

DEVELOPMENT OF POLYURETHANE BASED FOAM DRESSING IMPREGNATED WITH
SILVER NANOPARTICLES AND ASIATICOSIDE FOR DERMAL WOUND TREATMENT

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บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmaceutical Technology

Department of Pharmaceutics and Industrial Pharmacy

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2017

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การพัฒนาแผ่นปิดแผลชนิดโฟมพอลิยูรีเทนที่มีอนุภาคนาโนซิลเวอร์และเอเชียติโคไซด์สำหรับรักษา
บาดแผลที่มีความลึกระดับที่สอง



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีเภสัชกรรม ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2560

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

Thesis Title DEVELOPMENT OF POLYURETHANE BASED FOAM
DRESSING IMPREGNATED WITH SILVER
NANOPARTICLES AND ASIATICOSIDE FOR DERMAL
WOUND TREATMENT

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ACKNOWLEDGEMENTS

My sincere gratitude goes first to my advisor, Professor Garnpimol C. Ritthidej for the continuous support of my Ph.D. research, for her patience, motivation and knowledge. I felt exceedingly lucky to have an advisor who understood, profoundly cared and responded to my questions. I also would like to thank Associate Professor Vimolmas Lipipun, my co-advisor for her reinforcement, precious guidance and for always responding to all my questioning.

The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship and the Agricultural Research Development Agency (ARDA) for financial support throughout my Ph.D. program were acknowledged.

Appreciation was expanded to Assistant Professor Nontima Vardhanabhuti for her encouragement in my academic endeavor. I would like to thank my thesis committee members, Professor Chomchark Chuntrasakul, Associate Professor Ubonthip Nimmannit, Assistant Professor Vorasit Vongsutilers and Assistant Professor Jutarat Kitsongsermthon for giving such a valuable time and all suggestions to improve my thesis.

I would like to deeply appreciate Assistant Professor Phingphol Charoonrut, Dr. Yada Akkhawattanangkul and Assistant Professor Tawewan Tansatit, Faculty of Veterinary Science, Mahidol University, Professor Pornprom Muangman, Dr. Kusuma Chinaroonchai and nurses at Trauma unit, Siriraj hospital, Mahidol University for their precious advices and supports in animal and clinical studies. I also thank all participants for their important contributions.

I would especially be obligated to South City Group Co., LTD., P. Chongcharoen Co., LTD. and Bangkok Biomaterial Center for supports in every part of research work.

I would like to extend my thanks to friends and staffs for their helps and motivation.

Finally, I would like to thank my father whose memory will be in every step of my effort and my beloved family for their supports and understanding during my Ph.D. study.

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LIST OF ABBREVIATIONS

Ag	Silver
Alg	Sodium alginate
AS	Asiaticoside
Bl	Blank, Foam without natural polyols
BMI	Body mass index
CMC	Sodium carboxymethylcellulose
CHMW	Chitosan high molecular weight
CLMW	Chitosan low molecular weight
CS	Corn starch
DSC	Differential scanning calorimetry
ECM	Extracellular matrix
EDX	Energy-dispersive X-ray spectroscopy
FTIR	Fourier-transform infrared spectroscopy
Ge	Gelatin
HPLC	High-performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
MC	Methylcellulose
MDI	Methylene diphenyl diisocyanate
PBS	Phosphate buffer saline solution
PGS	Pregelatinized starch, starch 1500
PPG	Polypropylene glycol
SEM	Scanning electron microscopy
TDI	Toluene diisocyanate
UTM	Universal testing machine
WVTR	Water vapor transmission rate
ZOI	Zone of the inhibition

CHAPTER I

INTRODUCTION

A wound is defined as a disruption of the skin barrier, caused by an accident, trauma, surgery or other medical disorders; such as bed sores, cancer, venous ulcer and diabetic ulcer. Foreign bodies can invade the discontinuous tissue and produce both local and systemic infections. As such, without any protective barrier, the harmful thermal, mechanical and chemical influences could also potentially harm the skin directly. Consequently, discussions on wounds are now major public health topics worldwide. Dowsett et al [1] reported that the cost of wound treatment rises every year; for example, in the United Kingdom, costs will have increased by more than 200 million pounds sterling from 2014 to 2019.

Wounds are categorized into two main groups - acute and chronic, which differ in the period of healing. However, acute wounds can develop into chronic wounds when they are not treated properly. These wounds may cause morbidity, long-term disability, and even death. They can also disrupt a patient's daily life, work, and quality of life. Patients are not only affected by the wound but also experience pain and stress. Thus, it has been suggested that pain from a wound can have an effect on the healing, which results in an adverse effect on the patient's quality of life [2]. Chase et al [3] reported that patients with chronic leg ulcers had limitations in their physical functions and vitality with most patients feeling moderate to severe pain. Pains affect all aspects of everyday life including physical activities, sleep, and social activities. In delayed surgical wounds, patients express negative feelings including frustration and powerlessness. Therefore, a wound is associated with physical and psychosocial functioning and wellbeing.

Consideration about the wound healing outcomes and assessment is the most important step in wound care. Management of wounds poses a great challenge to surgeons and all other associated professionals in this field. Lack of knowledge in diagnosis and understanding of treatment along with ineffective clinical practices can delay healing. This, in turn, increases the risk of complications, which add to the cost of treatment. For this reason, some major factors should be considered; for example, the type (acute or chronic), depth and size of the lesion. Risk factors should also be considered; such as, nutritional status, age, systemic diseases and medications, which impede the healing process. In addition, acute wound treatment can be categorized into three groups - a first, second and third degree wound. A first degree or epidermal wound includes sunburn that can heal by itself. A second-degree wound has etiology in its dermis layer; as such, topical and advanced wound dressings are the proper modalities. Reepithelialization is the main mechanism of complete wound closure. A third-degree wound has etiology in the muscle and adipose tissue layer, and this type of wound can heal imperfectly.



In this study, the second-degree wound or dermal wound was considered because this wound type could be healed perfectly without any surgery needed. The appropriate dressing for each wound type can facilitate the wound's closure. All conventional wound dressings; such as gauze, are used in routine clinical practice; however, they have numerous disadvantages. Another choice is an advanced wound dressing, which has high performance. However, the commercial ones are imported, and the cost is rather high. The price per piece of 10 x 10 cm² polyurethane foam dressing without silver costs approximately 250 Thai Baht while dressing with silver costs more than 500 Thai Baht. Some patients could not afford this, especially those in the middle-income salary bracket. Thus, foam dressing has been developed to be an alternative for wound management.

Apart from dressing properties, the prevention of infection and facilitation of the wound's closure is also important. Silver is an antimicrobial agent, and asiaticoside is extracted from *Centella asiatica*, a medicinal plant from the Apiaceae family. This

plant is found in abundance in most tropical and subtropical countries including India, Sri Lanka, China, and Thailand. It is also well-known for wound healing properties. Using this herb does not only promote wound healing but also supports agriculture. In Thailand, this herb was mentioned as a five-products champion in the National Master Plan on Thai Herbal Development 2017-2021 [4].

This study is an innovation of polyurethane foam dressing containing hydrophilic polyols, silver, and asiaticoside to treat second-degree trauma wounds. These two active compounds, which have two desirable effects in wound management, were also added in this formulation. The properties would conform to ideal wound dressing properties as follows:

1. Polyurethane dressing, which has several porous sizes, permits exudates absorption and air permeability. Adding hydrophilic polyol compounds would improve the capacity of water absorption and maintain moisture within the dressing. Moreover, foam dressing is soft and flexible, so it can be used to prevent further trauma and applied to various parts of the body.
2. Silver, which is a potential antimicrobial agent, treats and prevents wound infection.
3. Asiaticoside will facilitate the wound healing process by accelerating the proliferation of fibroblasts and increase the collagen synthesis.

Up to now, there has been no patent claiming about a foam dressing containing antibiotics plus asiaticoside. Apart from the foam characteristics, this project aimed to approach two main activities: infection management and acceleration of wound healing. The commercial brands contain only antibiotics without a compound, which increases the rate of wound healing. Adding asiaticoside would thus make the dressing superior to other commercial products; therefore, this is an innovation in the field of wound management, especially in Thailand. Patients would have a chance to access a new dressing at a reasonable price rather than have to purchase imported commercial brands.

The objectives of the present study were:

1. To produce polyurethane based foam dressing with hydrophilic natural polyols containing silver nanoparticles and asiaticoside.
2. To obtain foam dressing with satisfied physical properties, releasing profiles and compatibility and also presents efficacy and safety in preclinical and clinical studies.



CHAPTER II

LITERATURE REVIEW

Trauma injury

In some countries, trauma injury is a major issue regarding public health since it can occur to anyone at any time. The World Health Organization (WHO) reported that more than five million people die of this injury each year [5]. Every minute, more than nine patients are affected by trauma injury with the total percentage of fatalities being about 1.7 times more than the total percentage from HIV/AIDS, tuberculosis and malaria combined. All causes of injury have health consequences beyond physical injury, as these have an impact on a patient's life. A person might have a reduction in his/her work productivity that could result in him/her becoming unemployed followed by psychological distress. As such, tens of millions of people who suffer from injuries would require hospitalization, emergency/intensive care and specialized treatment. Thus, it can be seen that there is a positive relationship of injury severity and psychological distress among the Thai people [6]. The treatment of a wound does not only improve the healing, but also reduce the total cost of treatment including the actual cost and hidden cost.

Skin and wound healing

The skin is the human body's largest organ, which supports physiologic regulation. It covers a surface and has area range from 1.5 m² to 2 m² [7]. It acts as a physical barrier that prevents water loss and protects against mechanical, chemical and foreign matter invasions from the environment. Skin also helps to maintain body temperature by keeping warm with fat tissue in a subcutaneous layer and

vasoconstriction, as well as accelerating heat loss with sweat production and vasodilation.

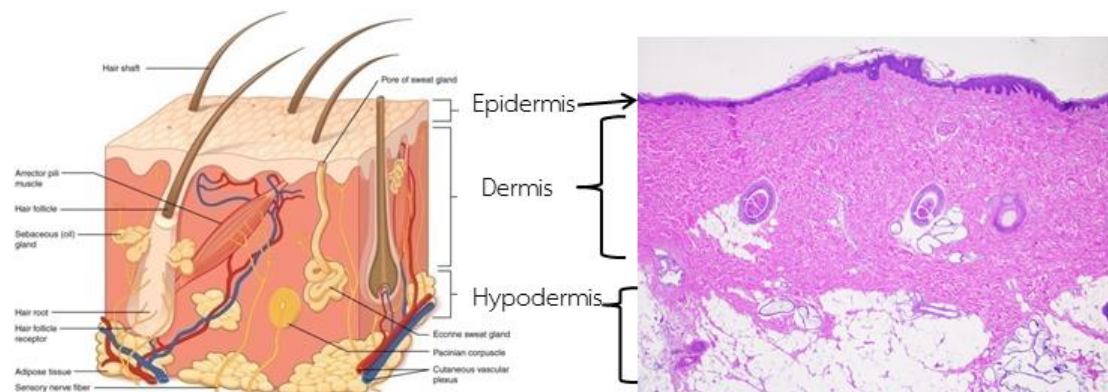


Figure II-1. Normal skin structure [8]

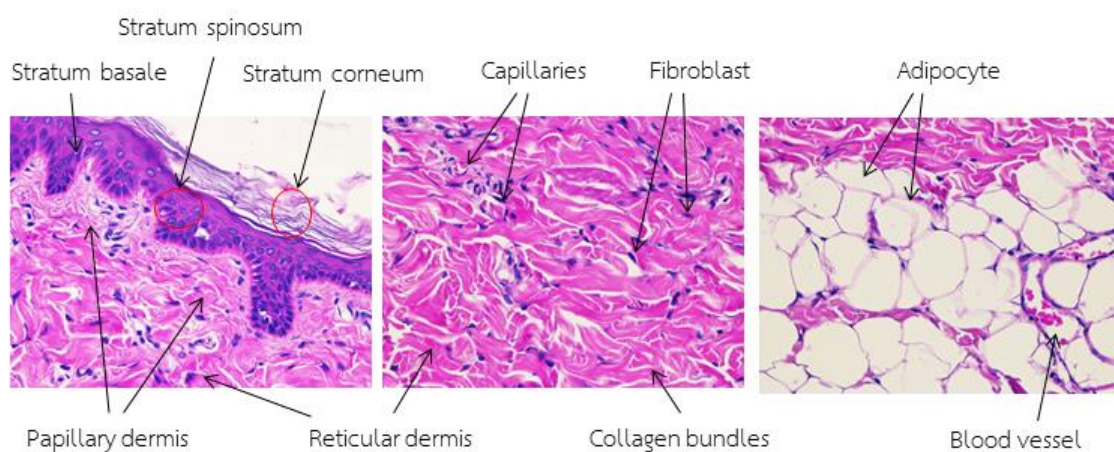


Figure II-2. Normal skin structure.

The skin can be categorized into three layers: the epidermis, dermis and subcutaneous layer [9, 10]. Each layer has its individual functions. (Figure II-1 to Figure II-2)

1. The epidermis layer is the outer layer of the skin. Its thickness relies on the location of the skin. The function of the epidermis involves fluid loss protection, mechanical prevention, chemical injury and microbial invasion. The epidermis is a stratified, squamous epithelium that consists mainly of keratinocytes, which are

differentiated from the stratum basale. These cells transform to become slightly flattened, move upward and differentiate in the stratum spinosum. The cells condense accumulated granules, which consist of aggregated keratin filaments. Finally, the flattened keratinocyte without a nucleus can be found in the stratum corneum before shedding. The stratum corneum plays a vital role, as the skin barrier defense system because it is the first layer contacting the external environment. The other cells which can be found in this layer are melanocytes and Langerhans cells. The Langerhans cells are an important part of the body's immune response. Pigment from melanocytes plays an important role in protecting the skin from ultraviolet radiation.

2. The dermis layer is beneath the epidermis layer and thicker than the epidermis. It is the scaffold structure consisting of collagen and elastic fibers in an extracellular matrix. The dermis composes of two layers: the papillary dermis and reticular dermis. The papillary dermis, located in the upper layer, consists of loose connective tissue while the reticular dermis is located in the deeper layer and comprises dense connective tissue. The extracellular matrix also contains blood and lymphatic vessels, nerve bundles, hair follicles and sweat glands. The nerves detect sensation, and the capillaries supply nutrients and oxygen to the cells. Different from the epidermis, the dermis contains lots of viable cells; such as, fibroblasts, dendritic cells, and macrophages.

The fibroblast is the major cell in the dermis, which produces and secretes elastic fibers and procollagen. The elastic fibers provide the skin elasticity. Procollagen, which is produced in the endoplasmic reticulum, is then transformed into collagen by the procollagen peptidases during exocytosis [11]. The collagen molecules are assembled to be collagen fibrils. The skin collagen consists of primarily type I and type III collagen (85% and 15% of the total collagen, respectively). The crosslinked collagen will provide the skin's strength.

3. The subcutaneous fat layer contains adipocytes and connective tissues that have larger blood vessels and nerves. It acts as a main storage site for fat and therefore energy. It also acts as a minor thermoregulatory.

From the skin structure which is divided into three layers according to depth, a wound can also be classified into three groups [12] as follows:

1. Epidermal wound (first-degree wound) has only pathology in the epidermis layer. This wound can heal itself within one week.

2. Dermal wound (second-degree wound or partial thickness wound) involves the epidermis and dermis layer, and is characterized by blebs, a pink-red color, moist and pain. It could be subdivided to be superficial partial thickness wound and deep partial thickness wound which both still retain viable cells. The appropriate treatment would facilitate wound closure within two to three weeks by epithelialization.

3. Full thickness wound (third-degree wound) involves the subcutaneous fat layer. There is no remaining viable tissue thus, it cannot heal by itself. The wound closure from the secondary intention would need to be considered.

Because the skin is the outer organ of the body, it tends to experience injury easier than other organs. Thus, wounds lead to loss of skin barrier properties. This can result in dehydration, loss of body temperature, and risk of infection. The term “open wound” is defined as the result of an injury of the skin which breaks the tissue. It can be classified into two groups depending on the healing time of a wound: acute or chronic. A trauma wound is defined as a skin injury occurring suddenly after a trauma; such as, abrasions, cuts, lacerations, puncture and burn wounds [13]. It can damage both the skin and underlying tissues which affect organ movement. However, acute wounds heal normally without any complications while chronic wounds take a longer time to heal and may have some complications.

