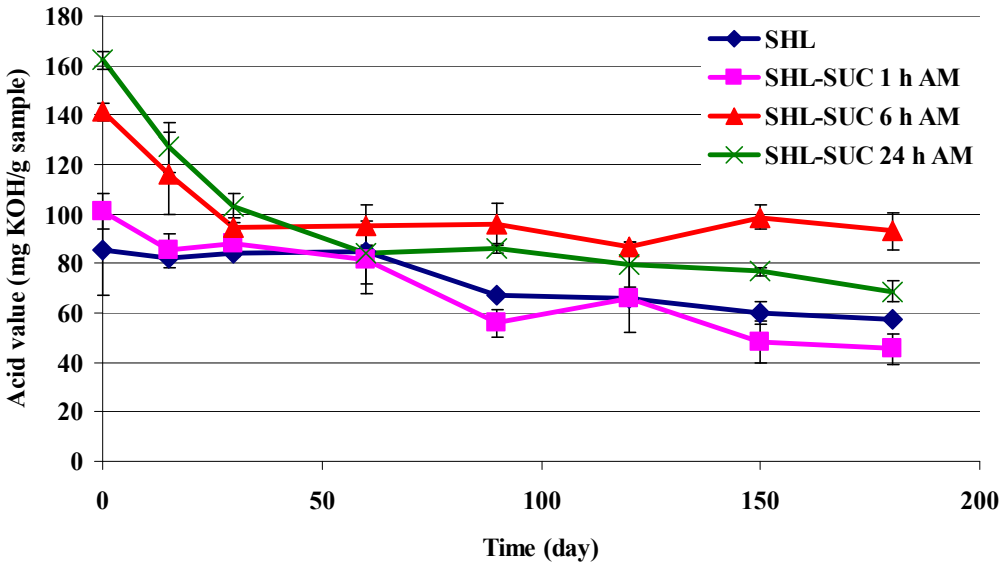

Figure 56 Change of acid value of SHL and SHL-SUC films after storage at 40°C, 75% RH for 6 months.

The stability of SHL and SHL-SUC film by monitoring the change of percentage dissolved at pH 6.8 was also studied. The percentage 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 percentage dissolved of SHL film gradually decreased until completely undissolved at 90 days. SHL-SUC films showed extremely reduction after 15 days (Figure 57). 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 12 h AM. The result well agreed with the percent insoluble solid, suggesting the solubility enhancement although the polymerization was not completely protected by succinate moieties.

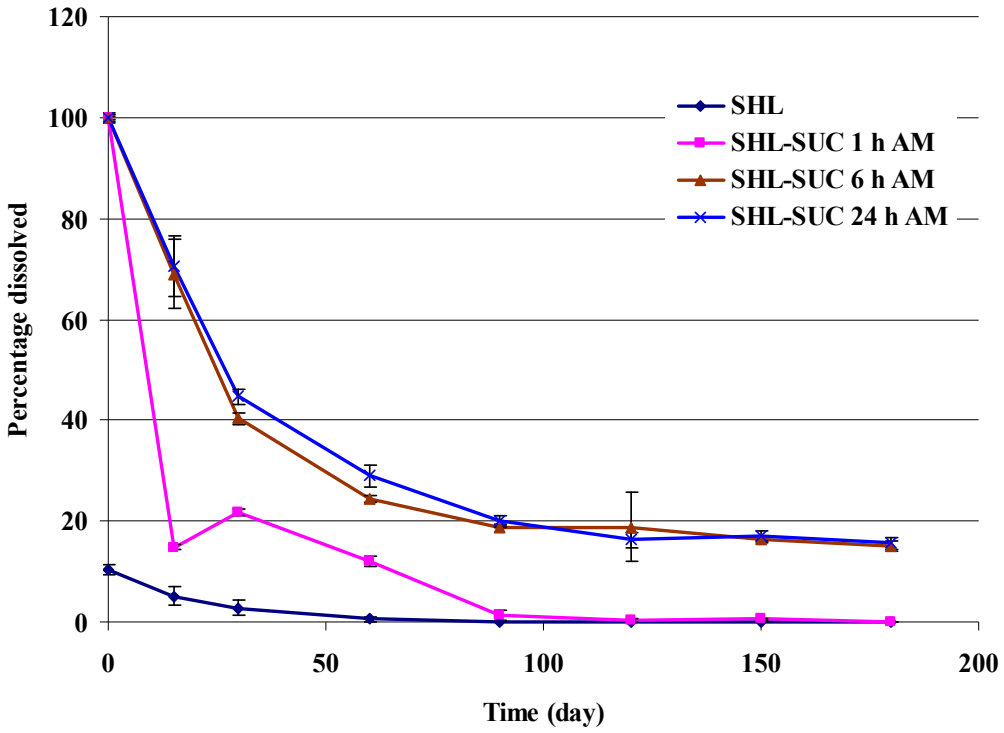

Figure 57 Change of percentage dissolved of SHL and SHL-SUC films in buffer pH 6.8 after storage at 40°C, 75% RH for 6 months

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 58). 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 59. 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 converse 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, therefore, the slower polymerization might related with the higher ratio of carboxylate form.

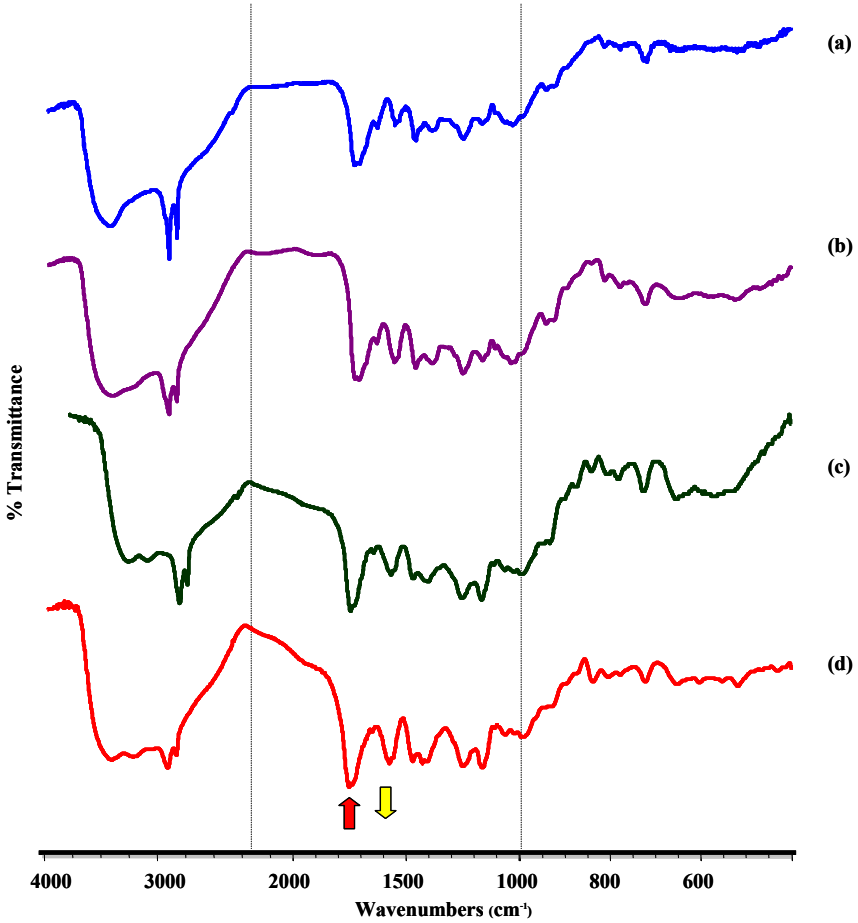

Figure 58 FTIR spectra of SHL and SHL-SUC films at initial; SHL (a), SHL-SUC 1 h AM (b), SHL-SUC 6 h AM (c) and SHL-SUC 24 h AM (d)

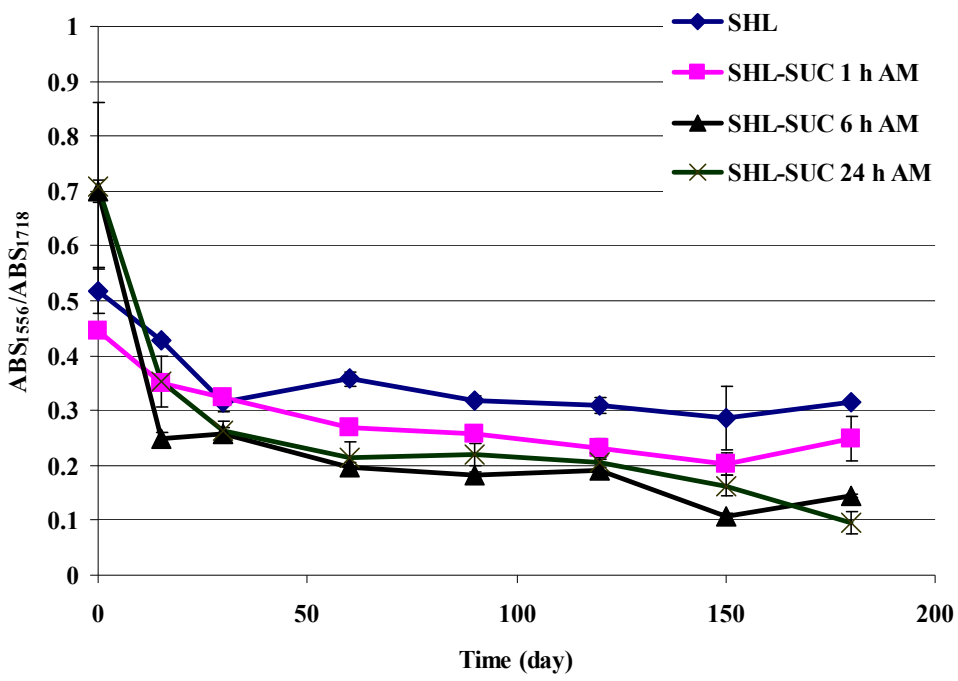

Figure 59 Change of ABS_{1556}/ABS_{1716} of SHL and SHL-SUC films after storage at 40 ºC, 75% RH for 6 months

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 60-63. 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 demonstrated. 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 $^{\circ}C$ 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 observed. 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.

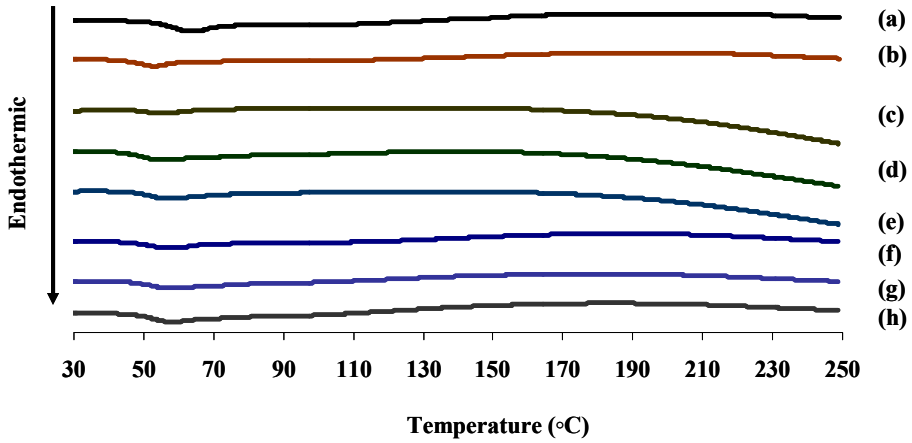

Figure 60 DSC curves of SHL films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

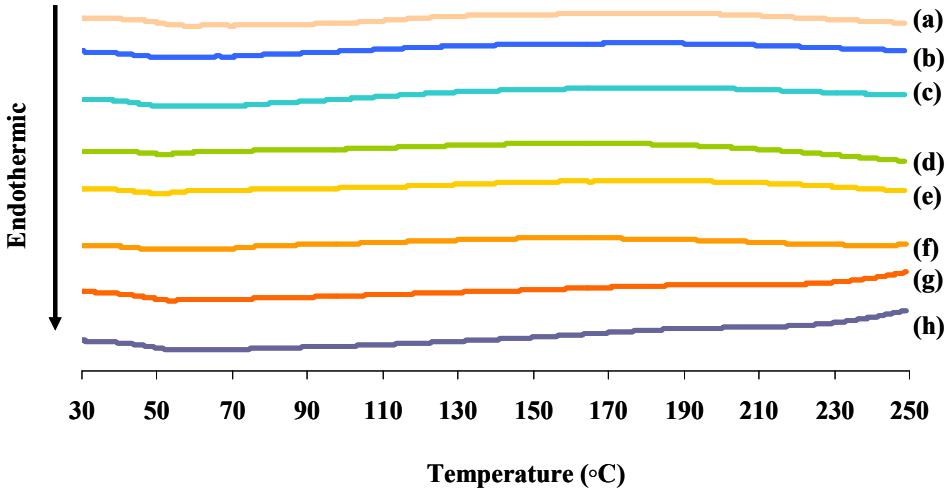

Figure 61 DSC curves of SHL-SUC 1 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

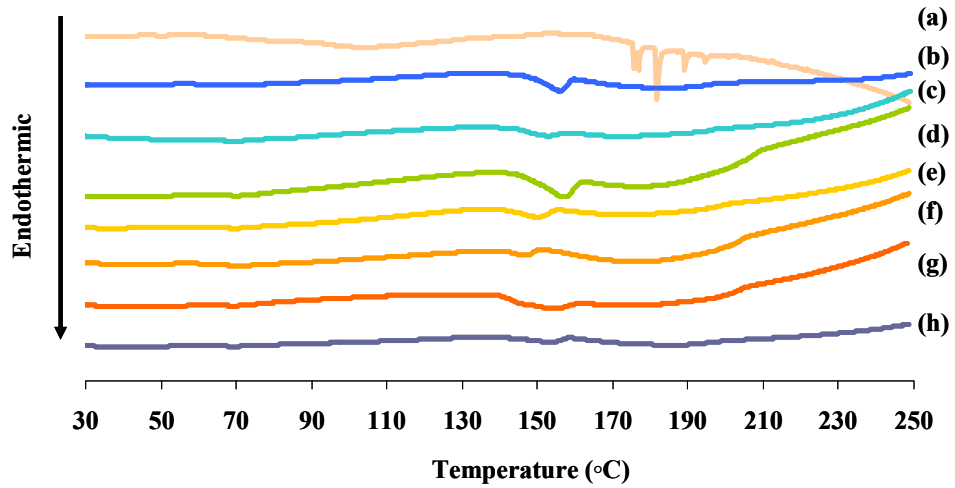

Figure 62 DSC curves of SHL-SUC 6 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

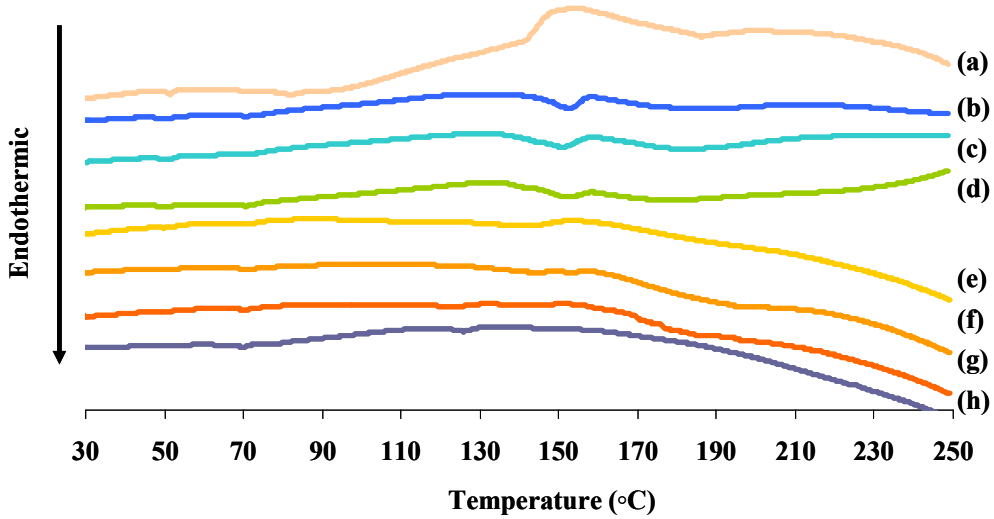

Figure 63 DSC curves of SHL-SUC 24 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

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 65. SHL demonstrated similar halo pattern for all storage time although the insoluble solid was dramatically increase. 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 64 i). Figure 65-57 shows 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.

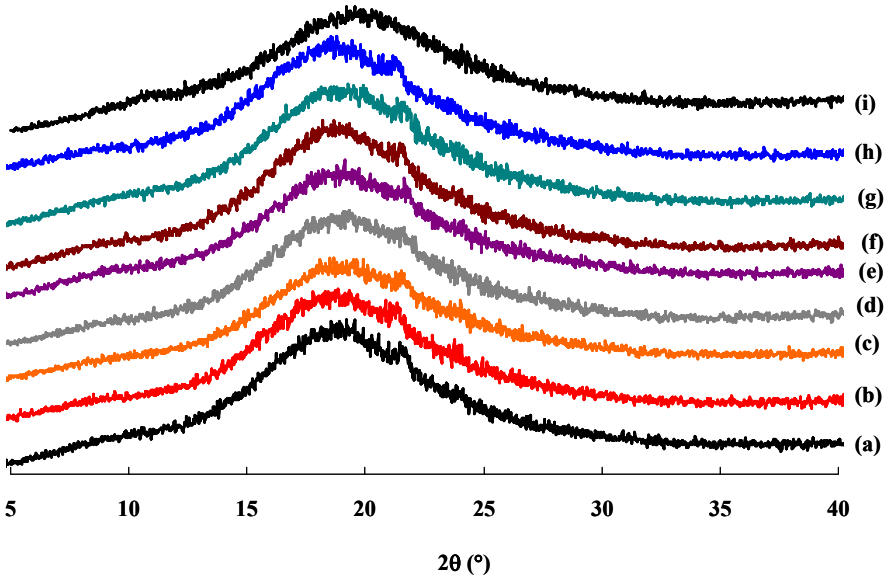

Figure 64 Powder X-ray diffraction patterns of SHL films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g), 180 days (h) and polymerize SHL (i)

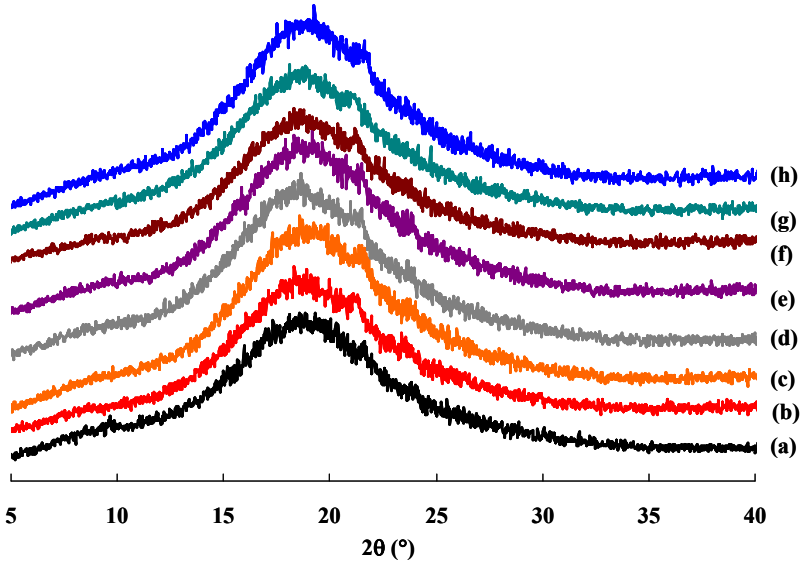

Figure 65 Powder X-ray diffraction patterns of SHL-SUC 1 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