Mechanism of wound healing

Wound healing is an important issue to treat trauma patients so to save life, decrease the chance of morbidity, and improve their activity in daily life. An understanding of the normal wound healing process could prevent an acute wound turning to becoming a chronic wound. Normal wound healing can be divided into overlapped four steps: hemostasis, inflammation, proliferative and remodeling [14] The

phases of wound healing are continuously occurring to repair and restore the cellular structure and tissues. (Figure II-3)

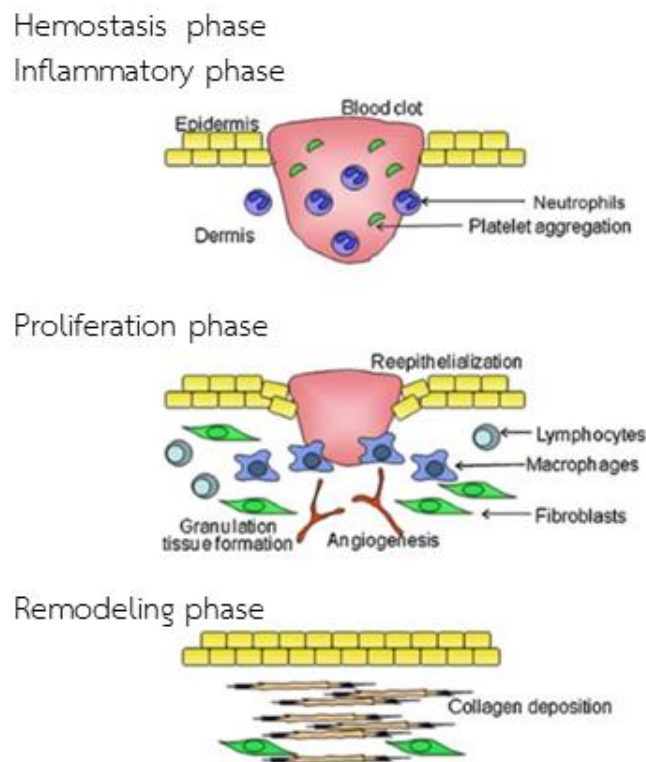


Figure II-3. Phases of wound healing [15]

1. Hemostasis / Coagulation phase occurs immediately after injury. Blood vessels begin constricting to decrease the amount of blood flow to the wound and eliminate blood loss. The activated platelets then adhere in the damaged tissue and aggregate together to form a platelet plug that temporarily blocks the blood flow. Fibrinogen is cleaved by thrombin to become fibrin and then cross links the framework and stops the bleeding. The platelets also secrete adenosine diphosphate (ADP) to activate thrombocytes. The thrombocytes and white blood cells release growth factors and cytokines; such as, a platelet-derived growth factor (PDGF), platelet factor IV, and transforming growth factor- β (TGF- β) into the local wound's environment. PDGF is a chemotactic for fibroblasts and TGF- β is

a potent modulator of fibroblastic mitosis, which lead to collagen fiber construction in later phases [16].

2. The inflammatory phase begins within 6-8 hours after hemostasis and extends for 2-3 days. Most characteristics of this phase are pain, swelling, the skin changes to a red color and heat. The main components, which leak from the vessels and influx to the wound, involve the polymorphonuclear leukocytes (PMNs) and macrophages. These cells eradicate bacteria, remove the debris and cleanse the wound. They also release various growth factors; such as PDGF, cytokines and interleukin (IL)-1 and tumor necrosis factor (TNF). The macrophages promote the proliferation of endothelial cells to form new capillaries, smooth muscle cells and the fibroblast to create the extracellular matrix.
3. The proliferation phase consists of many phenomena that include ECM deposition, angiogenesis, and reepithelialization. In the ECM deposition, the fibroblasts are activated, migrated and proliferated to produce collagen fibers. In normal wound healing, Type III collagen predominantly occurs and is then replaced by Type I collagen. The procollagen, the triple helix of tropocollagen, is secreted from the fibroblasts to the extracellular space. The peptidase enzymes cleave the terminal peptide chains and let the procollagen transform into collagen fiber [17]. The glycosaminoglycans (GAGs) and fibronectin are also produced by the fibroblasts and contribute to the ECM deposition. The new capillaries' formation requires the migration, mitosis, and maturation of endothelial cells. The basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are believed to regulate angiogenesis. After being renewed, the capillaries can provide oxygen and nutrients to the other cells. These can accelerate the wound healing effect. The keratinocytes also divide and increase in number and move to the center of the wound until the wound's closure. Reepithelialization is the process of the migration of epithelial cells from the periwound to the center. The

epidermal growth factor (EGF) is believed to play a main role in this aspect. The proliferation phase can last for two to three weeks.

4. The remodeling phase starts after two to three weeks since the injury and may take six months to two years. The proteinase enzymes; such as, matrix metalloproteinases (MMPs) are known as degraded wound tissue like collagen I, III, IV, and VII. The collagen rearrangement would increase the wound's strength by cross linking, degrading and replacing with more organized collagen. The wound would also create a contraction, which is believed to be regulated by myofibroblasts. These cells produce pull forces that shrink the edges of the wound together in order to close it.

From these steps, the primary goal in wound management is to facilitate the healing with good functions and aesthetic results. Inappropriate treatment leads to delayed healing, increasing the complications, length of hospital stay, and cost of treatment. This may result in hypertrophic scar healing. There are two factors involved in wound healing: local and systemic.

1. Local factors

- *Oxygenation* Cell: Metabolism normally uses oxygen for energy consumption. Oxygen can activate new capillaries' formation, proliferation and migration of epithelial cells, fibroblasts and collagen synthesis. The wound, which prolongs oxygen starvation, would delay healing [18, 19].
- *Infections*: Bacteria and foreign bodies can invade the wound easily. The body responds to these matters by inflammation, which is part of the healing process. The infection can affect chronic inflammation. Thus, the bacteria might form a biofilm to cover the wound bed so to increase bacterial resistance. The acute wound may become a chronic wound [20, 21]

2. Systemic factors

- *Age* [14]: An elderly person's skin structure may deteriorate compared to adult healthy skin. Consequently, the inflammatory process might be changed, and the function of the inflammatory cells, epithelial cells and

fibroblasts might decrease. This might affect a temporal delay in wound healing.

- *Nutrition* [22, 23]: Malnutrition can impede the rate of wound closure. It prolongs the inflammatory phase by reducing the proliferation of fibroblasts and formation of collagen. It also decreases the wound's tensile strength and increases the rates of infection. Essential amino acids; such as, L-arginine that affected to immunity, play an important role in the immune regulation by involving the immune response and inflammation [24]. Vitamin A, C and Zinc can help in collagen synthesis.
- *Comorbidity*: Diabetes, obesity and vascular disease can decrease the wound healing rate. People are also at risk of getting a new wound because of an insufficient blood supply [25]. In diabetes foot ulcers, the keratinocytes and fibroblasts present an absence of migration, proliferation, and incomplete differentiation, so it would be hard to close the wound [26].
- *Medications*: Steroids and immunosuppressive drugs might cause the immune system to become defective. Also, radiation may damage the normal tissue. The blood vessels would be injured and lead to hemorrhaging easily. The granulation tissue formation would thus be slowed down [27].
- *Alcohol consumption and smoking*: Chronic alcohol consumption can reduce immunity and may increase a risk factor for infection [28]. Nicotine from cigarettes may alter the oxygen supply by decreasing the tissue's blood flow. Moreover, carbon monoxide could bind the hemoglobin to be greater than that of oxygen resulting in the oxygenated hemoglobin in the bloodstream would be decreased [29].

Wound treatment

Generally, there are several modalities to treat the wound including topical antibiotics, wound dressing and surgery [30].

1. Topical antibiotics are usually prepared in cream, solution, and emulsion, which composes antibiotics; such as, povidone-iodine, chlorhexidine, silver sulfadiazine and neomycin. This prevents bacterial infection. Normally, it is recommended to change the dressing every day, so the patient may feel inconvenient and uncomfortable.
2. Wound dressings are frequently used with topical antibiotics. They can prevent trauma and other bacterial invasion.
3. Surgical procedures are the last method to use for wound treatment, especially for a deep wound with necrotic tissues. Debridement, removal of necrotic and infected tissue, and a skin graft whereby healthy skin is transplanted onto the wound are used to enhance secondary wound closure.

A wound dressing can be classified into two groups: temporary and permanent. Temporary wound dressings object to facilitate wound healing. This dressing type would be removed and changed until the wound's closure. On the other hand, permanent wound dressings are embedded in the wound's cavity in order to replace the missing skin tissue. As such, a surgical procedure is involved to close the wound through secondary intention. However, this research focused on a dermal wound which could heal by itself; therefore, a temporary wound dressing was selected for development in the study.

In selecting a temporary wound dressing, the wound should be assessed in size, depth, amount of exudate, presence or absence of infection or necrotic tissues, condition of the surrounding skin, and patient's well-being [31]. Nowadays, there are two types of wound dressings as follows:

1. Traditional wound dressing including gauze, gamgee™, cotton wool and bandages can be used as primary, secondary, or as part of several dressings. These dressings are dry and allow the evaporation of moisture leading to a dehydrated wound.

Concept of moist wound healing

Winter G (1962) studied the healing in superficial thickness wounds in a pig's model [32]. He reported that the dry wound contained the scab, which impeded the migration of the epithelial cells. In a moist wound, they move freely across a moist, vascular wound surface. Other studies also confirmed the advantage of a moist environment in which this condition can accelerate the inflammatory response and stimulate cell proliferation and wound healing in deep dermal wounds [33].

2. Advanced wound dressings have been developed to improve the defects of a traditional wound dressing. Their important characteristic is to retain a moist environment around the wound to accelerate wound healing. This can prevent cellular dehydration and stimulate collagen synthesis, epithelialization and angiogenesis. Applying an occlusive dressing over the wound can reduce the loss of fluid through evaporation.

The commercial dressings can be sub-classified according to their materials; such as, thin films, hydrocolloids, alginates and foam sheets. (Table II-1)

Table II-1. Strengths and weaknesses of an advanced wound dressing [34, 35]

Type	Strength	Weakness
Film	<ol style="list-style-type: none"> 1. Can be used as a primary or secondary dressing. 2. Transparency. Wound is visible without removing the dressing. 3. Waterproof. 	<ol style="list-style-type: none"> 1. Has limited absorbent properties. 2. Cannot apply on infected wounds. 3. Removal can be difficult.
Hydrocolloids	<ol style="list-style-type: none"> 1. Appropriate for low exudates. 2. Reduces further trauma from changing the dressing. 3. Are easily removed. 	<ol style="list-style-type: none"> 1. High exudate can leak from the dressing. 2. Sticky; may not be convenient.
Alginates	<ol style="list-style-type: none"> 1. Suitable for medium to high Exudates. 2. Some have hemostatic properties. 	<ol style="list-style-type: none"> 1. Dressing has low strength. It can tear. 2. May cause discomfort on dry wounds.
Foam	<ol style="list-style-type: none"> 1. Medium to high exudates. 2. Good air permeability. 3. High absorption. 	<ol style="list-style-type: none"> 1. May require secondary dressings. 2. After absorbing the exudate, it may not be close to the wound.

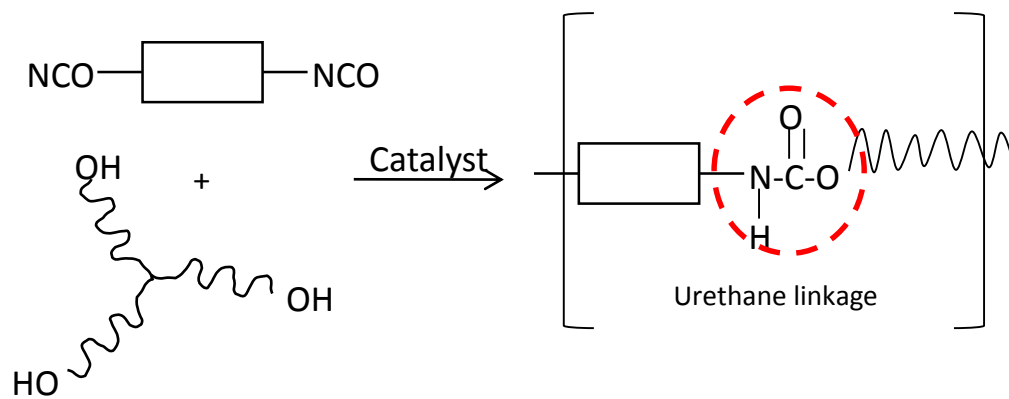
Wound dressings are medical items, which are still imported into Thailand. Most of them have a high cost of treatment. The Thai Customs revealed that the total value of imports of wound dressings had gradually increased every year from 2012 to 2017. In January 2018, there was nearly 10 million baht of imported wound dressing [36]. This shows the importance by patients to access these products. If there is a new wound dressing that can be produced in Thailand, this would help the patients. Up to now, there has been no dressing that could commit to all of the ideal wound dressing

characteristics. To develop a dressing locally, there are some ideal criteria that researchers should take into consideration as follows: [37]

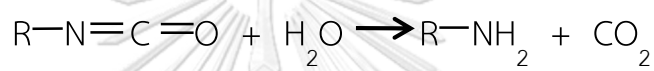
1. Capable of maintaining the humidity at the wound's site.
2. Non-toxicity and non-allergenic.
3. Capable of protecting the wound from further trauma.
4. Impermeable to bacteria.
5. Will allow gaseous exchanges.
6. Thermally insulating.
7. Comfortable and conformable.
8. Requires only infrequent changes.
9. Affordable.

Polyurethane [38]

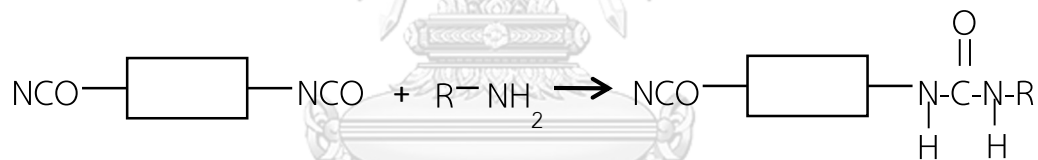
Polyurethanes are the synthetic macromolecules containing a repeated urethane linkage. They were first synthesized by Otto Bayer in 1938 then progressive development was continuously performed and the applications have been increasing. Because of their excellent mechanical properties, stability and good biocompatibility, they are widely used in every field of life; such as, automotives, furniture, footwear and clothing. Polyurethanes have also been developed and used in the medical and pharmaceutical fields; such as, artificial hearts, feeding tubes, catheter tubes, surgical drains and wound dressings. Polyurethanes have been typically made by the reaction of a polyol with a diisocyanate and the adding of other additives; such as, chain extenders, catalysts, and blowing agents. The main reactions of polyurethane were presented in Equations II-1 and II-2. The gelling reaction resulted from the polyols binding to an isocyanate to get urethane linkage (-NH-COO-). The reactivity of the hydroxyl group decreased in the order of the primary hydroxyl > secondary hydroxyl > phenol, respectively. The blowing reaction was caused by the isocyanate reacting with water to produce carbon dioxide gas. However, the NH_2 group of nucleophilic reactants (from Equation II-2) could react to the NCO group of electrophiles (from Equation II-1) that would generate the urea linkage (Equation II-3).



...Equation II-1



...Equation II-2



...Equation II-3

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The properties associated with their structures presented a linkage of the backbone. (Figure II-4) The polyurethanes as segmented polymers consisted of both soft and hard segments. The long chain polyols or soft segment acting as a backbone provided flexibility to the polymer. The diisocyanate combined to form the hard segment, which acted as a cross link which provided the foam's strength.

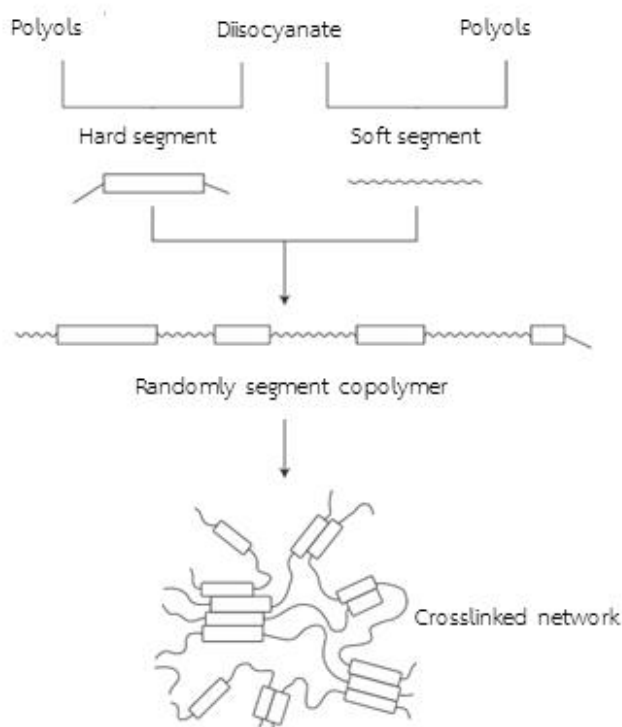


Figure II-4. Polyurethane crosslinked network [38]

Polyols

Polyols are a major part of polyurethane production and have a molecular weight between 400-5000 g/mol. They also contain the 2-8 hydroxyl groups at the end of the chain [39]. The length of the chain will affect the polyurethane properties as the soft segment. Increasing the molecular weight will create a flexible elastomer while decreasing the molecular weight will produce hard plastics.

Isocyanate

The isocyanates can be either aromatic or aliphatic monomers. The aromatic isocyanates are more reactive than the aliphatic ones, and they are also less stable in the light. The electron withdrawing group of the aromatic ring can increase a partial positive charge on the isocyanate carbon, so that the reaction can occur easily. In addition, the reactivity of an isocyanate group also depends on their substituents, stereochemistry and steric effect of the isocyanates. The 2,4 toluene diisocyanate (2,4 TDI), which has the NCO groups at a para position, is more reactive than the 2,6 toluene

diisocyanate (2,6 TDI) which presents the NCO groups at the ortho position [39, 40]. The most important aromatic isocyanates, which are used in the polyurethane industry, are toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI).

Silicone surfactant

The silicone surfactants are non-ionic surfactants, which emulsify incompatible ingredients by reducing surface tension. They can help the creation of bubbles during the mixing process. They also stabilize the cell wall when the foam is rising. In a formulation without a surfactant, the foam cells may be coalescent resulting in the foam's collapse.

Catalysts

The catalysts are used for the acceleration of the reaction time of the isocyanates with the polymer formation. They can change the relative speed of each reaction, so this can change the structure and properties of the foam. The most commonly used catalysts for the reaction with isocyanates are amine and tin catalysts. Tertiary amines; such as, triethylene diamine (DABCO) can accelerate the reaction of the isocyanate groups with water. The organometallic compounds; such as, tin catalysts involve the reaction of the isocyanates with the hydroxyl groups.

Polyurethane Foam Dressing

Foam dressing is a wound dressing, which has a high capacity of exudates absorption and provides a moist environment. It prevents exudates pooling and macerating the periwound environment. Wound exudates can impede healing, and bacteria can damage healthy granulation tissue. Moreover, doctors may increase the time between the dressing changes. The foam dressings have various sizes of small open cells which pull the exudates into the cells. Foam dressings are flexible, so they can prevent further trauma and can be cut to fit any body parts; such as, fingers, neck, toes, or ears. Payne et al [41] assessed the differences in treatment costs and cost-effectiveness between an advanced foam dressing and saline-soaked gauze in patients with leg ulcers. They summarized that the cost of treatment using a foam dressing was

cheaper than the saline-soaked gauze, and foam dressing provided more cost-effective treatment than saline-soaked gauze. Opananon et al [42] reported that wound treatment with a foam dressing showed lower pain scores than the 1% silver sulfadiazine cream. There was also a significantly lower number of wound dressing changes, nursing time, and shorter healing time. The commercial brand of foam dressings are Allevyn[®], Askina[®] Calgitrol, Mepilex[®] Ag and Urgocell[®] Ag.

The patents of polyurethane foam dressing

Lock et al (1986) [43] claimed that wound dressing was made of a synthetic plastic material, which had a water permeable property. It was produced by the polymerization of polyoxyethylene polyol with a polyisocyanate, the presence of a cross-linking agent or catalyst and without water. The polyol is a polyoxyethylene glycol having an average molecular weight of approximately 3000 and polyisocyanate is toluene diisocyanate (TDI). The approximate composition is follows: polyols 100 parts, catalyst 0.1-1.0 parts, silicone oil 1-10 parts and TDI 25-50 parts.

Honeycutt et al (1988) [44] stated that a foam polyurethane membrane was non-adherent, and had a thin film layer for maintaining the wound close to the body's temperature. The polyurethane prepolymer was selected from the group consisting of a polyethylene glycol diol with available isocyanate groups, a polyoxyethylene diol with available isocyanate groups, a mixture of a polyester and a diisocyanate, and a mixture of a polyether, a diisocyanate, and a polyoxypropylene diol capped with at least two diisocyanate end groups. The foam membrane with the thin film layer had a moisture vapor transmission rate greater than 0.30 grams/inch² /day and the density was less than 0.6 g/cm³.

Herrington et al (2004) [45] asserted that flexible polyurethane foam was made from the reaction in the presence of a blowing agent, a polyisocyanate and an active hydrogen containing composition comprising modified vegetable oil-based polyol. The ratio of this formulation was 1 to 40 parts by weight of a modified vegetable oil-based

polyol per 100 parts of an active hydrogen containing composition and polyether polyols.

Csati et al (2013) [46] declared that polyurethane foam comprised of at least one isocyanate and a polyol blend, which composed of at least one petroleum-based polyol and at least one natural oil-based polyol. To produce a polyol prepolymer, one natural oil-based polyol and one petroleum-based polyol in a ratio of between about 20:80 and about 80:20 based on the total weight that were mixed with the first isocyanate. For the foaming reaction, the polyol prepolymer was mixed with water at water concentrations of up to about 20% based on the total weight of the polyol prepolymer and water.

Braun et al (2012) [47] claimed that the foam contained prepolymer. It composed of the first polyol that was a triol having a molecular weight from 500 to 3,000 and a second polyol that was a diol having a molecular weight from 4000 to 12,000 mixed with isocyanate to get an isocyanate-terminated prepolymer. It also contained a weight of 0.5-4% of the surfactant. The final foam composition formed an open cell structure foam having open cells in the range of from 60% to 95% by weight.

Meike et al (2015) [48] mentioned about the composition for hydrophilic aliphatic polyurethane foams, which was composed of 45-80 parts by weight of hydrophilic polyisocyanates, 5 to 50 parts by weight of heterocyclic oligomers of low molecular weight aliphatic diisocyanates, 1 to 50 parts by weight of water, 0 to 1 part by weight of catalysts, 0 to 10 parts by weight of surfactants, and 0 to 20 parts by weight of alcohol and isocyanate-functional prepolymer.

Aou et al (2016) [49] claimed about the production of resilience polyurethane foam should contained 70-150 isocyanate index, 50.0-99.9 percent of at least one polyol, 0.1-50 percent of additives which including at least one catalyst, one surfactant, water not more than 2 percent. The aqueous phase would be dispersed in mixing of

liquid phase and solid phase at room temperature and atmospheric pressure. The liquid phase was water and the solid phase was an acid or an acid-modified polyolefin.

Natural hydrophilic polyols

Natural hydrophilic polyols have outstanding properties in water absorption. Because of their polar functional groups, they tend to react with water or other polar substances. They have been used in the pharmaceutical field for decades. The various advantages of natural plant-based materials include the following [50]:

1. *Biodegradability and eco-friendly*: They are obtained from natural sources and the manufacturing processes are not complicated. The fragmentation of the macromolecules to a lower molecular weight occurs as a result of some reactions; such as, photodegradation, oxidation and hydrolysis, and degradation by microorganisms.
2. *Biocompatibility and non-toxicity*: The reports of their toxicities are low compared to a synthetic polymer. They can conduct their functions without an undesirable host response. The Food and Drug Administration (FDA) approved several natural polymers as a medical ingredient. There are numerous research studies examining their applications in the pharmaceutical field.
3. *Inexpensive cost*: The sources of natural polyols are abundant, especially in agricultural countries. Thus, the cost of production is less than synthetic polymers.

The natural polymer can be classified according to the source of origin: plant and animal.

1. Natural polyols from plants

- a. Starches [51] are macromolecules, which are made from α -D-glucose units consisting of two forms of molecules: the linear amylose and the branched amylopectin. (Figure II-5) The ratio of the amylose/amylopectin depends on the plant source. They have lots of hydroxyl groups on the starch chains: at C-2 and C-3 of each glucose residue containing the secondary

hydroxyl groups and at C-6 containing the primary hydroxyl group when it is not linked. Starches which are deposited in granules show a hydrophilic property. They have strong intermolecular binding through hydrogen bonding. With the heating process, the water molecules can easily penetrate in the tight regions of amylopectin, which contains amylose. The amylose chains begin to dissolve and cause swelling. The advantages of starch, which is widely used in many fields, are its abundance, inexpensiveness and complete degradability through hydrolysis and enzyme degradation. Compared to other conventional polymers, starches may provide high viscosity in the manufacturing process.

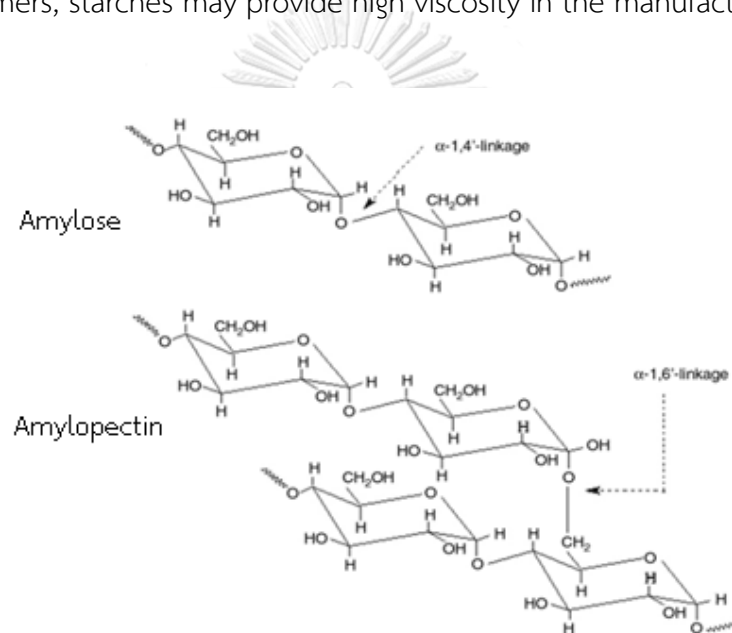
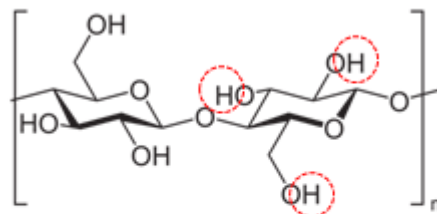


Figure II-5. Starch structure [52]

b. Celluloses [53] are a very important polysaccharide because they are the most abundant organic compound. They consist of a linear chain of $\beta(1\rightarrow4)$ linked D-glucose units. (Figure II-6) Unlike starches, celluloses have no coiled or branched structures. Cellulose molecules are arranged in a parallel position and link other chains together with hydrogen bonds. This can form microfibrils with a high tensile strength. Celluloses have a hydrophilic property and are strongly solvated because the three hydroxyl groups can react with water. They also partially or fully bind together to substitute groups to

afford derivatives with useful properties like cellulose esters and cellulose ethers (-OR); such as, methylcellulose (MC), hydroxypropylmethyl cellulose (HPMC) and carboxymethyl cellulose (CMC). In hydration, water penetrates into the polymer chains that rely on the integrity of the polymer network, which is influenced by the hydroxyl group and size of the substituted group. After that, the polymer chains are strained and shifted to reduce the strength leading to new hydrogen bonds [54].



HPMC ; R = H or CH₃ or CH₂CH(OH)CH₃

MC ; R = H or CH₃

CMC ; R = H or CH₂COOH

Figure II-6. Cellulose structure [55]

c. Alginate is a linear copolymer of a (1-4) glycosidic linkage between two monomers: β -D-mannuronic acid (M) and α -L-guluronic acid (G). (Figure II-7) They are extracted from the algae using a base solution and then reacted with acid to result in alginic acid. Sodium alginate is the sodium salt of alginic acid. The physical properties of alginates depend on the relative proportion of the three types of blocks: M, G, and MG. To form hydrogel, the common method is ionic cross linking. The G-blocks of alginate are believed to be involved in the intermolecular cross linking with divalent cations; such as, a calcium ion to form an egg box model [56], and they also bind to the guluronate blocks of the adjacent polymer chains to become more mechanically stable [57]. Alginate with a high M blocks content also binds to the cation, but it produces soft and more fragile gels. This is because of the straight arrangement of the M blocks

polymer. Alginate is widely used in many biomedical applications, especially in the areas of drug delivery because of its gelling, viscosifying and high-water intake properties. It is also included in the monograph in the European Pharmacopeia and the United States Pharmacopeia. Alginate is also used in wound dressing products; such as, Kaltostat[®], Algisite[®] M and Urgosorb[®].

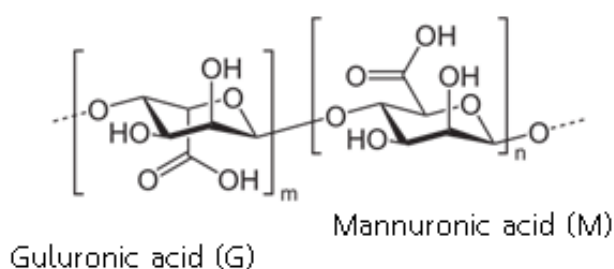


Figure II-7. Alginate structure [58]

1. Natural polyols from animals

a. Gelatin is a natural water-soluble protein derived from the partial hydrolysis of collagen, which is mainly found in humans and animals as bone, skin and connective tissue. It contains a composition of various amino acids and sequence depending on its source, but normally contains large amounts of glycine, proline and hydroxyproline as shown in Figure II-8 [59, 60]. Similar to collagen, the amino acid composition compacts to the chemical properties. In the solution, a gelatin structure is a mixture of α -chains (one polymer/single chain), β -chains (two α -chains covalently cross linked) and γ -chains (three covalently cross linked α -chains). The mechanism of the gelatin is the reversion of coiled helix polypeptide chains. Under a high temperature solution, these coiled helices tend to unfold and turn to form a helical conformation again after cooling, which results in a three-dimensional gel [61]. From this characteristic, thermal instability may be the limit or advantage of a gelatin application. Nowadays, it is used in a wide range of food, cosmetic and pharmaceutical products; such as, jelly, soft sweets, beverages, toothpaste, capsules and tablets.

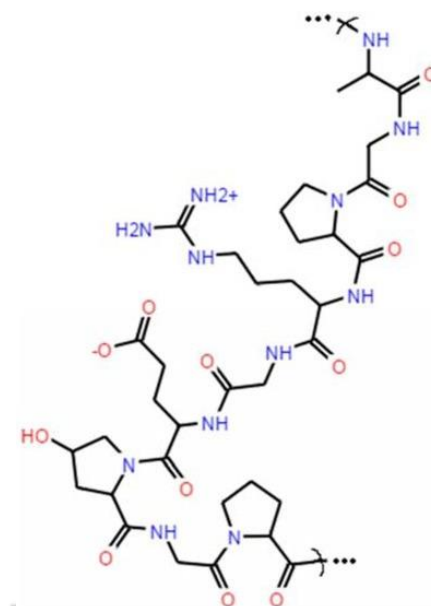


Figure II-8. Gelatin structure [62]

b. Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units. (Figure II-9) It is normally derived from the deacetylation of chitin, which is a structural component in the exoskeleton of crustaceans and insects. It contains the primary and secondary hydroxyl groups on each unit, and the amine group on each deacetylated unit. This macromolecule has -NH_2 groups and presents a weak base property. It is insoluble in water but it can dissolve in dilute acidic solutions by the protonation at amine group [63]. However, the polar functional groups within this polymer present a hydrophilic property. Chitosan can be depolymerized to reduce its molecular weight and viscosity in order to improve its solubility in aqueous media. It is an excellent excipient because of its low toxic, stable, biodegradable properties. Chitosan is used as an ingredient in wound dressing products; such as, Tegisorb™ and Chito-Seal® because of their hemostatic property [64].

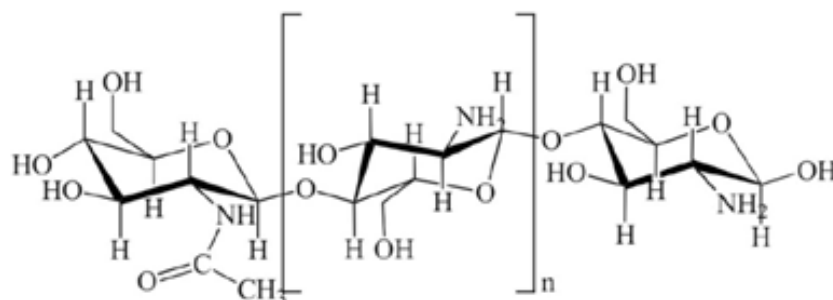


Figure II-9. Chitosan structure [65]

Silver

Apart from major foam dressing properties; such as, high exudates absorption, air permeability, further trauma protection, another issue that should be considered is controlling infection. Silver has been used as an antimicrobial agent in wound management since the 18th century. Moreover, it has been used in medical devices including dental work and catheters [66, 67]. Soluble silver salts and silver nitrate have also been reported for treating aphthous ulceration [68, 69]. Several wound care products have incorporated silver for use as a topical antibacterial agent [70-72] and also in wound dressings; such as, Aquacel[®] Ag Extra[™], ACTICOAT[°], Algicell[®]Ag, Askina[®] Calgitrol, Mepilex[®] Ag and Urgotul SSD[®]. Various forms of silver, which are available in silver products, may be summarized as follows:

1. Silver salts are more stable when a positively charged silver ion is incorporated with negatively charged ions (AgCl, AgNO₃, Ag₂SO₄).
2. Silver compounds; such as, silver sulfadiazine are produced by the substitution of a silver atom in silver nitrate for a hydrogen atom in a sodium sulfadiazine molecule.
3. Silver nanoparticles are incorporated within the dressing. They can be delivered to kill microorganisms through the wound's dressing. (Figure II-10)

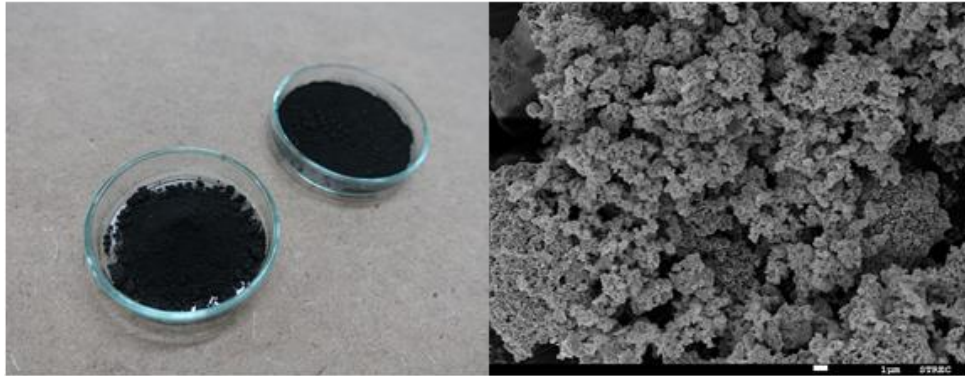


Figure II-10. Silver nanoparticles

Mechanism of Action

Elemental silver (Ag^0) does not have any antibacterial properties while silver cation (Ag^+) has a highly reactive activity. A silver ion will mainly interact with the thiol (sulfhydryl) groups of amino acids and other compounds; such as, cysteine and sodium thioglycolate [73, 74]. The possible mechanisms of silver in antibacterial properties are as follows:

1. **React with bacterial cell membranes:** The electrostatic attraction between the positive charge of silver and negative charge of a bacterial cell membrane leads to silver attaching onto the cell membranes [75]. After the silver ions have bound together, the cell membrane will be disrupted [76, 77]. The transmission electron microscopy photographs showed the treated bacteria with a silver solution rupturing, so the cell's contents were released into the surrounding environment [78-80].
2. **Binding with DNA:** Silver may also penetrate the cells and affect the cellular function. Singh et al [81] reported that the synthesized silver nanoparticles could inhibit quorum sensing process of *P.aeruginosa* which led to down-regulate virulence gene. It immediately binds to negatively charged proteins, RNA, and DNA of bacteria. Silver interacts with ribosomes that results in the inhibition of translation and protein synthesis [79]. These cause an interruption of the cell's membrane, enzyme inhibition and interruption of the DNA strands.