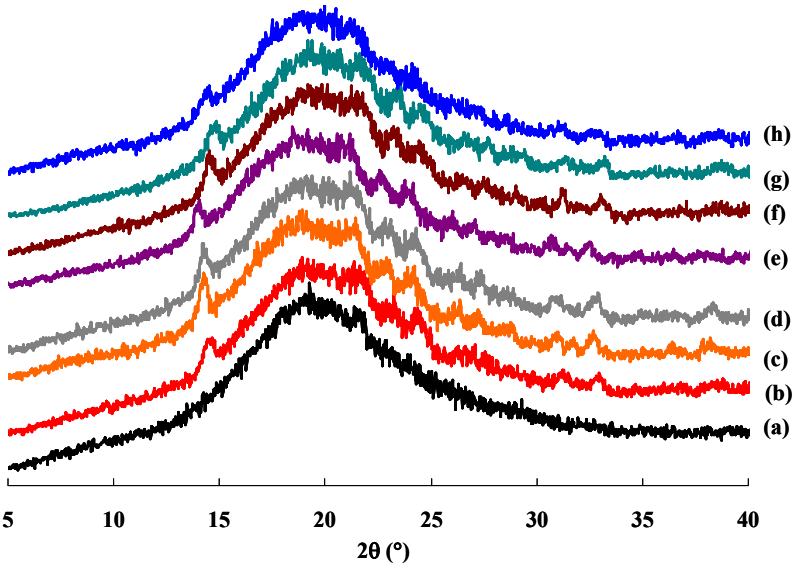

Figure 66 Powder X-ray diffraction patterns of SHL-SUC 6 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

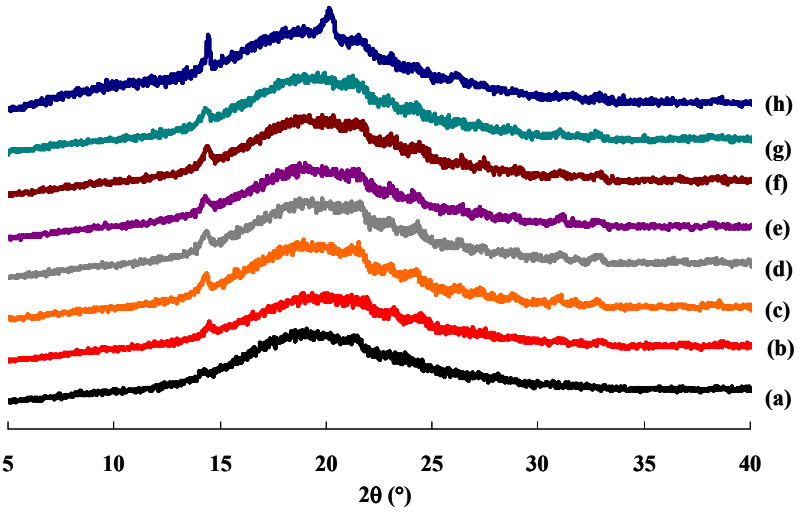

Figure 67 Powder X-ray diffraction patterns of SHL-SUC 24 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

5.1.2 Stability study of shellac phthalate film

Figure 68 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 [10]. The AV of SHL and SHL-PHT film upon storage is shown in Figure 69. 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 68). 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.

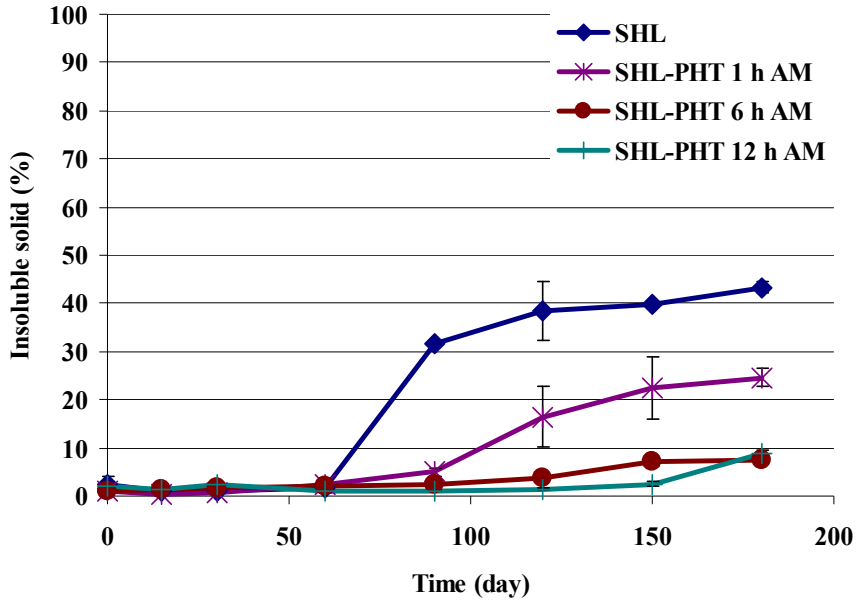

Figure 68 Change of percent insoluble solid of SHL and SHL-PHT films after storage at 40°C, 75% RH for 6 months

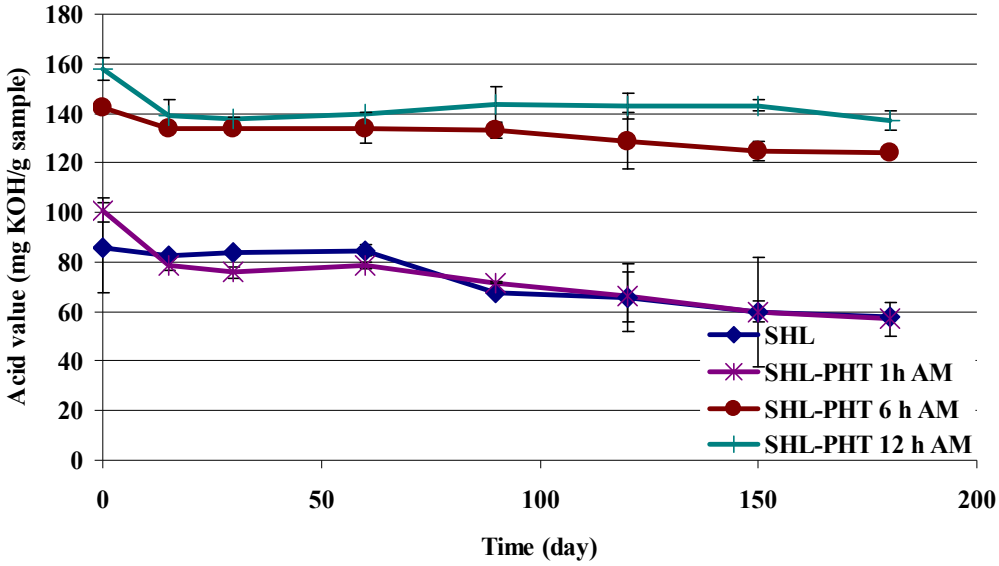

Figure 69 Change of acid value of SHL and SHL-PHT films after storage at 40°C, 75% RH for 6 months

Figure 70 demonstrates the change of percentage dissolved of SHL and SHL-PHT film at pH 6.8 during storage. The percentage 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 percentage dissolved was higher and the extended time, in which solubility decreased, was observed.

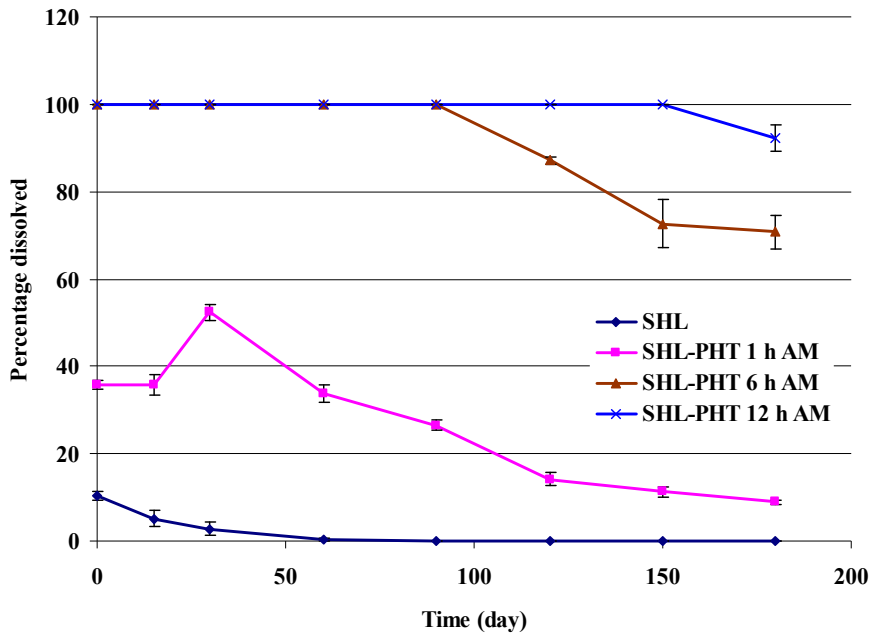

Figure 70 Change of percentage dissolved of SHL and SHL-PHT films in buffer pH 6.8 after storage at 40°C, 75% RH for 6 months

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 71. 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 72. 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.