The silver ion would bind the purine and pyrimidine base pairs resulting in the disruption of the double helix structure [82].

3. **Catalytic oxidation:** The antibacterial efficacy of silver relies on the ability of producing ROS and free radical species; such as, hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and the hydroxyl radical ($OH\bullet$). An excessive amount of generated free radicals destroy the respiratory chain, which causes necrosis, and eventually, cell death [82, 83].

Apart from its antibacterial properties, silver is also used as an antimicrobial agent, which controls yeast and mold [84].

Silver nanoparticles are defined as solid particles with a size in the range of 10-100 nm. The antimicrobial property of silver nanoparticles is superior to other silver types because of their large surface area, which can contact microorganisms more than others [85]. The silver nanoparticles act as a silver ion reservoir, which presents high stability. The particles can release lots of silver atoms producing a high silver concentration. Clinical studies have shown the superior wound healing effects of nanocrystalline silver dressings over silver compounds in the wound treatment [86-88]. It is currently used in various types of wound treatments; such as, trauma wound, burn, chronic ulcers and pemphigus.

The toxicity of silver

There are numerous *in vitro* studies that have reported the toxicity of silver nanoparticles on hepatoma cells [89], human lung epithelial cells [90] and in studies on animals [91, 92]. The toxicity of the silver nanoparticles depends on the size and ion fraction in the silver nanoparticles suspension [91, 93]. The previous study showed that the intraperitoneally administration of silver nanoparticles 10 nm in diameter could cause more toxicity to mice than other sizes. Its toxicity seemed to less than silver ion compounds [89, 94]. Moreover, it can be delivered to the wound bed, but is difficult to be absorbed to the systemic circulation because it is readily bound to proteins and other negative charge ions within the complex wound fluid [95].

Asiaticoside

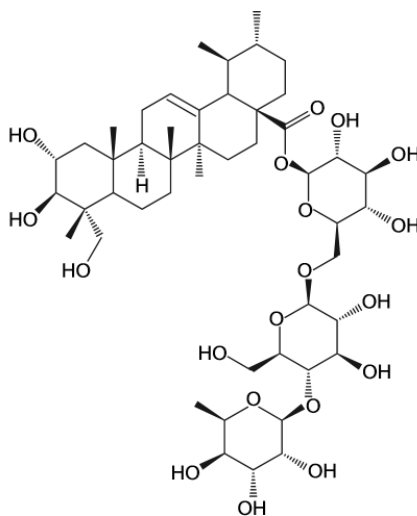
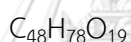


Figure II-11. Asiaticoside structure [96]

General characteristics [97]

General formula



Chemical Family

Triterpene glycoside

Molecular weight

959.13 g/mol

Melting point

235-238 °C

Solubility

Soluble in ethanol, methanol, insoluble in water.

One objective of wound treatment is accelerating the wound's closure. One famous herbal extract in wound healing research is Gotu kola (*Centella asiatica*). The extract has already proved to stimulate the proliferation of fibroblasts, increase collagen synthesis, decrease the activity of metalloproteinases, and thus increase collagen deposition. It also inhibits the inflammatory phase of wound healing [98-100]. Asiaticoside (Figure II-11), an active compound from *Centella asiatica*, was well-known in facilitating wound healing. It enhances normal human skin cell migration, attachment and growth. The asiaticoside could increase the amount of mRNA of collagen type I and type III in fibroblasts and also protein levels of procollagen type I and type III [101]. The asiaticoside significantly induced the Type I collagen synthesis in the human dermal fibroblast by the activation of the Smad pathway. Type I collagen synthesis is

stimulated by transforming the growth factor β (TGF- β). After the TGF- β binds to its receptors, the receptor regulated Smad proteins are phosphorylated and then translocated to the nucleus. In the nucleus, they perform as regulators of the target genes expression; such as, the Type I collagen gene [102].

There are many publications confirming the potency of asiaticoside in wound healing. Shukla et al and Shetty et al [103, 104] applied *Centella asiatica* extracts on hard to healed wounds. The results showed that there were increased hydroxyproline content, tensile strength, collagen content and epithelialization leading to facilitating the healing. Kimura et al [100] reported that the application of cream containing asiaticoside could repair burn wounds. This could be explained by the asiaticoside might enhance angiogenesis during the wound healing process in the consequence of the stimulation of VEGF production. Orally asiaticoside showed anti-inflammatory activity in lipopolysaccharide-treated rats. These effects could relate to the suppression of pro-inflammatory mediators; such as, the TNF- α and IL-6 levels, COX-2 protein expression and PGE2 production [105]. In the clinical study, administration of two capsules of 50 mg asiaticoside three times a day could shorten the healing process of wounds in diabetic patients. The results showed that the *Centella asiatica* extract capsule had an effective activity in the wound healing promotion and also suppressed scar formation [106]. Recent pre-clinical and clinical studies of a topical application containing asiaticoside in wound healing properties are shown in Table II-2.

Table II-2. Pre-clinical and clinical studies of an application containing asiaticoside in wound healing properties.

Studies	Objectives	Methods	Conclusion
Kimura et al [100]	To examine the effect of asiaticoside on cytokine production and macrophages in the regenerating of a mice wound model.	Topical ointment containing 10^{-12} to 10^{-8} % (w/w) asiaticoside applied on the wound daily. The cytokine and monocyte levels were investigated.	The enhancement of healing might be caused from angiogenesis. This is a result of the MCP-1 expression in the keratinocyte and IL-1 β expression in the macrophage.
Paolino et al [107]	To test the therapeutic effect of asiaticoside-loaded non-transformable vesicles.	The rats were divided to receive the treatment of asiaticoside or a vesicle with asiaticoside. Then, topical treatments were covered with non-occlusive patches. The application was performed two times daily. After seven, 14 and 28 days, the animals were sacrificed to determine the degree of the collagen synthesis.	The asiaticoside vesicle showed great skin permeation and increased collagen synthesis.

Studies	Objectives	Methods	Conclusion
Somboonwong et al [108]	The healing activities of the centella asiatica extract in an incision and partial thickness burn wound model.	The rats were divided to receive various types of centella asiatica extract. The solution was applied daily. The appearance and wound healing were evaluated for 14 days.	All types of extracts promote wound healing in both the incision and burn wounds.
Sawatdee et al [109]	The efficacy of the <i>Centella asiatica</i> extract in the excision wound healing rat model.	The 200 mm ² of excised full thickness wounds were created. The extract was sprayed on the wound, five puffs (~2.5 mL) once daily for 14 days compared to the povidone iodine solution.	A spray containing <i>Centella asiatica</i> extract complex was successfully developed for the treatment of fresh wounds.
Verma et al [110]	To elucidate the effect of asiaticoside on the different stages of healing of skin wounds, in a freshwater teleost <i>Cirrhinus mrigala</i> .	A skin wound approximately 2 mm in diameter was excised using a sterile disposable biopsy punch. The wounds were intraperitoneal injected with asiaticoside solution, vehicle and nothing over 30 days. Histology examination was determined at different stages.	The wounds in fish treated with asiaticoside showed significantly increase in cellular proliferation and also decrease in apoptosis in both the epidermis and dermis compared to vehicle control.

Zhang et al [111]	To develop a porous microsphere for the topical delivery of asiaticoside to increase its absorption and improve its therapeutic effects.	Rats with full skin wounds were divided into four groups randomly, including blank, blank microspheres, the asiaticoside solution, and asiaticoside-loaded microspheres groups. The wound's site appearance was observed every four days and reported as the wound closure rate.	Asiaticoside-microspheres could promote the proliferation, migration of the keratinocytes <i>in vitro</i> . They considerably accelerated the reepithelialization, collagen synthesis and angiogenesis.
Zhu et al [112]	To prepare asiaticoside loaded in nanofibers with sodium alginate, chitosan, and PVA. To determine the improved healing effect of nanofibers on deep partial thickness burn injuries.	Sprague-Dawley rats were burned and then divided into five groups: control, centella triterpenes cream, the high dose of asiaticoside loaded nanofibers group, middle dose and low dose, middle (d), and low doses (e). The study was performed for 21 days.	Its healing effect with deep partial thickness burn injuries was significant. The positive expression of VEGF, cluster of differentiation 31 (CD31), and proliferating cell nuclear antigen (PCNA), and down regulation of TNF and IL-6.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Foam preparation

To prepare the foam, the following materials were acquired: polypropylene glycol (PPG, MW 3000), silicone copolymer surfactant (Dabco DC5810), amine catalyst (Dabco 33-LV), tin catalyst (T9), and toluene diisocyanate (TDI) from Air Products and Chemicals Company Limited (Pennsylvania, USA). Methylene chloride was also obtained from Fisher Scientific, (New Hampshire, USA) Other hydrophilic polyols that would be used were gelatin (Cartino Gelatin Company Limited, Samut Prakan, Thailand); corn starch (National Starch and Chemical (Thailand) Limited); Pregelatiized starch (starch 1500) and hydroxypropyl methylcellulose (Methocel[®] E5) (Colorcon Asia Pacific Pte. Ltd., Singapore); methylcellulose and sodium carboxymethylcellulose (Dai-ichi Kogyo Seiyaku Company Limited, Tokyo, Japan); chitosan MW 200,000 (Seafresh Chitosan (Lab) Company Limited, Thailand); sodium alginate (Acros Organics, Geel, Belgium) and chitosan low molecular weight (MW 50,000) (Sigma-Aldrich Chemical Company, Missouri, USA).

2. Active Ingredients

The 20 nm sized silver nanoparticles (pharmaceutical grade) were bought from Guangzhou Hongwu Material Technology Company Limited, Guangzhou, China (Batch No. HW-P160819).

The 95% of asiaticoside powder (pharmaceutical grade) was bought from Xian Lyphar Biotech Company Limited, Shaanxi, China (Batch No. LYPH150927).

3. Miscellaneous

Methanol HPLC (Burdick & Jackson[®], Seoul, Korea)

Acetonitrile HPLC (Burdick & Jackson[®], Seoul, Korea)

70% Nitric acid (Ajax Finechem, Auckland, New Zealand)

Thiazolyl Blue tetrazolium Bromide (Sigma-Aldrich Chemical Co., Missouri, USA)

Lysozyme from chicken egg white 70000 U/mg (Sigma-Aldrich Chemical Co., Missouri, USA)

Dulbecco's Modified Eagle's Medium (Gibco[™]DMEM, No. 11995, Thermo Fischer Scientific, Massachusetts, USA)

Fetal bovine serum (Gibco[™]FBS, Thermo Fischer Scientific, Massachusetts, USA)

Antibiotic-Antimycotic agent (Gibco[™] Anti-Anti (100X), Thermo Fischer Scientific, Massachusetts, USA).

0.25%w/v Trypsin-1 mM EDTA solution (Gibco[™] Trypsin-EDTA (0.25%) (1X), Thermo Fischer Scientific, Massachusetts, USA)

Mueller Hinton II Agar (Becton, Dickinson and Company, Le Pont de Claix, France)

DMSO (Fisher Scientific UK Limited, Leicester, UK)

Askina[®] calgitrol (B.Braun, Melsungen, Germany)

Mepilex[®] Ag (Mölnlycke Health Care, Gothenburg, Sweden)

Allevyn[®] (Smith & Nephew, Inc., Massachusetts, USA)

Normal saline solution (Thai Otsuka Co. Ltd., Samutsakorn, Thailand)

All other chemicals are of reagent grade.

Instruments

1. Balance
 - a. (4-digit) Model A200S (Sartorius, Goettingen, Germany)
 - b. (5-digit) Model XP205 (Mettler Toledo, Ohio, USA)
2. Stirrer Model RW20 digital (IKA[®], Staufen, Germany)
3. Oven

- a. Model UM 400 (Mettmert GmbH+ Co. KG, Schwabach, Germany)
 - b. Model BM 600 (Mettmert GmbH+ Co. KG, Schwabach, Germany)
4. Magnetic stirrer Model POLY15 (VARIOMAG[®], Florida, USA)
5. High performance liquid chromatography Model LC-20AB, Detector model SPD-20A (Shimadzu Scientific Instruments, Kyoto, Japan)
6. Atomic absorption spectroscopy Model AA280FS series (Varian, California, USA)
7. Fourier Transform infrared spectroscopy Model Spectrum[™] One (Perkin-Elmer, Massachusetts, USA)
8. Scanning electron microscope Model JSM-7610F (JEOL, Tokyo, Japan)
9. Differential scanning calorimeter Model DSC822^e (Mettler Toledo, Ohio, USA)
10. Water bath
 - a. Model polystat cc1 (Huber, North Carolina, USA)
 - b. Model B 22 (Mettmert GmbH+ Co. KG, Schwabach, Germany)
11. Centrifuge Model 5810 (Eppendorf, Hamburg, Germany)
12. Sonicator Model S 79H (Elma Schmidbauer GmbH, Singen, Germany)
13. Inverted microscope Olympus Culture Microscopes Models CKX31, Olympus Life Science, Tokyo, Japan
14. Microplate reader Model VICTOR3 (Perkin Elmer, Massachusetts, USA)
15. Laminar air flow Model 1.2 (Thermo Scientific, Massachusetts, USA)
16. CO₂ Incubator Model 3111 (Thermo Scientific, Massachusetts, USA)
17. Foam vertical cutting machine Model IS-M, Albrecht Bäumler GmbH & Co. KG, Freudenberg, Germany
18. Ultrapure water producer Model Micropure UV/UF (Thermo Scientific, Langeselbold, Germany)
19. Vortex Genie-2 Model G560E (Scientific Industries, Inc, New York, USA)
20. pH meter Model FiveEasy[™] FE20-1 (Mettler-Toledo, Schwerzenbach, Switzerland)
21. Vernier caliper (Mitutoyo digimatic caliper series 500, Tokyo, Japan)
22. Universal Testing Machine (Shimadzu, model EZ-S 500 N, Osaka, Japan).
23. Gamma Chamber 5000, BRIT, Mumbai, India

Laboratory supplies

1. HPLC column (HALO-5[®] C18 column (250x4.6 mm), 5 μ m, Advance Materials Technology, USA)
2. Cell culture plates (Costar[®] 24 Wells Cell Culture plate, Corning Inc, New York, USA)
3. Cell culture plates (Costar[®] 96 Wells Cell Culture plate, Corning Inc, New York, USA)
4. 25, 75 cm² flasks, Canted Neck (Corning Inc, New York, USA)
5. 2, 5, 10 ml pipettes (Costar[®] steripette, Corning Inc, New York, USA)

Methods

Part I: Foam preparation and characterizations: *In vitro* studies

This part could be divided into 2 sections:

- Section one: Preliminary study of the 2% of natural polyols. The characterizations which performed following;
 - 1) Morphology and density
 - 2) Absorption test
 - 3) Desorption test
 - 4) Tensile strength test
 - 5) Compression test
 - 6) Water vapor transmission rate (WVTR) test
 - 7) Foam integrity test
- Section two: Determination of the 2-12% of the three types of selected natural polyols. The characterizations which tested in this section were similar to section one. The fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) analysis were also tested in selected concentrations.