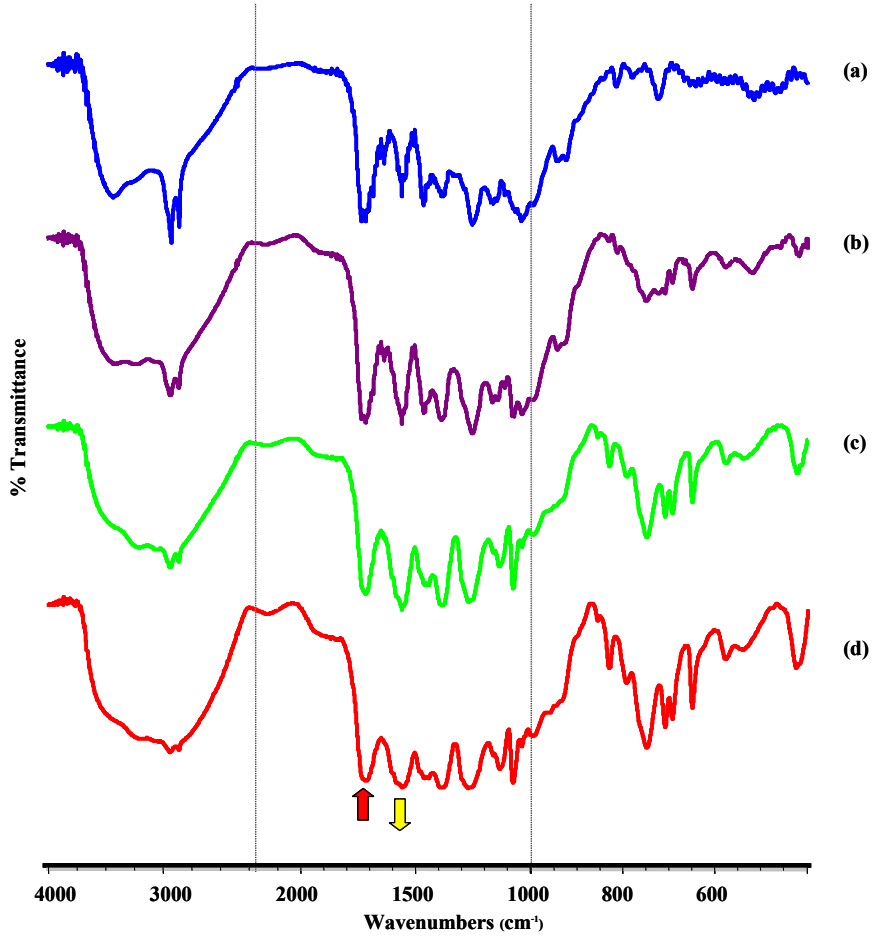

Figure 71 FTIR spectra of SHL and SHL-PHT films at initial; SHL (a), SHL-PHT 1 h AM (b), SHL-PHT 6 h AM (c) and SHL-PHT 12 h AM (d).

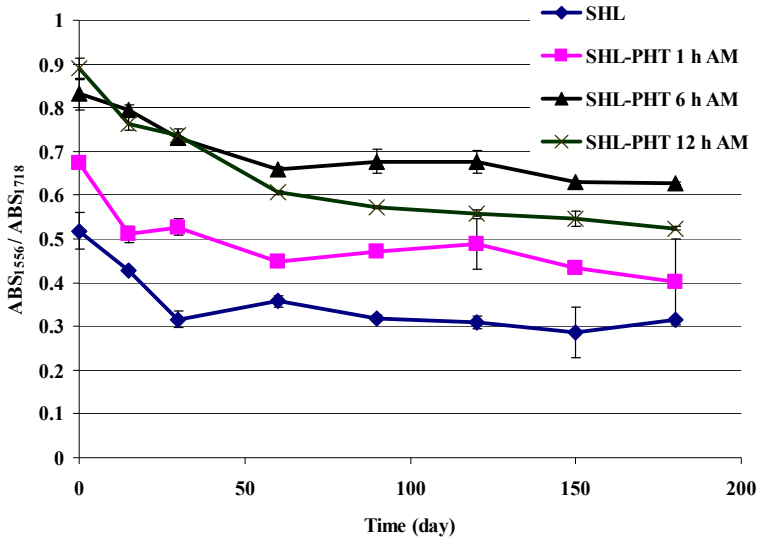

Figure 72 Change of ABS_{1556}/ABS_{1716} of SHL and SHL-PHT films after storage at 40°C, 75% RH for 6 months

The thermal behavior of SHL-PHT film upon storage at 40 ºC, 75 % RH for 6 months was investigated by DSC. Figure 73-75 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 $^{\circ}$C 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 monitored by DSC.

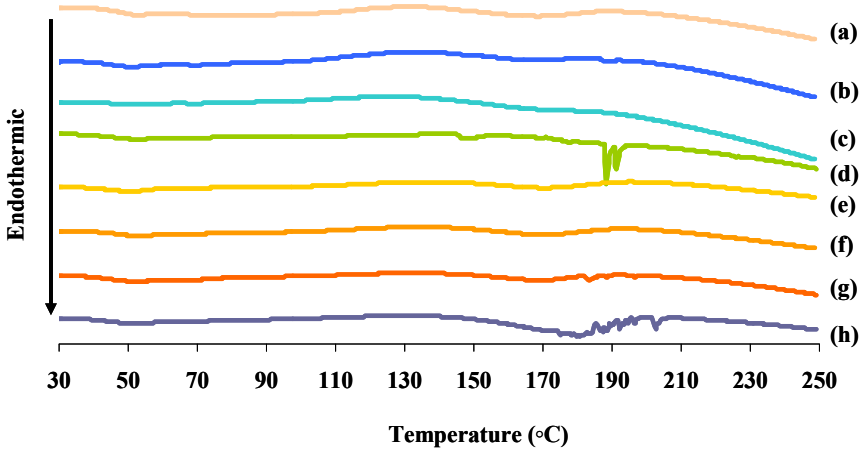

Figure 73 DSC curves of SHL-PHT 1 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

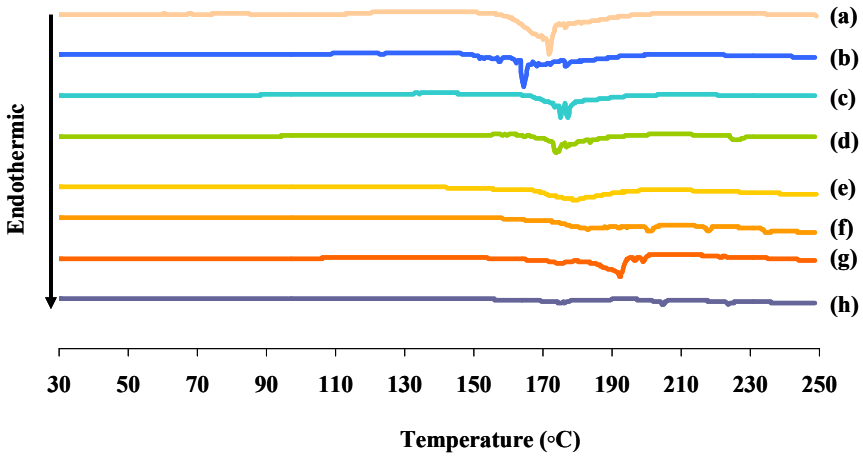

Figure 74 DSC curves of SHL-PHT 6 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

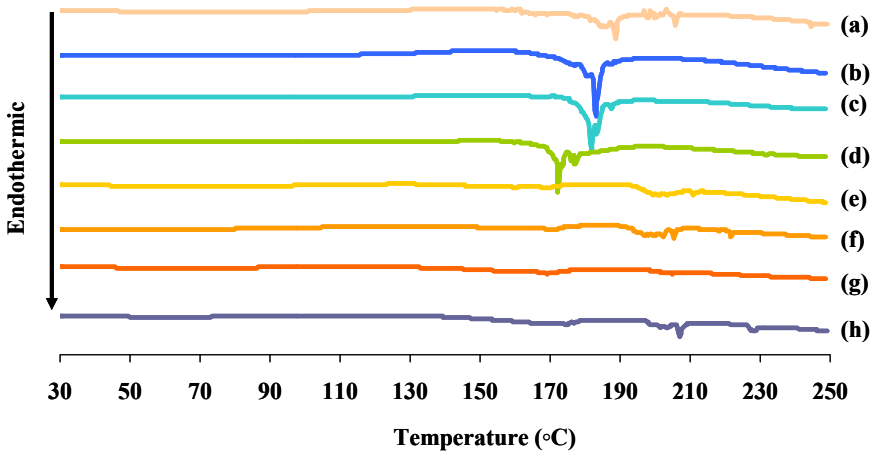

Figure 75 DSC curves of SHL-PHT 12 h AM films after storage; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 days (g) and 180 days (h)

Figure 76 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 77-78), 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 68). 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.