1. Preparation

Polyurethane foam is produced by the reaction of polyether polyols with the diisocyanate. This reaction needs water as a blowing agent, amine catalyst, and tin catalyst (T9), as the catalysts in order to complete the foaming process. First, the polyols, deionized water, silicone copolymer surfactant and amine catalyst were mixed together in a plastic cup and vigorously stirred by a five-degree four-winged impeller at 500 rpm for two min to ensure a good dispersion of the reagents. Then, the mixture was cooled in an ice bath until the mixture's temperature reached 20 degree Celsius. Second, the toluene diisocyanate was also cooled in the ice bath until reaching 20 degree Celsius. Third, the tin catalyst was added to the mixture and stirred at 1900 rpm for one minute. The TDI was added and stirred for 5-7 seconds. The mixture was poured into a mold, which was covered with waxed paper at 25-30°C. The foam would be created within 10 seconds. Then the loaf of foam was placed on the shelf for 72 hr to confirm complete curing. The loaf of polyurethane foam was sliced by a foam cutting machine to obtain the foam sheet. The thickness of the sheet was 6.0 ± 0.5 mm. The blank was the polyurethane foam without any hydrophilic natural polyols. The foam with the hydrophilic natural polyols was the formulation in which the natural polymer was added to the mixture with PPG 3000 and other reagents in the first step. The hydrophilic natural polyols would be varied at 2 percent by weight as follows:

- Pregelatinized starch (PGS)
- Corn starch (CS)
- Hydroxypropyl methylcellulose (HPMC)
- Methylcellulose (MC)
- Sodium carboxymethylcellulose (CMC)
- Chitosan MW 200,000 (CHMW)
- Chitosan MW 50,000 (CLMW)
- Sodium alginate (Alg)
- Gelatin (Ge)

The composition of the blank polyurethane foam is described in Table III-1. For the second section of the foam preparation with 2-12% of natural polyols, the silicone

surfactant was increased to 1.5 g and methylene chloride was increased to 3.0 g of the polyols.

Table III-1. The composition of the polyurethane foam.

Ingredients	Weight (g)
PPG 3000	100
Silicone surfactant (DABCO DC5810)	1.3
Methylene Chloride	2.0
Purified water	2
Catalyst (DABCO 33LV)	0.2
Catalyst (T9)	0.13
TDI	31.71
(+/-) Natural polyols	2

All formulations (blank and hydrophilic natural polyols were added in the polyurethane foams) would be characterized by their physical properties including morphology, absorption test, desorption test, tensile strength test, compression test and water vapor transmission test.

2. Characterization

2.1) Morphology and density

This method adapted from that of Mandru et al and Lee SM et al [113, 114]. The scanning electron microscopy (SEM) determined the structure and pore sizes of the foam sheet. The samples were cut with a blade and attached on the stub. Then, the sample was coated with gold. The surface and cross-sectional observation were performed using a scanning electron microscope (JSM-7610F; JEOL, Tokyo, Japan), working at 10 kV, 15x magnification. The pore sizes were calculated from approximate 300 pores of cross-sectional view using the Image J program [115].

The foams were measured in width, length, and thickness using a Vernier caliper (Mitutoyo digimatic caliper series 500, Tokyo, Japan) in millimeters. Then, the samples

were weighed and recorded in grams. The density was calculated and reported as g/cm³ unit [116].

2.2) Absorption test

The prominent property of the foam dressing was water absorption. It is recommended to be used in moderate to heavy amounts of exudate. This study determined the absorption capacity of the polyurethane dressing. The wound dressings were cut to a size of 2.5 x 2.5 cm² and preconditioned in a desiccator with silica gel for 24 hr. After that, the initial weight of the cut sample was measured (A) and held in a stainless steel mesh tea ball apparatus, which was then sunk in a beaker containing 120 ml of distilled water before incubation at 37±1°C for 24 hr. This volume is the excess volume which the tea ball apparatus could totally sink in the beaker. At each point in time, the weights of the samples were collected at one, 10, 30, 60, 120, 240, 480 and 1,440 min (B). The apparatus was taken out of the beaker and suspended for 10 seconds for free drainage. The excess water was wiped off with paper and returned back after weighing. Five samples per foam formulation were evaluated. Then, the percentage of water absorption was calculated according to Equation III-1. A and B were the weights of the apparatus with the sample at the initial and at time t after water absorption, respectively. Time was defined as the duration of each period of time [117].

$$\text{Water absorption (\%g)} = \frac{(B - A)}{A} \times 100$$

...Equation III-1

2.3) Desorption test

This study followed Parsons et al [118] research with some modifications. The dehydration behavior was determined by measuring the different weights of the samples between wet and dry conditions. The aim of this experiment was to determine the ability of dressings to retain moisture. The appropriate moist at the wound bed facilitates the migration of epithelial cells. The samples from the absorption test would be used in this study. The foam sheets would be taken out from the solution and

suspended for 10 seconds for free drainage and weighed again to obtain the wet mass (W_w). Subsequently, put onto aluminum foil and kept in an incubator for 24 hr at ambient condition. This condition would permit the dressing could retain the moisture within the structure and dehydrate under environment humidity. The weights of the samples were collected six times: 0.5, 1, 2, 4, 8 and 24 hr (dry mass, W_d). The calculation of the percentage of desorption was determined by the wet and dry mass of the samples (Equation III-2). Time was defined as the duration of each period of time.

$$\% \text{Desorption (\%g)} = \frac{W_d - W_w}{W_w} \times 100$$

...Equation III-2

After the absorption and desorption test, the weights of the samples before absorption and after desorption were calculated to determine the percentage of the weight, which is shown in Equation III-3. W_i and W_d referred to the mean weight before the absorption test and the mean weight after the desorption test, respectively.

$$\% \text{Loss (\%g)} = \frac{W_d - W_i}{W_i} \times 100$$

...Equation III-3

2.4) Tensile strength test

In changing the wound's dressing, some nurses might pull the dressing before covering the wound. A dressing which is weak in strength would be torn easily. The sample was cut in a dumbbell shape of a certain size [119]. The test sample which had a tear point would be excluded. The diameter and gauge length were 10 and 60 mm, respectively. The initial thickness, gauge length, and diameter of the sample would be measured and recorded using a Vernier caliper (Mitutoyo digimatic caliper series 500, Tokyo, Japan). The sample was installed on the machine in a cross-sectional direction between the grips of the instrument. The mechanical properties were determined with a Universal Testing Machine (UTM) (Shimadzu, model EZ-S 500

N, Osaka, Japan) with a load capacity of 500 N and cross-head speed at 50 mm/min. Five specimens per sample were tested and reported as the mean value of those observed. The results were reported as tensile strength [85] and the percentage of elongation.

2.5) Compression test

The objective of this study was to evaluate how a material reacts when it is compressed. The wound dressing, which presented high compressive strength would protect the wound from compression or trauma. This study was applied from ASTM standard D3574 [119] using a UTM. The test sample of dimensions 25 mm × 25 mm × 6 mm were cut, and the initial thickness was determined. The sample was placed between the horizontal plates of a compression device; each plate had a surface area more than the test sample. The compression plates were arranged and the space between them was adjusted to the required deflected height. The samples were compressed to 75% of their original thickness with a speed of 2 mm/min, after that the samples were removed. The compressive strength values were reported at 25, 50 and 75 strains, respectively. Five specimens per sample were tested. The value was reported as the mean value of those observed.

2.6) Water vapor transmission rate (WVTR) test

The WVTR test is important for wound dressing material because this helps to understand the moisture permeability of the material and environment. The liquid, which was inside the wound layer, vaporizes and transports to the atmosphere. If the wound dressing material does not allow the moisture vapour into the atmosphere, it will create an infection and periwound maceration. Differ from desorption test, this study intends to determine the permeability of dressing which permits air to the environment. This experiment was adapted from an EN 13726 – 2: 2002 method in order to determine the water vapour transmission rate [120]. The foam sheet with a 35.5 mm diameter round shape was attached with paraffin to the mouth of a container, which was filled with 25 ml of distilled water. The oven was preheated at 37°C for two hr with silica gel. The test sample container was weighed at the initial time (W_{t1}) then

kept in the oven at $37\pm 1^\circ\text{C}$, $40\pm 5\%$ RH for 24 hr. The relative humidity was controlled by silica gel and it was replaced in case measured value was out of range. The test containers were weighed at 0.5, 1, 2, 4, 8 and 24 hr, and the results (W_{t_2}) were recorded. The different weights ($W_{t_1} - W_{t_2}$) were calculated from two weights between the different time periods. The WVTR was calculated by Equation III-4 and Equation III-5 where A, r, and T were the area of the test sample in m^2 , the radius of the test area in m, and time in min (min), respectively.

$$\text{WVTR (g/m}^2\text{/min)} = \frac{(W_{t_1} - W_{t_2})}{A(t_1 - t_2)}$$

...Equation III-4

$$A = \pi r^2$$

...Equation III-5

2.7) Foam integrity test: Enzyme degradation test

This study aimed to determine whether foam dressing could be degraded by lysozyme, which was normally found in wound exudates. Adapted from Wang et al and Tangsadthakun et al [121, 122], dry samples in approximately $1.0 \times 1.0 \times 0.6 \text{ cm}^3$ in size were weighed (W_0). This test was performed two times. First, the foam with 2% of natural polyols was tested to clarify their integrities in the preliminary study. Second, foam without natural polyols, foam with HPMC, sodium alginate and chitosan at 4% and 6% of the concentration were tested to confirm their properties. The samples would be suspended in six-well plates containing 7 ml of phosphate-buffered saline (pH 7.4) and lysozyme (concentration $1.6 \mu\text{g/ml}$) for 4, 8, 24, and 48 hr. The comparison group was a phosphate-buffered saline (pH 7.4) without enzyme. All samples were incubated at 37°C . After a period of time, the dressing was removed from the solution and then washed three times with the same volume of distilled water. The dry weight of the sample was measured after drying at 50°C for 48 hr (W_1) to examine the percentage of degradation (Equation III-6).

$$\% \text{ Degradation} = \frac{(W_0 - W_1)}{W_0} \times 100$$

...Equation III-6

2.8) Fourier transform infrared spectroscopy (FTIR)

This study was performed to object the interaction between the hydroxyl group of natural polyols and isocyanate group of TDI. Functional group interaction of prepared foam dressing was evaluated using an FT-IR spectrophotometer Model Spectrum™ One (Perkin-Elmer, Massachusetts, USA) with an ATR method [123]. The samples (Bl and selected natural polyol foam) were kept in a desiccator before testing. They were cut and adhered to the sample holder using double adhesive tape. The spectra were recorded in the region between 4000 to 515 cm^{-1} and were composed by 64 scans with a spectral resolution of 4 cm^{-1} .

2.9) Differential scanning calorimetry (DSC) analysis

This experiment used to detect the interactions between foam ingredients as well as natural polyols of foam dressing and its degradation by determination of the amount of heat which was required to increase temperature between sample and reference. The thermal analyses of pure solid content, foam material with or without selected natural polyols in 6% concentration were determined by Model DSC822^e (Mettler Toledo, Ohio, USA) [124]. Approximately 5 mg of the sample was packed in a 40 μl pin-holed aluminum pan and then was weighed with a five-digit digital balance. The sample was heated from 30°C to 300°C at a rate of 10°C.min⁻¹. A nitrogen flow rate of 60 ml.min⁻¹ was used. All samples were tested as a duplicate.

After evaluation the physical characteristics, the type and concentration of the hydrophilic polyols added in the polyurethane foam were selected for three formulas. The criteria to be considered as the high absorption capacity, low desorption rate, good WVTR, good tensile strength and compressive strength profile. These formulations would be used in the next step. After the preliminary study, three natural polyols were selected to be further evaluated in the second section. The researchers varied the concentration of these natural polymers between 2% to 12% and evaluated the physical properties again. After the assessment of the characteristics, only the 2 concentrations of those three natural polymers were chosen to add to the active ingredients.

3. Preparation and determination of the polyurethane foam dressing impregnated with silver nanoparticles and asiaticoside

3.1) Preparation of the polyurethane foam dressing impregnated with silver nanoparticles

The active ingredients were loaded into the foam sheets of 10x10 cm² through an absorption process. From the information of the commercial products, it was claimed that the silver concentration was 0.083-1.41 mg/cm² [125, 126]. In this study, silver nanoparticles of 0.4, 0.6, 0.8 and 1.0 mg/cm² were used.

The foam dressing was measured for the exact width and length using a Vernier caliper. Then the silver amount per sheet was calculated. The silver powder was weighed and dispersed in a glass tube containing deionized water. The volume of water was half the optimal water absorption volume and equal in each preparation. The test tube containing the silver suspension was covered with paraffin film and sonicated for 2 hr to obtain the silver suspension. After that, the silver suspension was mixed for 10 seconds and poured onto the tray. The glass tube was rinsed three times at the same volume. The total volume of deionized water used in silver dispersion was less than the optimal fluid absorption volume of foam. The silver suspension was mixed in the tray again using a plastic paddle. The suspension was completely loaded by the foam dressing during the absorption and compression process in both sides. Then, the foam impregnated silver was dried in the oven (Model UM 400, Memmert GmbH+ Company KG, Schwabach, Germany) at 40°C for 48 hr. The silver releasing profiles were measured and the concentration, which still had efficacy and no cell toxicity, would be used in the next study.

According to a previous study, the range of asiaticoside used in the alginate dressing had a concentration of 2.5-10.0% [127]. Moreover, it was reported that the polyester dressing with a 5% concentration of *Centella asiatica* extract was associated with shorter hospital stays compared with standard wound dressings [128]. In the asiaticoside impregnation, 5% of the powder was calculated according to the foam's weight. The asiaticoside powder was added in selected dispersion after sonication. The

mixed suspension was then poured onto the tray and foam sheet was absorbed the mixture as described above.

3.2) Active compounds content determination

Silver content

This experiment was adapted from Kulthong et al (2010) [129]. The solution from the sample sheet of 1 x 1 cm² was mixed with 1 ml of 50%v/v of nitric acid and boiled in a water bath (Model B 22 (Memmert GmbH+ Company KG, Schwabach, Germany) at 70°C for two hr in order to break down the polyurethane foam and dissolve all the silver content. Then, 0.5 ml of acid solution was added to 2 ml of distilled water. The solution was centrifuged at 3000 rpm for five min in order to precipitate foam residues. One milliliter of supernatant was pipetted into 4 ml of water. The amount of the silver ion was determined by a flame atomic absorption spectrophotometer (Varian model AA280FS, California, USA) and reported as percent content (Equation III-7) [130]. The standard concentration was prepared from a standard silver solution (Merck KGaA, Darmstadt, Germany). The atomic absorbance was measured under the following conditions:

Lamp current	3 mA
Wavelength	328.1 nm
Flame type	Air/Acetylene
Air flow	13.20 L.min ⁻¹
Acetylene flow	1.8 L.min ⁻¹

$$\% \text{ Content} = \frac{\text{Actual amount of drug in experiment}}{\text{Amount of theoretical drug}} \times 100$$

...Equation III-7

Asiaticoside content

The determination of the asiaticoside content was modified from Hengsawas et al [131] which had been already validated by the HPLC method including specificity, linearity, accuracy, and precision. The factors which differed from the previous study were column's brand name (HALO-5[®] and Hypersil[®] BDS) and wavelength of detection

(220 and 210 nm). The cubed foam was suspended in 5 ml of 100% methanol. After mixing the samples for 20 seconds, they were shaken in a water bath at 30°C for one hour. The foam samples were changed to another solution, mixed for 20 seconds and shaken for one hour. The total solution was mixed and filtered through a 0.45 µm membrane filter prior to injection. The asiaticoside content was determined using a High-performance Liquid Chromatography (Shimadzu model LC-20AB, Shimadzu Scientific Instruments, Kyoto, Japan) with a UV detector (Shimadzu model SPD-20A, Kyoto, Japan). The mobile phase was water-acetonitrile with linear gradient conditions of water 70, 0, 70 and 70% of water in pump A, and 30, 100, 30, 30% acetonitrile in pump B at the time intervals of 0, 12, 15 and 30 min, respectively. The asiaticoside was operated over a range of elution periods of 3.5-3.8 min. The foam without active compounds was already tested to confirm there was no peak in asiaticoside's elution period.

Column	: HALO-5 [®] (C18) column (250x4.6 mm), 5 µm (Advance materials technology, USA)
Detector	: UV detector at 220 nm
Injection volume	: 20 µL
Flow rate	: 1 ml/min
Mobile phase	: Water – acetonitrile linear gradient conditions

3.3) Releasing of active compounds

The releasing study was applied using the static Franz diffusion cells method [132]. All Franz cells were fixed onto a magnetic stirrer. Deionized water and PBS pH 7.4 with 10% of methanol were used as receptor media for the content determination of the silver and asiaticoside, respectively. The addition of methanol was to facilitate the releasing of asiaticoside because this compound is water-insoluble [96]. The receptor compartment of a 12.0-14.0 ml medium was maintained at 37°C and magnetically stirred using a 1 cm length magnetic bar at a speed of 250 rpm. The receptor compartment of each medium was maintained at 37°C and continuously magnetically stirred. A round shape dressing sample was placed between the donor

and the receptor compartment. At various time intervals, the solution of 4 and 3 ml were sampled for determination of silver and asiaticoside releasing profiles from the receptor, respectively and replaced with the fresh solution with the same volume to maintain the fluid level. For determination the amount of silver release, the sampling solution was mixed with 1.0 ml of 25%v/v nitric acid to dissolve the silver nanoparticles prior to exposure to an atomic absorption spectroscopy as in Item 3.1. The amount of asiaticoside release was quantified by using HPLC as in Item 3.2.