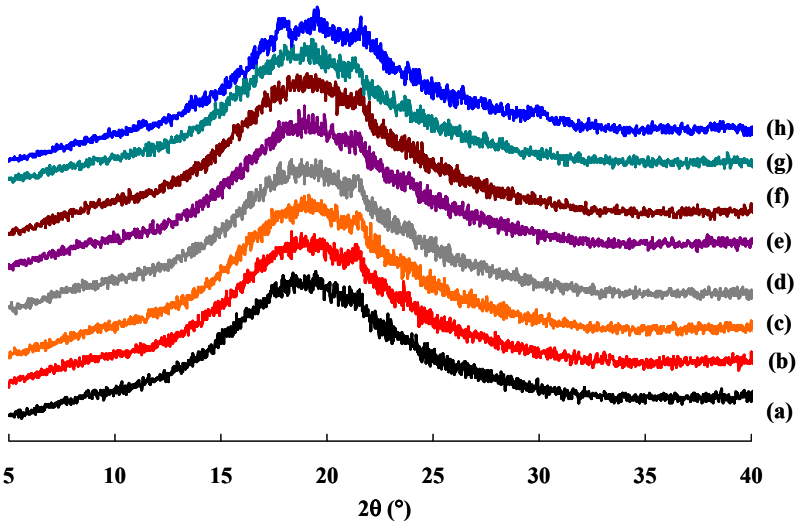

Figure 76 Powder X-ray diffraction patterns of SHL-PHT 1 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 day (g) and 180 days (h)

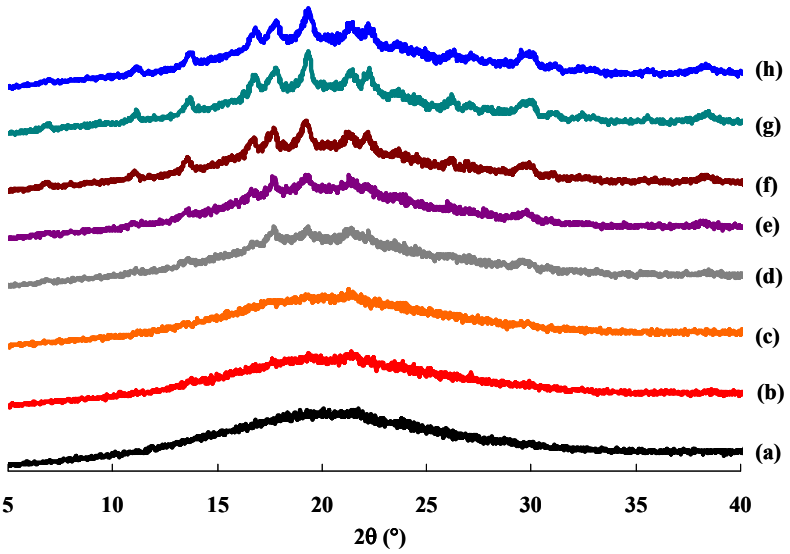

Figure 77 Powder X-ray diffraction patterns of SHL-PHT 6 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 day (g) and 180 days (h)

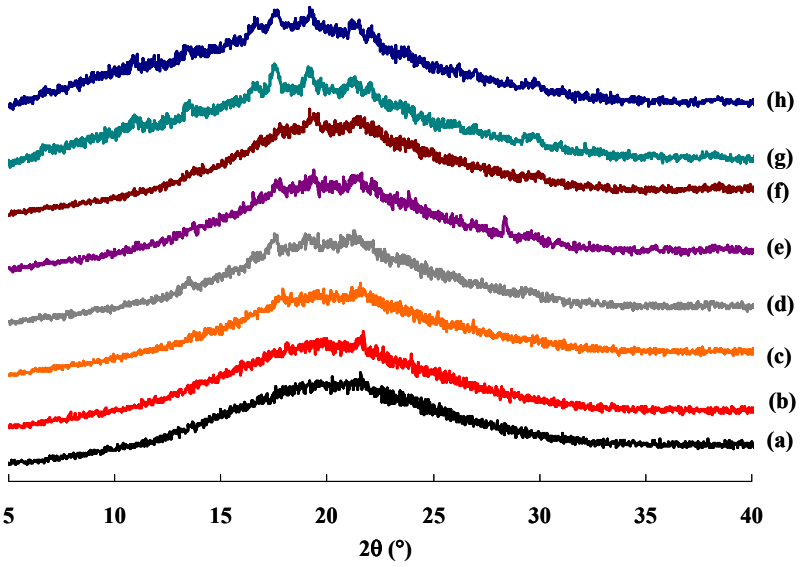

Figure 78 Powder X-ray diffraction patterns of SHL-PHT 12 h AM films; initial (a), 15 days (b), 30 days (c), 60 days (d), 90 days (e), 120 days (f), 150 day (g) and 180 days (h)

5.2 Stability study of shellac and shellac ester coated tablet

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 tablet were periodically withdrawn and then evaluated for tablet properties.

5.2.1 Stability study of shellac succinate coated tablet

The SHL and SHL-SUC 1 h AM coated tablet with the coating level of 6 mg/cm^2 and SHL-SUC 6 h AM and SHL-SUC 24 h AM coated tablet 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 tablet properties of SHL coated tablet after storage are shown in Table 9. 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 tablet 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 10-12, weight and hardness of SHL-SUC coated tablet were not significantly changed after kept in stability chamber. For disintegration results, the SHL-SUC coated tablets showed similar result as SHL coated tablet. 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 9 Tablet properties of SHL coated tablet 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	1.0469±0.0086	16.9±2.0	> 2 h	112.7±13.6
15	1.0575±0.0083	13.2±0.3	> 2 h	101.4±2.3
30	1.0510±0.0072	13.0±0.6	> 2 h	157.1±12.4
60	1.0580±0.0080	12.6±0.6	> 2 h	> 3 h
90	1.0577±0.0087	12.9±1.7	> 2 h	> 3 h
120	1.0537±0.0088	13.6±1.0	> 2 h	> 3 h
150	1.0529±0.0079	12.7±1.7	> 2 h	> 3 h
180	1.0573±0.0083	14.6±0.7	> 2 h	> 3 h

Table 10 Tablet properties of SHL-SUC 1 h AM coated tablet 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	1.0414±0.0093	11.4±0.6	> 2 h	12.3±0.5
15	1.0372±0.0083	11.0±1.1	> 2 h	33.1±13.5
30	1.0351±0.0111	11.3±0.7	> 2 h	58.6±16.2
60	1.0394±0.0118	11.0±1.4	> 2 h	> 3 h
90	1.0402±0.0098	10.8±0.6	> 2 h	> 3 h
120	1.0373±0.0077	10.8±1.2	> 2 h	> 3 h
150	1.0362±0.0089	10.9±0.2	> 2 h	> 3 h
180	1.0384±0.0094	11.0±2.3	> 2 h	> 3 h

Table 11 Tablet properties of SHL-SUC 6 h AM coated tablet 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	1.0579±0.0098	13.1±1.8	> 2 h	7.0±0.2
15	1.0323±0.0083	16.4±0.5	> 2 h	12.4±1.2
30	1.0633±0.0105	18.9±1.0	> 2 h	85.6±28.9
60	1.0613±0.0118	16.4±1.6	> 2 h	> 3 h
90	1.0581±0.0106	14.5±2.1	> 2 h	> 3 h
120	1.0619±0.0084	13.0±2.3	> 2 h	> 3 h
150	1.0616±0.0123	16.9±1.3	> 2 h	> 3 h
180	1.0611±0.0103	15.1±2.6	> 2 h	> 3 h

Table 12 Tablet properties of SHL-SUC 24 h AM coated tablet 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	1.0562±0.0069	18.6±2.0	> 2 h	4.1±0.3
15	1.0692±0.0151	12.5±1.1	> 2 h	3.9±0.4
30	1.0586±0.0071	12.2±0.8	> 2 h	7.4±0.5
60	1.0700±0.0091	13.0±0.6	> 2 h	23.5±7.9
90	1.0680±0.0081	10.4±0.9	> 2 h	167.8±9.1
120	1.0665±0.0085	11.1±1.0	> 2 h	165.7±12.6*
180	1.0674±0.0076	9.5±0.5	> 2 h	167.8±9.1

* 2 of 6 tablets dissolved within 3 h

The enteric coated tablet is 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 tablet are demonstrated in Figure 79. The percent drug released in phosphate buffer pH 6.8 of SHL coated tablet 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 80-82 demonstrates the dissolution profile of SHL-SUC coated tablet upon storage. The drug released of SHL-SUC 1 h AM, 6 h AM and 24 h AM coated tablet was remarkably declined after storage up to 15, 30 and 60 days, respectively. The results confirm the polymerization of SHL-SUC, but SHL-SUC 24 h AM coated tablet showed better stability (> 40% drug released) when storage up to 180 days, as compared to SHL and other SHL-SUC coated tablet (< 10% drug released).

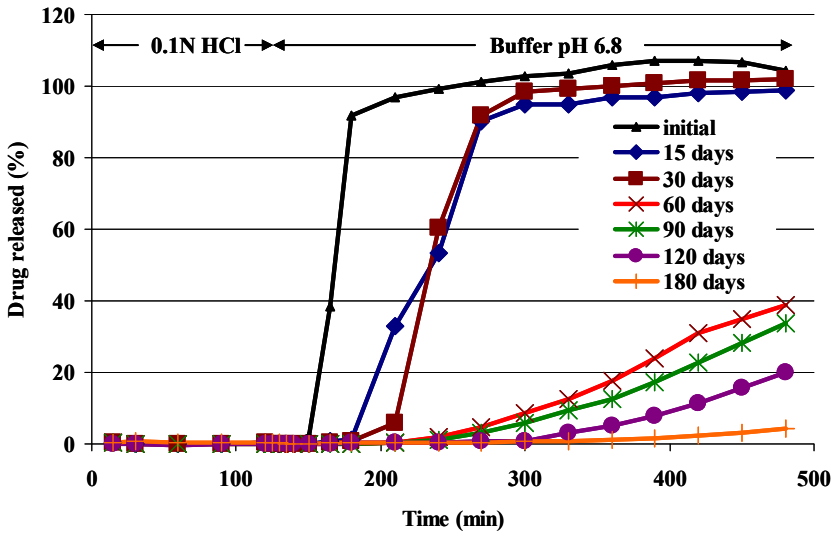

Figure 79 Dissolution profile of SHL coated tablet after storage

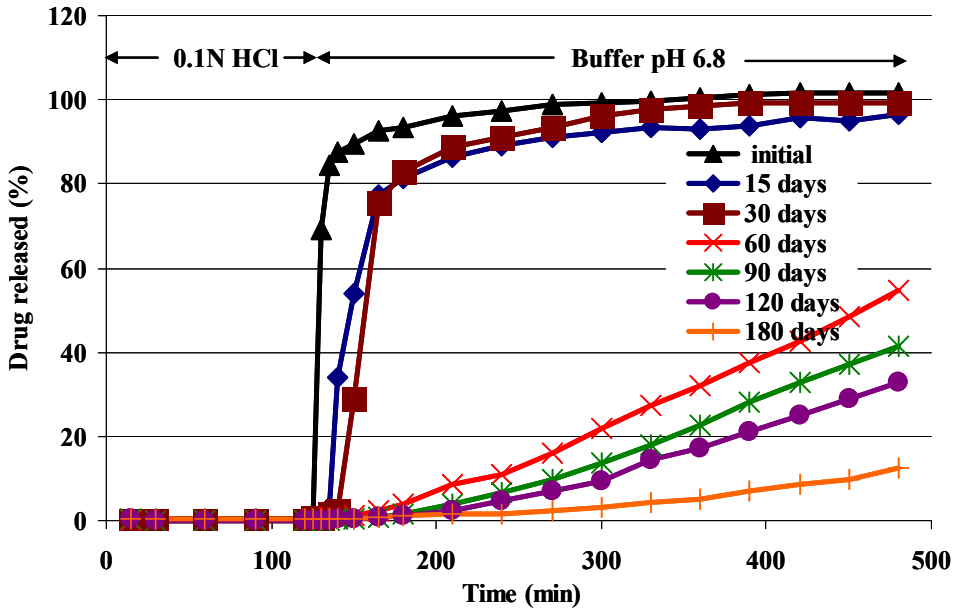

Figure 80 Dissolution profile of SHL-SUC 1 h AM coated tablet after storage

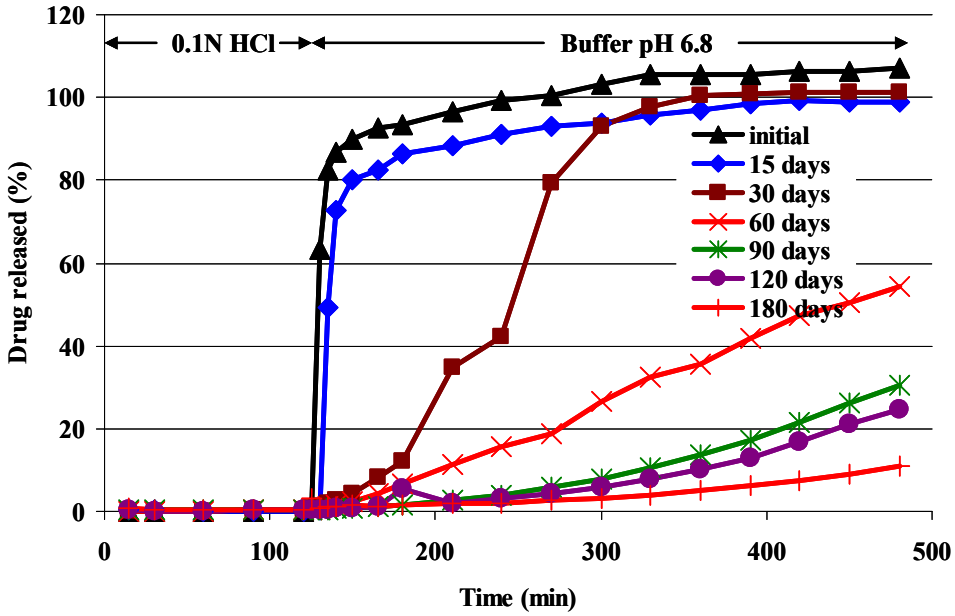

Figure 81 Dissolution profile of SHL-SUC 6 h AM coated tablet after storage

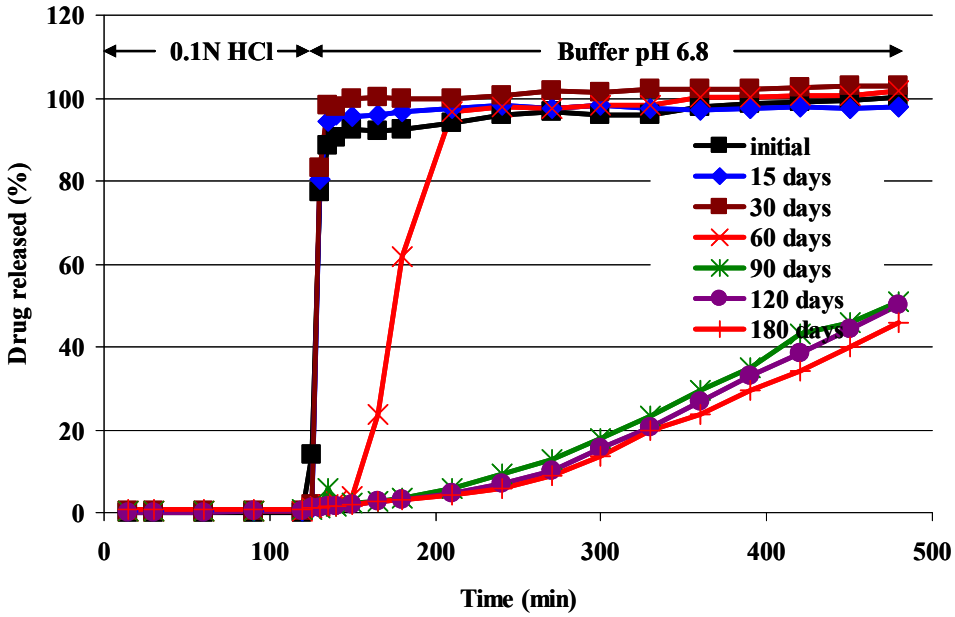

Figure 82 Dissolution profile of SHL-SUC 24 h AM coated tablet after storage

5.2.2 Stability study of shellac phthalate coated tablet

The SHL, SHL-PHT 1 h AM, SHL-PHT 6 h AM and SHL-PHT 12 h AM coated tablet 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 tablet were periodically withdrawn and comparatively assessed.

The tablet properties of SHL coated tablet after storage are demonstrated in Table 13. The change of weight, hardness and disintegration time of enteric coated tablet 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 14-16 shows the tablet of SHL-PHT AM coated tablet during storage. For SHL-PHT 1 h AM, the disintegration time of the coated tablet 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 tablet demonstrated better stability. The coated tablet 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 tablet was relatively higher as compared to SHL-SUC coated tablet.

Table 13 Tablet properties of SHL coated tablet 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	1.0492±0.0421	38.9±4.0	> 2 h	> 3h
15	1.0498±0.0465	37.7±4.5	> 2 h	> 3h
30	1.0490±0.0503	39.3±6.5	> 2 h	> 3h
60	1.0508±0.0430	37.5±2.4	> 2 h	> 3h
90	1.0502±0.0137	39.0±1.3	> 2 h	> 3h
120	1.0482±0.0317	36.4±4.4	> 2 h	> 3h
150	1.0499±0.0221	38.5±2.5	> 2 h	> 3h
180	1.0500±0.0545	39.0±2.4	> 2 h	> 3h

Table 14 Tablet properties of SHL-PHT 1 h AM coated tablet 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	1.0760±0.0241	39.7±6.0	> 2 h	91.2±4.5
15	1.0786±0.0188	40.8±3.9	> 2 h	> 3h*
30	1.0781±0.0271	40.8±5.8	> 2 h	> 3h**
60	1.0742±0.0386	40.9±1.2	> 2 h	> 3h
90	1.0754±0.0224	40.3±1.8	> 2 h	> 3h
120	1.0755±0.0313	41.5±5.7	> 2 h	> 3h
150	1.0786±0.0253	40.9±3.2	> 2 h	> 3h
180	1.0785±0.0317	37.9±2.8	> 2 h	> 3h

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

Table 15 Tablet properties of SHL-PHT 6 h AM coated tablet 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	1.1170±0.0365	40.5±6.3	> 2 h	13.0±1.6
15	1.1163±0.0361	40.6±2.8	> 2 h	31.1±1.1
30	1.1168±0.0290	39.7±1.9	> 2 h	37.0±7.3
60	1.1164±0.0183	40.6±2.7	> 2 h	153.5±13.1*
90	1.1176±0.0271	40.1±2.2	> 2 h	172.4±40.3**
120	1.1175±0.0240	40.8±2.3	> 2 h	> 3 h
150	1.1174±0.0294	39.4±3.7	> 2 h	> 3 h
180	1.1168±0.0336	40.2±5.1	> 2 h	> 3 h

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

Table 16 Tablet properties of SHL-PHT 12 h AM coated tablet 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	1.1110±0.0322	40.4±2.7	> 2 h	14.9±2.23
15	1.1163±0.0186	40.8±3.6	> 2 h	29.5±1.1
30	1.1178±0.0198	39.4±3.0	> 2 h	38.3±3.2
60	1.1153±0.0434	40.9±5.4	> 2 h	121.5±13.7
90	1.1145±0.0289	39.7±2.1	> 2 h	162.4±23.0
120	1.1121±0.0171	39.8±4.3	> 2 h	> 3 h
150	1.1182±0.0419	39.6±3.1	> 2 h	> 3 h
180	1.1166±0.0482	41.1±6.6	> 2 h	> 3 h

The dissolution profiles of SHL and SHL-PHT AM coated tablet after storage are shown in Figure 83-86. SHL and SHL-PHT AM coated tablet 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 tablet showed faster polymerization, the aging effect might be accelerated with hot air during coating process. Although the instability of the SHL-PHT coated tablet was observed, the aging effect was slowly occurred as compare to SHL and SHL-SUC coating.