3.4) Stability test

According to the WHO's guideline and ISO 11137-2 [133, 134], the obtained polyurethane foam dressings were kept in an aluminum foil pouch. The aluminium foil pouch was completely sealed on four sides, so to protect the pouch from the air and humidity. All packaging was sterilized by gamma irradiation at a dose of 25 kGy according to ISO 11137-2. After that, they were stored in 30 ± 2 and $40\pm 2^\circ\text{C}$ and $75\pm 5\%$ RH for three and six months. After accelerating the aging time, the samples were tested for the active compound contents as mentioned in Items 3.2. The percent remaining was calculated from Equation III-8.

$$\% \text{remaining} = \frac{\text{Amount at t time}}{\text{Amount at post - radiation}} \times 100$$

...Equation III-8

3.5) Antibacterial test

The foam samples that were conducted in this study were as follows:

1. Foam dressing without natural polyols + Ag 1 mg/cm^2 + 5% of asiaticoside (BL-1Ag-AS)
2. Foam dressing with 6% of HPMC + Ag 1 mg/cm^2 + 5% of asiaticoside (H6-1Ag-AS)
3. Foam dressing with 6% of Alg + Ag 1 mg/cm^2 + 5% of asiaticoside (A6-1Ag-AS)
4. Foam dressing with 6% of CLMW + Ag 1 mg/cm^2 + 5% of asiaticoside (C6-1Ag-AS)

5. Foam dressing without any natural polyols and active ingredients (BI)
6. Foam dressing without any natural polyols + 5% of asiaticoside (BI-AS)
7. Commercial foam dressing with silver alginate 1.4 mg/cm² (Comparative I)
8. Commercial foam dressing with silver sulfate 1.2 mg/cm² (Comparative II)
9. Commercial foam dressing without silver (Comparative III)

The agar diffusion method was performed for determination of the antibacterial activity of the untreated and treated polyurethane dressings with silver nanoparticles and commercial silver dressings. This study was adapted from a previous study [135] using four bacterial strains commonly found in trauma wounds [136]: *Staphylococcus aureus* (ATCC® 6358™), *Bacillus subtilis* (ATCC® 6633™), *Escherichia coli* (ATCC® 25922™) and *Pseudomonas aeruginosa* (ATCC® 27853™). The bacteria were grown in a culture slant for overnight incubation, suspended in the broth and adjusted the turbidity to the 0.5 McFarland standards (1.5×10^8 cfu/ml). The broth of 100 µl was used to streak on the Muller-Hinton agar plates in three directions to form a confluent lawn. Each side of the 1 x 1 cm² samples absorbed the distilled water for 100 µl. then were applied on the agar in duplicate. The plates were left in an incubator at 37°C for 24 hr. The diameter of the clear zone surrounding the test dressing was measured for the zone of growth inhibition using a Vernier caliper (Mitutoyo digimatic caliper series 500, Tokyo, Japan) and recorded in millimeters [137]. All zones of the inhibitions (ZOI) were reported as a mean and standard deviation from three independent experiments.

3.6) Compatibility test: Cytotoxicity test

In vitro cell culture

Human fibroblasts (ATCC® CRL-2522™) were used in this study. The complete growth medium composed of Dulbecco's Modified Eagle's Medium (Gibco™ DMEM, No. 11995, Thermo Fischer Scientific, Massachusetts, USA) containing a saline solution, amino acids, 25 mM of D-glucose, 1 mM of sodium pyruvate, 10% of fetal bovine serum (Gibco™ FBS, Thermo Fischer Scientific, Massachusetts, USA), and 1% of Antibiotic-Antimycotic agent (Gibco™ Antibiotic-Antimycotic (100X), Thermo Fischer Scientific, Massachusetts, USA). The 15 ml of the cell suspension was the culture with media in

a culture flask. The cell culture grew at the temperature of 37°C and 5% CO₂ incubator until the cell density reached 70-80% confluent. For the cell subculture, the media was removed and the cell layer was rinsed with 0.25%w/v of Trypsin-0.53 mM EDTA solution (Gibco™ Trypsin-EDTA (0.25%) (1X), Thermo Fischer Scientific, Massachusetts, USA) in order to remove all traces of the serum that contained a trypsin inhibitor. After that, 2.0 ml of trypsin-EDTA solution was added to the flask and the cells were observed for the detachment under an inverted microscope (Olympus Culture Microscopes Models CKX31, Olympus Life Science, Tokyo, Japan). Fresh medium was added, and the cell suspension was aliquot to new culture vessels. The subculture was done every 48-72 hr until the cells were ready for further study.

Cytotoxicity test

The MTT assay is broadly used to determine the in vitro cytotoxic effects of drugs. It relies on the conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, yellow color) to formazan crystals (purple color) by mitochondrial succinate dehydrogenase, which is located in mitochondria. The total mitochondrial activity refers to the number of viable cells.

From a study by Burd et al (2007) [138], the number of fibroblasts were counted using a hemocytometer. The cultured flask was swirled to distribute the cells. Then the cell suspension was pipetted 0.5 ml and placed in a microcentrifuge tube. The 100 µl of the suspension was placed in a new tube and mixed with 400 µl of trypan blue solution. Only 100 µL of the new suspension was pipetted and applied to the hemocytometer gently and covered with a cover glass. The hemocytometer was placed on the inverted microscope with a 10X objective. The number of live cells, which were stained by the color were counted in five sets (four sets of corner squares and one set of a center square) of 16 small rectangles. Then the value was calculated by dividing by five and multiplying by 10⁴ and the actual number of cells were reported as cell/ml. The cell suspension was planted in the fibroblast growth medium on 24 well plates at a density of 5 x 10³/well. The plate was incubated in an atmosphere of 95% of air and 5% of CO₂ at 37°C for 72 hr to get 70% of the cells' confluences. The

complete growth medium was added into each well for 300 μl . At that time, the 1 x 1 cm^2 dressing samples, which had been soaked with 200 μl of the phosphate buffer solution, were then added to the culture well. Each group was continued as follows:

1. Control group: 500 μl of the complete growth media solution.
2. Phosphate buffer solution group: 300 μl of the complete growth media solution plus 200 μl of the phosphate buffer solution.
3. Blank group: 300 μl of the complete growth media solution with foam dressing without natural polyols and active ingredients was soaked with 200 μl of the phosphate buffer solution.
4. Foam with natural polyols (6% of HPMC, CLMW, and Alg) but without active ingredients groups: 300 μl of the complete growth media solution with foam dressing was soaked with 200 μl of the phosphate buffer solution.
5. Foam with asiaticoside group: 300 μl of the complete growth media solution with foam dressing with asiaticoside was soaked with 200 μl of phosphate buffer solution.
6. Foam with natural polyols (6% of HPMC, CLMW, and Alg) and active ingredients (silver and asiaticoside) groups: 300 μl of the complete growth media solution with foam dressing with natural polyols and active ingredients was soaked with 200 μl of the phosphate buffer solution.

After that, the cells were incubated for another 24 hr. The cell viability was determined by an MTT assay [139]. The dressing was then removed. The 0.5 mg/ml MTT solution was pipetted into each well. The plate was incubated at 37°C for four hr then the solution was removed. 0.5 ml of DMSO was added to dissolve the purple crystal. The absorbance was measured at 560 nm in triplicate (100 μl in three wells of 96 well plates), using a microplate reader (Perkin Elmer Victor3™ Model 1420-050, Massachusetts, USA). The percentage of the cells' survival was calculated from the following Equation III-9.

$$\% \text{ Survival} = \frac{\text{Absorbance of surviving fibroblasts of samples}}{\text{Absorbance of surviving fibroblasts of control}} \times 100$$

...Equation III-9

3.7) Sterility test

This study confirmed the sterilization process of foam dressing before an *in vivo* study. All foam dressings were sterilized by gamma radiation (Gamma Chamber 5000, BRIT, Mumbai, India) at 25 kGy [134], which is the recommended dose by the IAEA Standards for Tissue Banks (IAEA 2003) and the AATB Standards (AATB 2002) as the minimum dose for bacterial sterilization.

The sterility test that followed the United States Pharmacopeia (USP39) [140] consisted of two mediums: a fluid thioglycolate medium, which is primarily intended for the culture of anaerobe bacteria, and a soybean-casein digest medium, which is suitable for the culture of both fungi and aerobic bacteria. Three type of samples; foam without natural polyols impregnated with silver plus asiaticoside (Bl-1Ag-AS), foam with 6% of HPMC impregnated with silver plus asiaticoside (H6-1Ag-AS), and foam with 6% of Alg impregnated with silver plus asiaticoside (A6-1Ag-AS) were cut to 4 cm² (approximately 0.11-0.13 g). Control was performed using the Bl foam. Ten packages were tested in each medium. Thirty-five milliliters of each medium was pipetted into a glass tube using an aseptic technique. Then, the samples were directly inoculated into the culture medium. After immersion, the test tubes with the fluid thioglycolate medium were incubated at 32.5±2.5°C under anaerobe condition while the test tubes with the soybean-casein digest medium were incubated at 22.5±2.5°C. The incubation was 14 days, and then the medium was transferred to fresh tubes of the same medium and incubated for four days. If the results from the fresh medium were a clear solution, it could infer that these products complied with the test for sterility.

Part II: Efficacy and safety in animal model

1. Skin irritation of selected foam dressing on rabbits

This experiment was approved and performed by the Industrial Metrology and Testing Service Center, Thailand Institute of Scientific and Technological Research (TISTR) according to the OECD Guideline 2015 [141]. (Protocol No. TS-59001) Three rabbits were housed individually before the experiment within approximately $20\pm 3^{\circ}\text{C}$ and $50\pm 10\% \text{RH}$, for adaptation to minimize stress and physiologic alteration before the experiment. Conventional laboratory diets with drinking water were provided ad libitum. Twenty hr before the test, the fur on the dorsal area was removed and avoided abrading the skin. A piece (0.4 cm^2) of the tested dressing (A6-1Ag-AS) was soaked with 0.5 ml of normal saline solution before being applied on the skin. Then, it was covered with a sterile gauze patch and attached with adhesive tape. The skin area, which was applied with the dressing, was called the study group. On beside side, the control group was applied with a gauze patch and adhesive dressing. The experiment was left for four hr then removed and gently cleaned with cotton balls soaked with a normal saline solution. The redness and swelling response grading were between 0 - 4 points where 0 was defined as no serious skin reaction and 4 was a serious reaction. After dressing removal, the evaluations were recorded at 1, 24, 48 and 72 hr, respectively. If there was any skin reaction, a confirmation test with two more animals would be taken into consideration.

The grading of skin reaction following;

1. Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation	4

2. Oedema Formation

No oedema	0
Very slight oedema (barely perceptible)	1

Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised > 1 mm and extending beyond the area of exposure)	4

2. Efficacy and safety of prepared foam sheet on pigs

This experiment was approved by the Faculty of Veterinary Science - Animal Care and Use Committee (FVS-ACUC), Mahidol University (Protocol No. MUVS-2016-09-34). This study was performed by two experienced veterinarians between November 2016 to February 2017.

Five domestic farm pigs with an average weight of 20-25 kg were housed at the laboratory animal housing facility for a minimal acclimation period of seven days before the experiment. The animals were housed one per cage in a room with a 12-hour light/dark cycle with ad libitum access to drinking water and routine feeding. For all the surgical procedures, the animals were made to fast 12 hr before surgery. The animals were anesthetized prior to and during the infliction of the experimental wounds. The pigs were induced with tiletamine-zolazepam and intubated by the veterinarians. An ear vein catheter was placed through which normal saline was administered throughout the surgery, and anesthesia was maintained with isoflurane. The surgical interventions were performed under sterile conditions. In each pig, ten deep partial thickness of the excision wounds (the area about 225 mm²) were created along the markings using toothed forceps, a surgical blade and pointed scissors. The total number of experimental wounds was 50, with 10 per animal. All wounds were cleaned with a sterile normal saline solution following the program for each group. These ten created wounds were randomly assigned into five groups of treatments as follows:

Group I (comparative group I) was treated with a commercial foam dressing (Allevyn[®])

Group II (comparative group II) was treated with a commercial silver coated polyurethane foam dressing (Askina[®] Calgitrol)

Group III (study group I) was treated with foam dressing impregnated with silver and asiaticoside formula 1 (1 mg/cm² of silver nanoparticles and 5% of asiaticoside, BL-1Ag-AS)

Group IV (study group II) was treated with a hydrophilic alginate foam dressing impregnated with silver and asiaticoside formula 2 (6% of sodium alginate, 1 mg/cm² of silver nanoparticles, and 5% of asiaticoside, A6-1Ag-AS)

Group V (study group III) was treated with a hydrophilic alginate foam dressing impregnated with silver (6% of sodium alginate and 1 mg/cm² of silver nanoparticles, A6-1Ag).

All of the wounds were applied to the dressing, then covered with sterile gauze, and changed every one to two days [142]. During the observation of the wound healing, the wound area was periodically recorded with sterile plastic and a non-permanent fine point marker pen and calculated (in cm²) by using an image J program on 0, 4, 7, 14 and 21 days. The percent of wound closure was calculated from Equation III-10. A digital camera (SONY, model DSC TX9, Sony Company Limited, Japan) was used, facing down, 10 cm above the wound, collect the wound appearance. During this study, the animals were routinely checked for food and water consumption and mentation. The body weight, clinical signs, and skin irritation were also observed daily.

$$\% \text{Wound closure} = \frac{(\text{Wound area at day 0} - \text{Wound area at t days})}{\text{Wound area at day 0}} \times 100$$

...Equation III-10

Histological evaluation: Punch biopsy (diameter size about 6 mm) were taken at two time points: 7 and 14 days post-wounding at the wound's edge. The tissue was taken and fixed in 10% of buffered formalin solution. Each specimen was embedded in a paraffin block and a thin section (3 μm) was prepared and stained with hematoxylin-eosin and Masson's trichrome method. The tissue from the normal skin, which had a similar size to the wound tissues, would be collected in order to compare with the tissue from the wounds. After that, the slide was examined histologically under a light microscope model Nikon Eclipse E200 (Nikon Instruments, Tokyo, Japan).

The histologic examinations were modified from the studies of Abramov et al (2007) and Karayannopoulou et al (2011) at a magnification of 400x for the five areas [143, 144]. For the comparison of normal skin, the epithelium cell layer, amounts of the inflammatory cells and fibroblasts were counted and scored using the criteria presented in Table III-2.

Table III-2. Histological evaluation criteria

Parameters	Histological grading score			
	0	1	2	3
Epithelium cell layer	Normal	Mild increase	Moderate increase	Marked increase
Amount of inflammatory cell	Normal	Mild increase	Moderate increase	Marked increase
Amount of fibroblast	Normal	Mild increase	Moderate increase	Marked increase
Amount of new capillary	Less than 3 vessels	3-10 vessels	11-30 vessels	More than 30 vessels

Part III: Irritation test in human volunteers

A prospective randomized matched pair study was designed to evaluate the irritation sign of a selected foam dressing (from Part I) and commercial foam dressing (Askina[®] Calgitrol) in healthy volunteers with some modifications from Hasatsri et al (2015) and the US FDA/CDER Guidance (1999) [145, 146] and performed with the approval of the Ethics Review Committee for Research Involving Human Research Subjects, Health Sciences group, Chulalongkorn University (No. 051.1/60). The committee complies with the Declaration of Helsinki (1964), Committee of International Organization on Medical Science (CIOMS) and International Conference on Harmonization, Guidance on Good Clinical Practices (ICH GCP). The volunteers aged between 18-65 years were enrolled in this study. The exclusion criteria were skin disorder, immunosuppressive, steroid or antihistamine medication, wound or any

abnormal skin in the testing area, underlying diseases involving an immune disorder, liver or kidney disease, cancer, and allergic susceptible to active ingredients or any components. After the written informed consents were obtained, each participant received 2x2 cm² of two samples; the developed foam dressing with silver nanoparticles and asiaticoside (developed dressing group) and commercial foam dressing with silver (comparative dressing group), randomly applied on each upper arm and covered with a self-adhesive nonwoven fabric. At each point of time, the upper arm was cleaned using a cotton ball soaked with a normal saline solution, photographed and investigated using Mexameter® (Courage + Khazaka Electronic GmbH, Germany) prior to applying the new dressing. Subjects were instructed to continue changing the dressing daily and were also suggested not to apply other skin cosmetics or medications to the testing area. The pain and itching scores were assessed by the volunteers at the end of the study. This visual analog scale was between 0-10 points whereas 0 defined no discomfort and 10 defined as unbearable discomfort.