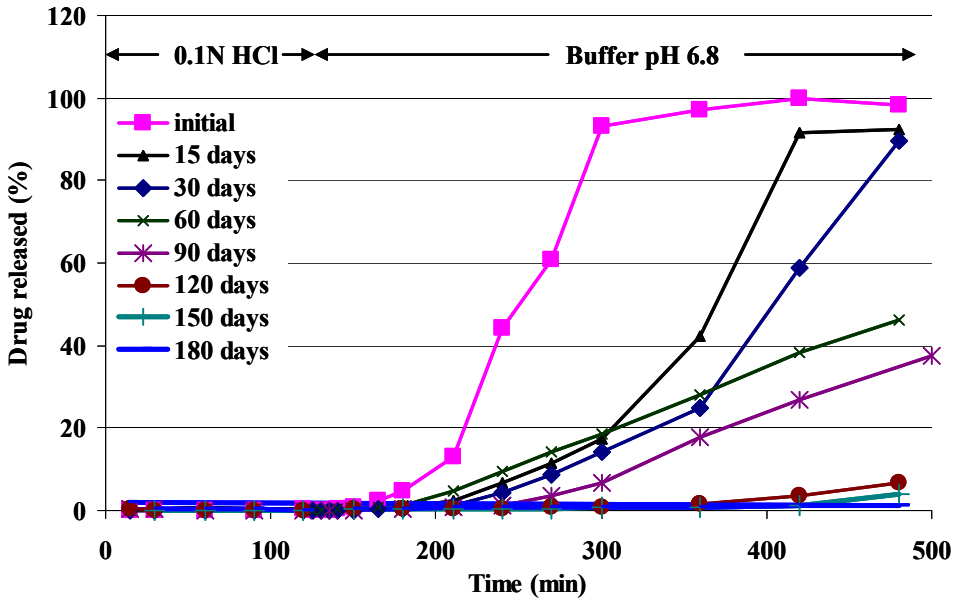

Figure 83 Dissolution profile of SHL coated tablet after storage

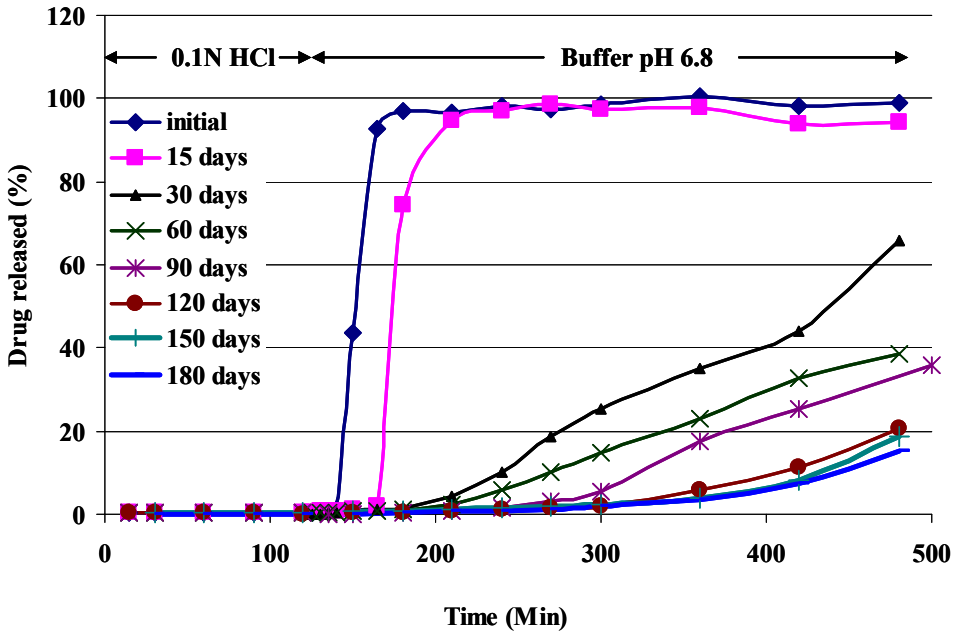

Figure 84 Dissolution profile of SHL-PHT 1 h AM coated tablet after storage

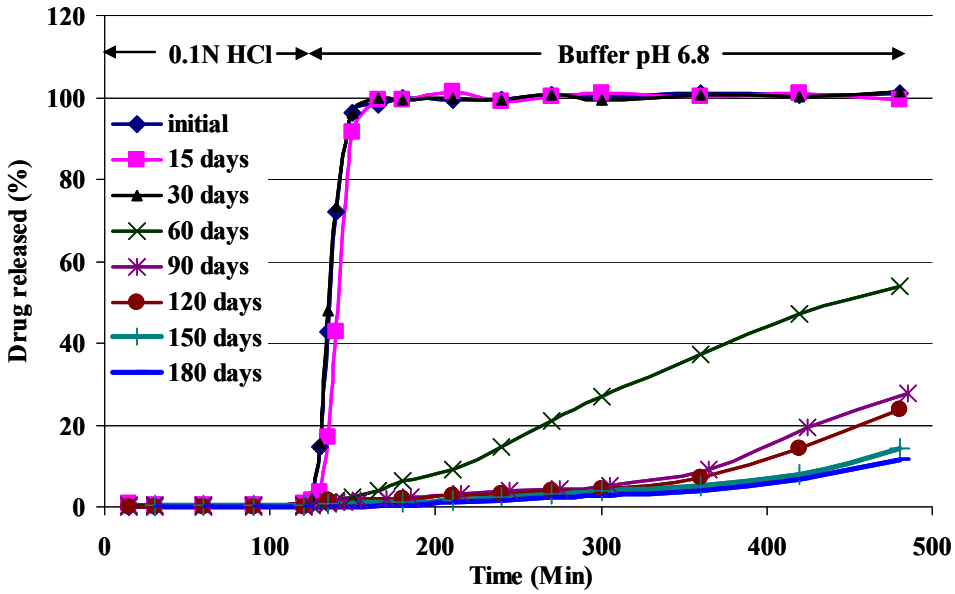

Figure 85 Dissolution profile of SHL-PHT 6 h AM coated tablet after storage

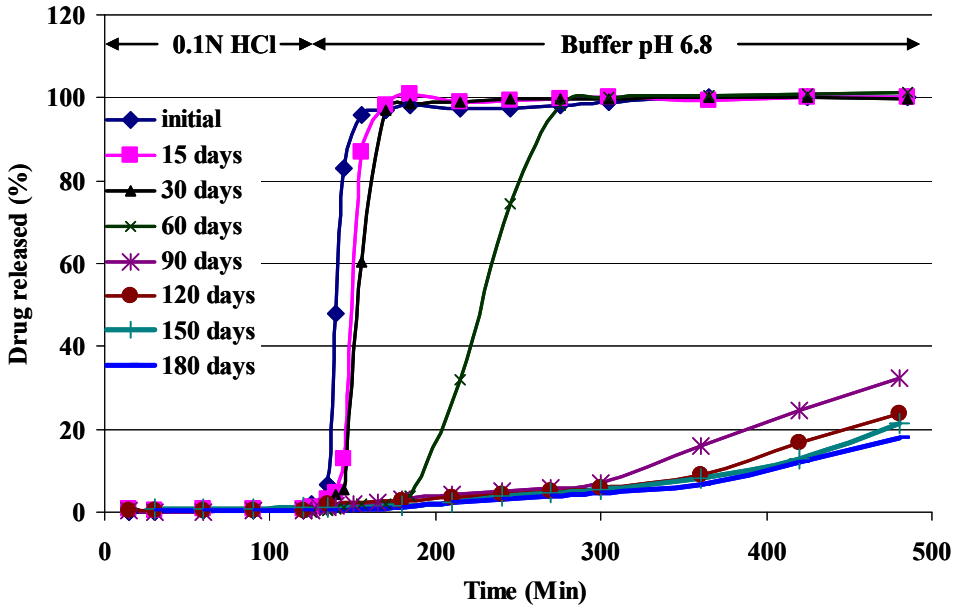

Figure 86 Dissolution profile of SHL-PHT 12 h AM coated tablet after storage

Figure 87 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 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 system, 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.

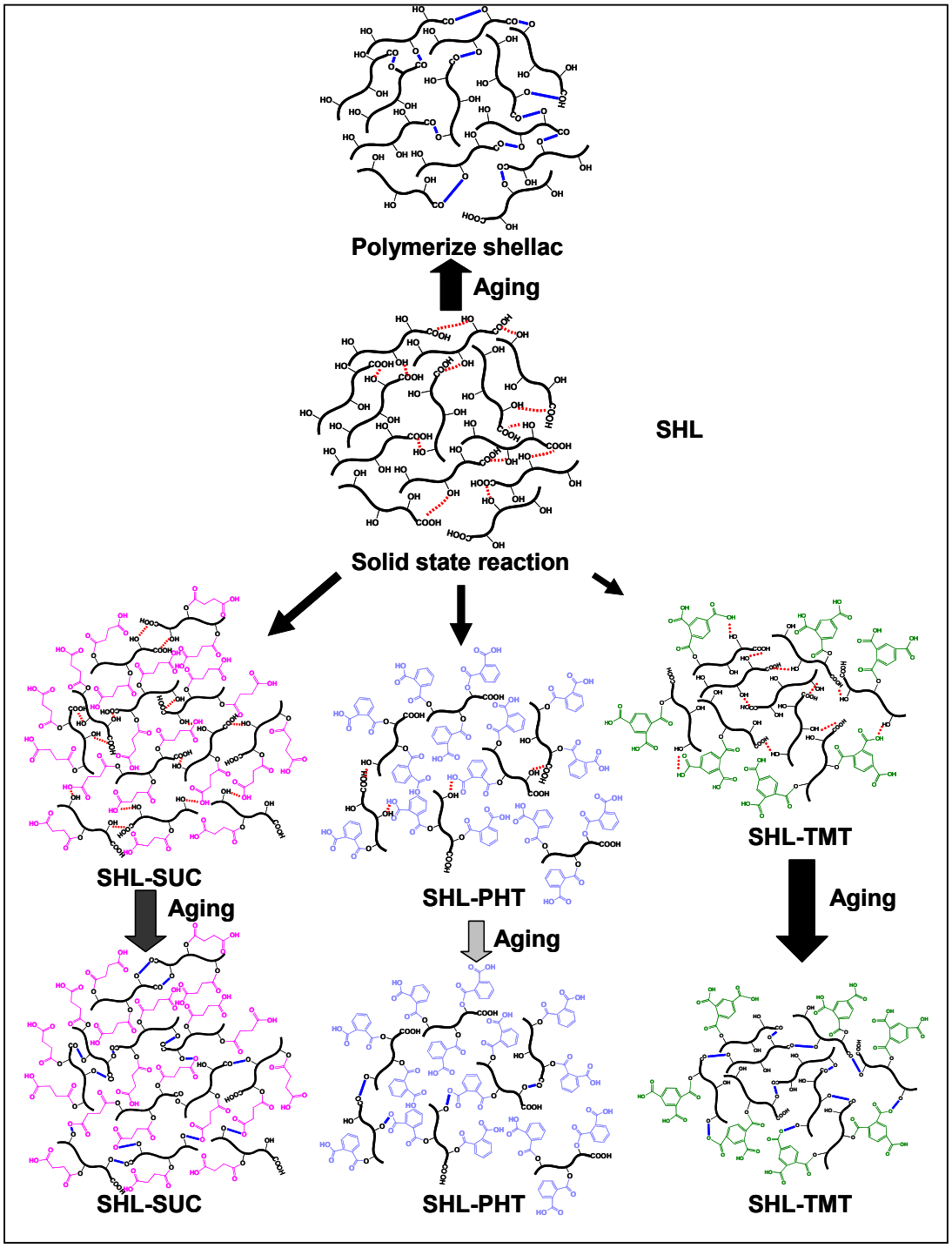

Figure 87 Proposed diagrams of shellac ester formation, aging effect and molecular arrangement of SHL and SHL-CAHs

Chapter IV

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. For SHL-SUC system, 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. From the previous studies, SHL had modified by patial hydrolysis with NaOH. The result showed that the acid value of hydrolysed SHL was increased. However, the acid value of shellac esters was higher as compared with hydrolysed SHL (Limmatvapirat et al. 2004: 41-9). 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. However, these signals were slightly changed in SHL-TMT system. 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. SHL was previously improved its enteric properties by forming salt with AMN and AMP. The modulus of 100:0 to 0:100 AMP:AMN salts of SHL film were in the range of 50-650 KPa, while that of SHL-SUC and SHL-PHT films were around 50-370 KPa. The high modulus resulted from the high puncture strength and the low percentage of elongation of 0:100 AMP:AMN. The data suggested that the flexibility of SHL-SUC and SHL-PHT film was as same as 100:0 to 40:60 AMP:AMN film of SHL. However, SHL-SUC and SHL-PHT showed better moisture protection (Limmatvapirat et al. 2007: 690-698).

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 hydrolysed SHL and SHL coated tablets were within 80 min and more than 2 h, respectively (Limmatvapirat et al. 2004: 41-9) 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 AM 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 tablet.

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.

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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 17 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 18 The percentage of drug released of SHL coated tablets (initial).

Time		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 19 The percentage of drug released of SHL-SUC 1 h AM coated tablets (initial).

Time		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 20 The percentage of drug released of SHL-SUC 6 h AM coated tablets (initial).

Time		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 21 The percentage of drug released of SHL-SUC 24 h AM coated tablets (initial).

Time		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 22 The percentage of drug released of SHL-PHT 1 h AM coated tablets (initial).

Time		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
	210	98.35	104.17	99.19	100.57	3.15
	240	97.71	100.20	96.86	98.26	1.73
	270	98.47	99.43	99.05	98.98	0.48
	300	0.15	0.41	0.59	0.38	0.22
	330	0.15	0.41	0.62	0.39	0.24
	360	0.18	0.45	0.63	0.42	0.22

Table 23 The percentage of drug released of SHL-PHT 6 h AM coated tablets (initial).

Time		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
	210	101.50	100.45	100.67	100.87	0.55
	240	100.77	100.82	100.67	100.75	0.07
	270	101.74	101.18	99.56	100.83	1.13
	300	0.31	0.15	0.07	0.18	0.13
	330	0.24	0.10	0.09	0.14	0.08
	360	0.34	0.13	0.15	0.21	0.12

Table 24 The percentage of drug released of SHL-PHT 12 h AM coated tablets (initial).

Time		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
	210	98.66	100.87	101.36	100.29	1.44
	240	98.53	100.87	100.54	99.98	1.27
	270	101.78	100.06	99.05	100.30	1.38
	300	0.12	0.20	0.19	0.17	0.05
	330	0.10	0.17	0.15	0.14	0.04
	360	0.15	0.45	0.22	0.27	0.16

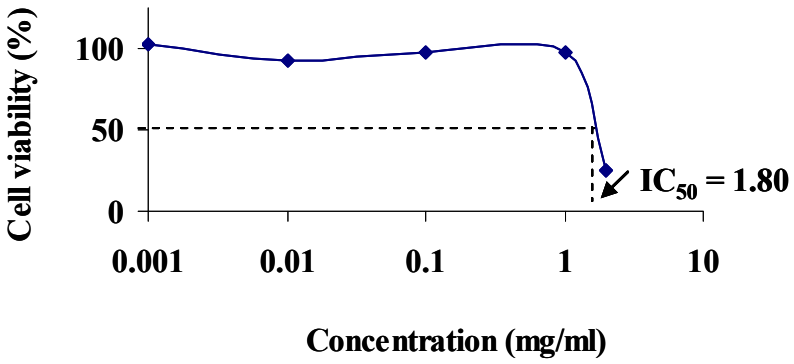

Figure 88 The effect of SHL on cytotoxicity incubated with Caco-2 cell.

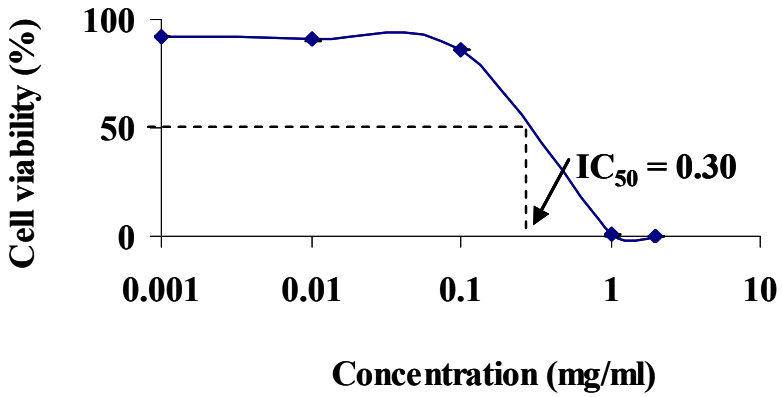

Figure 89 The effect of SHL-SUC 24 h AM on cytotoxicity incubated with Caco-2 cell

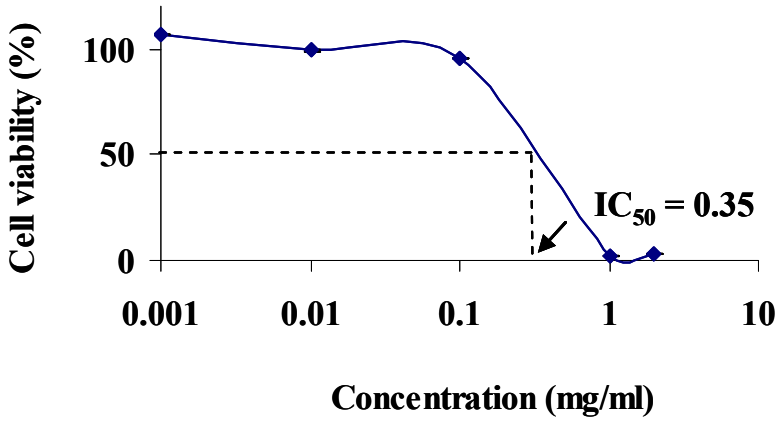

Figure 90 The effect of SHL-PHT 12 h AM on cytotoxicity incubated with Caco-2 cell.

Outcome of the Research Project

Papers

(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 J of Pharm and Biopharm, 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 (8): 1241-1244.

(4) 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. Thai Journal of Agricultural Science 2011, 4 (5), 224-229.

Conference Proceedings and Abstracts

(1) 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.

(2) Sangnim T, Panjapornpon D, Limmatvapirat C and Limmatvapirat S. Study of factors affecting solid state formation of shellac phthalate. The 3th Silpakorn University Research Fairs (SURF), 28-29 January 2010, Silpakorn University, Nakhon Pathom, Thailand.

(3) 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.

(4) 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.

(5) 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.

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