Part IV: Clinical efficacy test

A prospective, randomized, clinical trial was conducted under the approval of an institutional ethics review board of Siriraj Hospital, Mahidol University (COA No. Si 355/2017) who complied with the Committee of International Organization on Medical Science (CIOMS) and International Conference on Harmonization, Guidance on Good Clinical Practices (ICH GCP). It was also registered in Thai Clinical Trial Registry (TCTR). (TCTR number. TCTR20171228001) This study was subjected to evaluate the efficacy of the polyurethane foam dressing impregnated with silver nanoparticles and asiaticoside for the treatment of acute traumatic wounds. The sample size was calculated from Akita et al [147]. There was a total of 28 wounds included in this study. The patients were enrolled from July of 2017 through January of 2018.

Before being recruited into the study, the study protocols were explained to the patients. Patients meeting the following criteria were included in the study: aged between 18 and 60 years and had two acute traumatic abrasion wounds or dermal burn wounds on either arm or leg. The area of these wounds was less than 50 cm².

Main exclusion criteria included patients who had underlying diseases, which might impede wound healing; such as, diabetes, cancer, an immune disorder or immunosuppressive medication, chronic liver or kidney disease, and a history of allergic reaction to silver and herbs. Pregnant and lactating patients were also excluded. Informed, written consent was obtained from the participants and their demographic data were collected.

Their wounds were randomized to receive two treatments: either the polyurethane foam dressing impregnated with silver nanoparticles and asiaticoside (A6-1Ag-AS group) or the gauze dressing with 0.5% of chlorhexidine acetate (comparative group, Bactigras*). The gauze dressing was the standard treatment in the clinic and was a renowned dressing used in Thailand. At each dressing change, the wound's characteristics were assessed by a surgeon and an experienced nurse. The photographs were taken with a digital camera (SONY DSC TX-9, Tokyo, Japan). Thirty min after changing the dressing, the participants assessed the pain score for each wound using a 0-10 visual analog scale. The patients were then followed-up every two days for observation and dressing change until the wound's closure. The primary endpoint of the study was the day of the wound closure, which was evaluated by a surgeon and a nurse who were part of the treatment team. The percentage of reepithelialization, pain assessment, and adverse reaction were also investigated.

Statistical analysis

The mean \pm SD of the groups were calculated for each data set. The differences in all quantitative data; such as porosity, tensile strength, compression test, drug contents, the zone of inhibition, wound area and day of epithelialization in pig study were compared using one-way analysis of variance (ANOVA) and Tukey's or Dunnett's multiple comparison tests. The percentage of enzyme degradation between two groups, the redness in healthy volunteer study, the day of the wound's closure, the percentage of reepithelialization and pain score in the clinical study were evaluated using a paired t-test. The qualitative data; such as participants' demographic data and the dermatologic effect was expressed as a number, percentage value, and descriptive

information. The differences were considered statistically significant when p was less than 0.05. SPSS 22.0 (SPSS Co., Ltd., Bangkok, Thailand), which was used for the statistical analysis.



CHAPTER IV

RESULTS AND DISCUSSION

Part I: Foam preparation and characterizations: *in vitro* studies

In this part, there are two sections. The first involves foam with 2% of various types of natural polyols by comparing to foam without natural polyols. They are characterized the physico-mechanical properties according to Method No. 2. From this section, three natural polyols with a variety of concentrations ranging from 2-12% were selected to characterize their properties in the second section. After that, only two concentrations of each natural polyol type were selected to be undertaken by following Method No. 3.

Section One: Foam with 2% of various types of natural polyols by comparing to foam without natural polyols.

1. Preparation

This was a preliminary study about foam dressing without and with 2% of the nine types of natural polyols. These were foam without natural polyol (Blank; BL), pregelatinized starch (PGS), corn starch (CS), gelatin (Ge), sodium carboxymethylcellulose (CMC), sodium alginate (Alg), hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), chitosan low MW (CLMW), and chitosan high MW (CHMW). The appearances are shown in Figure IV-1. All formulations provided white and soft foam and could be flexible immediately after compression. Foam made from starch (PGS and CS) and Ge provided a little rough feeling.

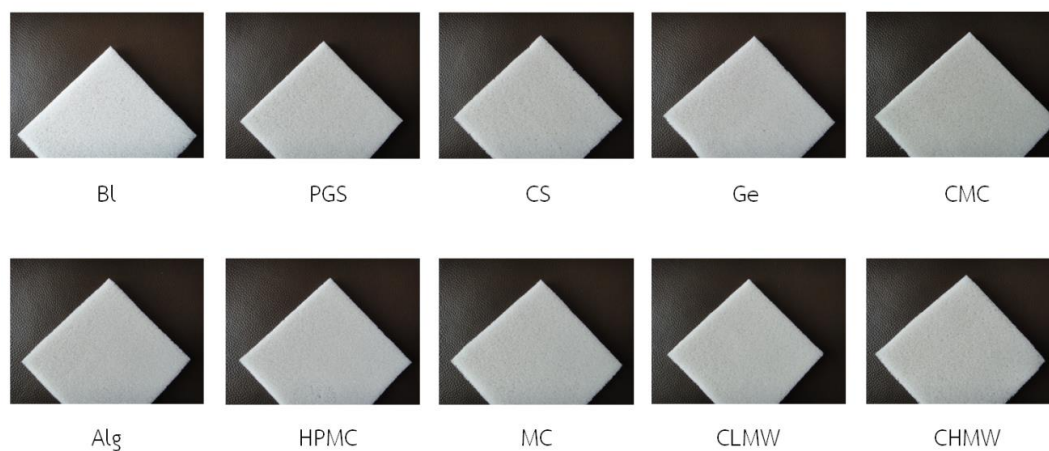


Figure IV-1. The appearance of foam dressing without / with 2% of natural polyols.

2. Characterization

2.1) Morphology and density

The average pore sizes were in a range of 0.276-0.369 mm² (Table IV-1). The Bl showed 0.328±0.601 mm². The SEM photomicrographs revealed dense, porous structure. The foam with natural polyols provided different sizes and densities. The formulation, which had the large pore sizes were Ge and CHMW (0.355±0.576 and 0.369±0.781 mm², respectively) while the Alg and HPMC showed the average low results (0.297±0.547 and 0.296±0.542 mm², respectively). The larger pore size of Ge and CHMW might be caused by the flaked powder which was bigger than fine powder of other natural polyols. It might hinder the creaming effect in the polymerization. The densities were between 40.76-44.78 g/cm³ and not related to porosity. The natural polyols containing foam showed density more than Bl foam except for chitosan foam. The OH groups of natural polyols which was added might interact with NCO group of isocyanate and produce urethane linkage. The NH₂ group containing chitosan might interfere with this polymerization [38] and produce the urea linkage. However, these results did not show any statistical differences between the groups ($p > 0.05$, ANOVA test). This might have been caused because only 2% was added in the formulation and could not affect the porosity and density. The SEM photographs from the top and side views are shown in Figure IV-2 and Figure IV-3. The top view showed a circular shape while the side view presented oval shape because of the preparation. The foam

mixture was poured into the tray and the blowing effect could occur in down to top direction. The side view would present a more circular shape in factory-scale which the foam mixture was poured onto the belt.

Table IV-1. The pore size and density of foam without / with 2% of natural polyols. Adding the natural polyols seemed to decrease porosity and increase density

Group	Bl	PGS	CS	Ge	CMC
Pore size (mm ²)	0.328±0.601	0.344±0.677	0.331±0.709	0.355±0.576	0.323±0.613
Density (mg/cm ³)	41.09±1.09	43.42±1.14	43.56±1.46	42.88±3.08	44.14±2.11
Group	Alg	HPMC	MC	CLMW	CHMW
Pore size (mm ²)	0.297±0.547	0.296±0.542	0.314±0.592	0.310±0.572	0.357±0.708
Density (mg/cm ³)	41.64±2.62	44.44±1.89	44.78±1.45	40.76±1.96	40.93±1.97

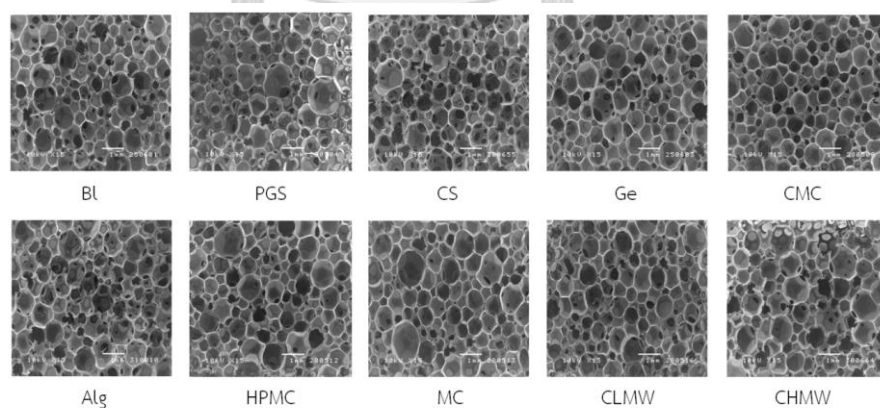


Figure IV-2. The pore structure of foam without / with 2% of natural polyols (top view).

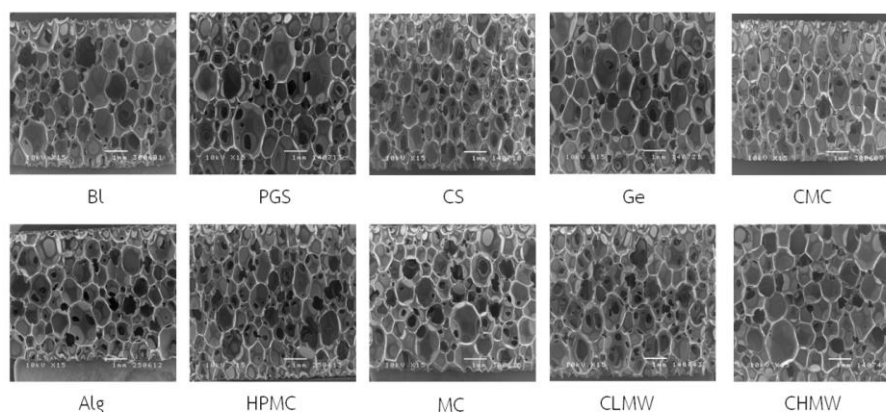


Figure IV-3. The pore structure of foam without / with 2% of natural polyols (side view).

2.2) Absorption test

The absorption test was conducted to determine the rate of water absorption of the foam at each point in time. The high slope of absorption profile inferred the rapid absorption rate in the initial time. Moreover, the foam had a high capacity of absorption that would present a high curve after a long period of time. As such, a good dressing should provide a high absorption capacity in order to prevent overwhelmed exudates. Figure IV-4 shows the percentage of absorption over 24 hr. All foam formulations immediately absorbed the fluid within one minute, which differed from other dressing types. The rate significantly decreased at 30 min and did not change after 240 min.

The natural polyol foam seemed to provide a higher percentage of absorption compared to Bl. There were no differences between the types of natural polyols ($p > 0.05$, ANOVA test). The PGS and CS had percent absorption less than other natural polyols (13.52 ± 2.34 and $13.56 \pm 3.01\%$ at 1440 min). Donovan et al [148] reported the 2-4 molecules water per hexose unit in starch was required for full hydration. The formulations which showed the high absorption profiles were the HPMC, CMC, Alg, and CLMW which presented 15.86 ± 4.94 , 15.75 ± 3.09 , 15.98 ± 4.22 and $15.22 \pm 3.88\%$ while Bl showed $13.07 \pm 2.35\%$ at 1440 min. It was reported that the water molecules which bound to polysaccharide one repeating unit approximately 6 ± 2 and 3-4 molecules in

sodium alginate and chitosan, respectively [149, 150]. This might be because the Alg foam could absorb water more than CLMW foam. The absorption of HPMC foam seemed higher than MC (15.86 ± 4.94 and $14.53 \pm 4.53\%$, respectively at 1440 min). Because of substitution of hydroxypropyl and methoxyl group of HPMC which differed from MC. The MC tended to aggregate together in the solution as bundles by folding of unsubstituted regions of cellulose structure while the hydroxypropyl and methoxyl group in HPMC were more polar and decreased intermolecular association so it was greater segmental mobility and improve hydrophilic property [151, 152].

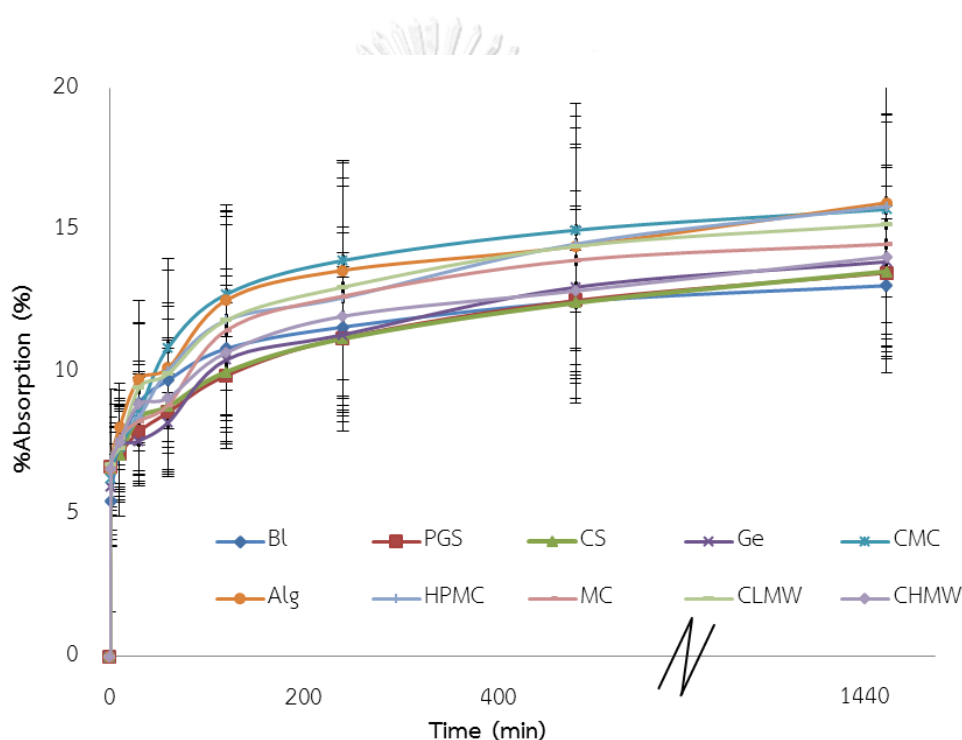


Figure IV-4. The percentage of absorption of foam without / with 2% of natural polyols. The foam with 2% HPMC, CMC, Alg, and CLMW provided high %absorption. ($p > 0.05$, ANOVA test)

2.3) Desorption test

A desorption test was used to determine the capacity the foam dressing could be retained moisture at the wound bed. The dressing was changed every one to three days depending on the amount of exudates. The foam which could not keep hydration would lead to a dry wound. Moreover, the dry condition might cause scabs over the

wound bed. These scabs would hinder the migration of epithelial cells. The desorption profiles are shown in Figure IV-5. The desorption rates were in a range of -2 to -5%/grams at 30 min then they increased the rate over a period of 480 min. There were no significant differences between the groups. ($P > 0.05$, ANOVA test) Foam without natural polyols seemed to provide a greater desorption rate than the natural polyols. This might be caused by lack of hydrophilic functional groups in Bl foam. Foams with the CMC, Alg, HPMC, MC, and CLMW formulation were likely to show a lower percentage of desorption than other natural polyols (-28.49 ± 3.01 , -27.47 ± 4.03 , -28.10 ± 5.72 , -28.24 ± 4.52 and $-28.95 \pm 4.21\%$, respectively at 480 min).

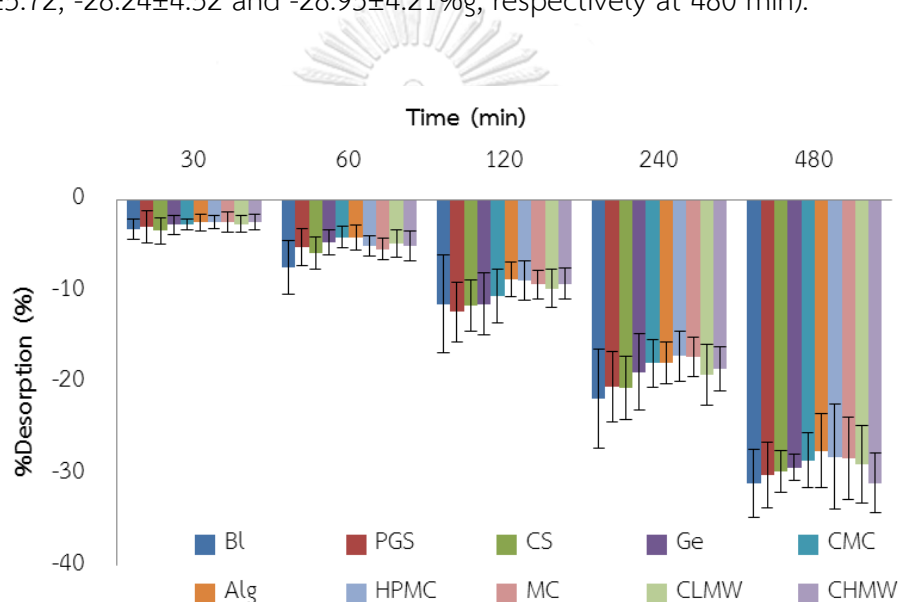


Figure IV-5. The percentage of desorption of foam without / with 2% of natural polyols. Foams with the 2% of CMC, Alg, HPMC, MC and CLMW formulation were likely to show a low %desorption.

2.4) Tensile strength test

The mechanical test including the tensile strength and elongation could infer the strength of the foam dressing. The dressing with low strength and %elongation might tear easily when changing the dressing or in a patient's movement. The natural polyols did not affect these mechanical test values (Figure IV-6). Foam with HPMC, MC and CLMW seemed to show high tensile strength (5.40 ± 0.38 , 5.38 ± 0.36 , 5.55 ± 0.56 and $5.51 \pm 0.41 \times 10^{-2}$ MPa, respectively) while Ge, CMC, Alg, and CLMW showed a high

percentage of elongation (163.60 ± 14.41 , 167.02 ± 20.49 , 157.99 ± 22.51 and $158.04 \pm 15.68\%$, respectively). ($p > 0.05$, ANOVA test) Adding the natural polymers might increase the strength by urethane polymerization otherwise it might decrease this result by an increment of the bulky branch group.

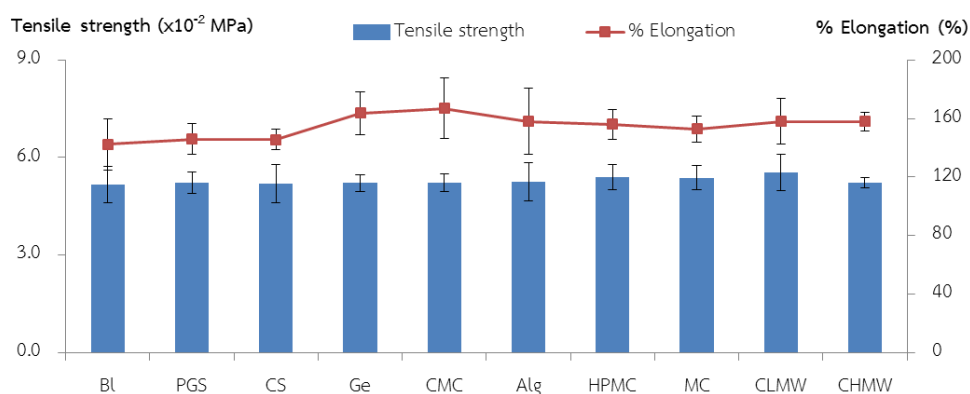


Figure IV-6. The mechanical strength of foam without / with 2% of natural polymers.

Foam with 2% HPMC, MC, and CLMW seemed to show high tensile strength while Ge, CMC, Alg and CLMW showed a high percentage of elongation.

2.5) Compression test

A compression test was used to determine the strength when the material received the compressive force. The 2% concentration of natural polyols did not affect the compressive strength significantly (Table IV-2). Foam with CMC, HPMC, and CS seemed to provide a high compressive strength while foam with Ge and CLMW provided low compressive results. ($p > 0.05$, ANOVA test) Adding the natural polymers might increase the compressive strength because the density was increased. Similar to previous studies [153-155], the compressive strength was related to foam density and/or viscosity. The hydroxyl groups in polyols were involved in the cross-linking structure of PU polymers and then results in foam with higher compressive strength. The compressive strength could also decrease in case of decreases in foam density and crosslink density which caused by less urethane linkage formation. The chitosan

group which presented low density showed quite low compressive strength, however, the viscosity and urethane linkage might increase their strengths.

Table IV-2. The compression strength of foam without / with 2% of natural polyols

Formulation	Compression strength ($\times 10^{-3}$ MPa)		
	25% strain	50% strain	75% strain
Bl	3.61±0.65	4.60±1.05	10.73±3.15
PGS	3.96±0.31	5.42±0.44	13.28±1.33
CS	4.37±0.40	5.99±0.58	14.49±1.18
Ge	4.01±0.21	5.31±0.37	12.12±1.77
CMC	4.75±0.94	6.52±1.38	15.29±2.72
Alg	3.99±0.52	5.58±0.38	13.33±0.99
HPMC	4.34±0.28	5.93±0.37	14.52±0.99
MC	4.30±0.25	5.50±0.87	13.40±1.35
CLMW	3.87±0.23	5.24±0.30	12.28±1.18
CHMW	3.99±0.50	5.47±0.62	13.95±1.71

2.6) Water vapor transmission test

Vapor permeability of the wound dressing might prevent the risk of wound maceration at the wound bed because of the vaporization of exudates. The dressing which could not permit air vaporization might slip from the wound bed. Moreover, the WVTR might help exudate absorption. The water from the lower side could permeate through to the upper side and let the exudate from wound bed penetrate into the foam. The WVTR of foam without/with natural polyols are shown in Figure IV-7. The rates were slow at 30 min and then increased at every point of time; however, the natural polyols did not change this property. The Bl which presented large porosity seemed to have a higher WVTR rate over the natural polyol groups. Foam with Ge, CMC, and Alg seemed to show a low WVTR.

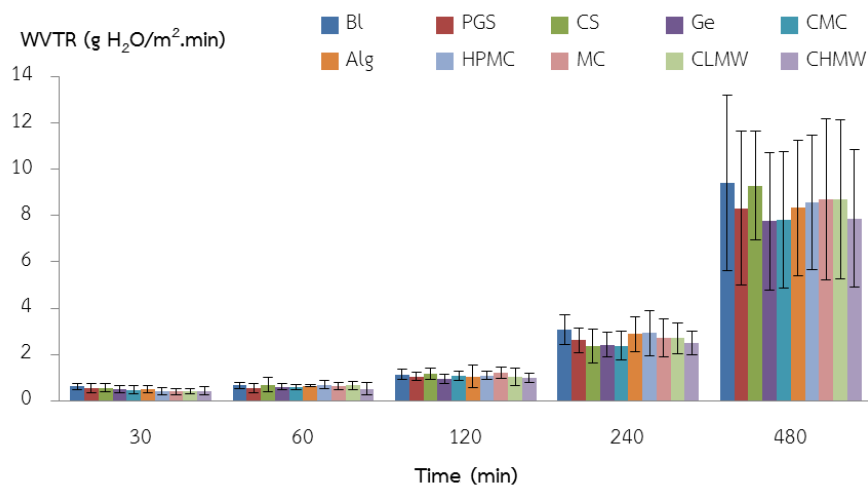


Figure IV-7. The WVTR of foam without / with 2% of natural polyols. The Bl seemed to have a higher WVTR rate over the natural polyol groups.

2.7) Foam integrity: Enzyme degradation test

The dressing would be applied on the wound bed which had exudate, electrolytes, and enzymes. The integrity of the foam dressing could confirm the dressing would not be degraded by the enzymes. Table IV-3 demonstrated the percentage of the enzyme degradation with and without an enzyme solution. The foam degraded in the enzyme solution did not differ from foam in a buffer solution. ($p > 0.05$, pair t-test) In each time point, the %degradation of all formulations were not significant. ($p > 0.05$, ANOVA test) Foam with natural polyols likely increased the percentage of degradation compared to foam without natural polyols. The Ge, CMC, and Alg foam seemed to provide greater degradation in both solutions than other natural polyols.

Table IV-3. The percentage of enzyme degradation of foam without / with 2% of natural polyols.

	4 hr		24 hr		120 hr	
	Lysozyme	PBS	Lysozyme	PBS	Lysozyme	PBS
Bl	0.21±0.07	0.19±0.14	0.39±0.21	0.35±0.22	1.64±0.51	1.27±0.34
PGS	0.30±0.23	0.21±0.15	0.45±0.18	0.43±0.28	1.98±0.80	1.72±0.43
CS	0.33±0.13	0.24±0.15	0.41±0.12	0.31±0.17	1.86±1.01	1.87±0.93
Ge	0.59±0.33	0.57±0.23	0.64±0.18	0.58±0.19	2.71±1.12	2.38±0.64
CMC	0.64±0.10	0.61±0.23	0.70±0.30	0.60±0.32	3.13±0.50	3.09±0.55
Alg	0.58±0.26	0.53±0.33	0.65±0.07	0.60±0.18	3.12±0.63	2.76±0.66
HPMC	0.55±0.18	0.47±0.19	0.42±0.09	0.38±0.11	2.61±1.19	2.51±1.33
MC	0.44±0.14	0.34±0.21	0.50±0.17	0.39±0.11	2.85±0.51	2.65±0.66
CLMW	0.31±0.16	0.28±0.11	0.42±0.29	0.37±0.07	1.68±0.45	1.71±0.93
CHMW	0.35±0.17	0.39±0.29	0.47±0.18	0.49±0.20	2.20±0.92	1.98±0.45

No significances were found in all experiments because the natural polyols were low concentration. From the results in Section one, the three natural polyols of HPMC, CLMW and Alg were selected according to the capacity of high absorption, low desorption rate, and good mechanical strength compared to other natural polyols.

The HPMC was chosen from cellulose group. It had greater water absorption capacity and lesser desorption profile compare to MC. It provided some smooth feeling more than CMC foam. The Alg was chosen because it presented high percent absorption and low percent desorption. CLMW showed the good absorption, desorption profiles over CHMW. For starches and Ge were not chosen because they provided some rough feeling, the percentage of absorption and other properties less than other natural polyols.

Section Two: A comparison of three selected natural polyols with various concentrations of 2-12% was undertaken.

1. Preparation

Foam with HPMC, CLMW and Alg were prepared in concentrations of 2-12% (2-12% HPMC, 2-12% CLMW and 2-12% Alg). Foams with CLMW had a little yellowish color. The effect of the degree of concentration to the foam's appearance could not be observed with the eyes. Foam had small pieces like flakes at a high concentration, especially at 8-12%. This might have been caused by the excess amount of the natural polyols.

2. Characterization

2.1) Morphology and density

The average pore sizes of all formulations were in a range of 0.253 to 0.327 mm². (Table IV- 4) The pore structures of the three polyols are shown in Figure IV-8 to Figure IV-10. The average pore sizes from all groups were similar to commercial wound dressings [114]. The foam with a high concentration of natural polyols seemed to produce smaller pore sizes (0.296±0.542 and 0.288±0.496 mm² in 2% and 12% of HPMC, 0.310±0.572 and 0.253±0.478 mm² in 2% and 12% of CLMW, 0.297±0.547 and 0.289±0.558 mm² in 2 and 12% of Alg). ($p > 0.05$, ANOVA test) This might cause by the opportunity of natural polyols could react with diisocyanate more than foam without natural polyols [156]. The polymerization might compact the foam structure. The concentration of polyols might increase the viscosity of the polymer solution. This might hinder the growth of air bubbles in the foam blowing reaction. [157, 158]. Foam with CLMW seemed to provide a larger pore size compared to other polyols.

The smaller pore size in high concentration could be observed by the higher density (44.44±1.89 and 45.14±1.85 g/cm³ in 2% and 12% of HPMC, 40.76±1.96 and 45.16±3.71 g/cm³ in 2% and 12% CLMW, and 41.64±2.62 and 44.72 g/cm³ in 2% and 12% of Alg). The concentration of natural polyols might increase foam density, however, there were no significant results. ($p > 0.05$, ANOVA test) (Table IV- 4) Adding the polyols powder in foam mixture could increase the weight of the sample so the density which was calculated by weight/volume was also increased. The density of foam might be an inverse relationship with pore size. Hu et al and Ryan et al [159, 160]

explained the porosity of foam was expressed by $P (\%) = 100(1 - D_{\text{foam}}/D_{\text{polymer}})$ where D_{foam} and D_{polymer} were the density of foam and actual density of the polymer, respectively. From this equation, it could be inferred that the density of foam would increase when the porosity decreased.

Table IV- 4. Pore sizes and densities of foam with 2-12% of natural polyols.

	2%HPMC	4%HPMC	6%HPMC	8%HPMC	10%HPMC	12%HPMC
Pore size (mm ²)	0.296±0.542	0.267±0.473	0.255±0.522	0.264±0.534	0.271±0.573	0.288±0.496
Density (mg/cm ³)	44.44±1.89	44.54±0.94	44.81±3.07	45.55±1.08	46.48±1.47	45.14±1.85
	2%CLMW	4%CLMW	6%CLMW	8%CLMW	10%CLMW	12%CLMW
Pore size (mm ²)	0.310±0.572	0.327±0.662	0.307±0.584	0.304±0.539	0.259±0.478	0.253±0.478
Density (mg/cm ³)	40.76±1.96	41.25±1.63	41.20±1.46	42.91±1.32	43.01±1.72	45.16±3.71
	2%Alg	4%Alg	6%Alg	8%Alg	10%Alg	12%Alg
Pore size (mm ²)	0.297±0.547	0.288±0.585	0.289±0.549	0.274±0.530	0.280±0.543	0.289±0.558
Density (mg/cm ³)	41.64±2.62	41.94±1.95	42.28±3.57	42.54±2.84	42.92±1.43	44.72±1.40

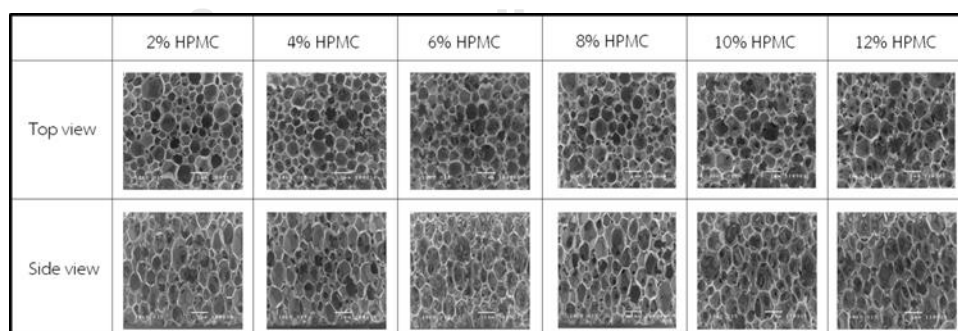


Figure IV-8. The pore structure of foam with a 2-12% concentration of HPMC.

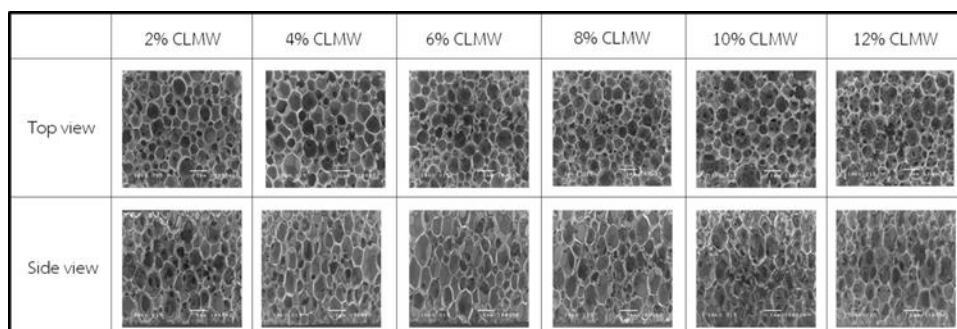


Figure IV-9. The pore structure of foam with a 2-12% concentration of CLMW.

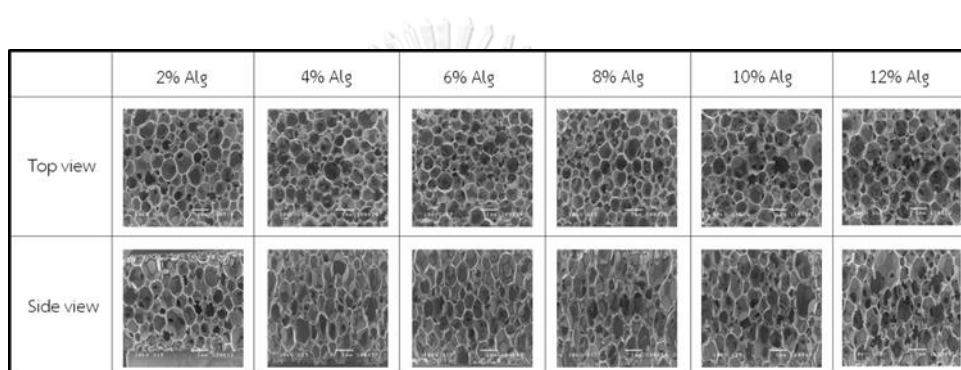


Figure IV-10. The pore structure of foam with a 2-12% concentration of Alg.

2.2) Absorption test

The percentage of absorption of Bl and 2-12% of HPMC, CLMW, and Alg are shown in Figure IV-11 to Figure IV-13. All formulations could absorb water at 1 min then they rapidly increased at 60 min. ($p < 0.05$, paired t-test) The percentage of absorption continuously increase over 24 hr. ($p < 0.05$, repeated measures ANOVA test) The natural polyols could facilitate water absorption compare to foam without natural polyols. The higher concentration of natural polyols could increase the capacity of absorption. This might be because of the increasing of the hydroxyl group which was left from polymerization would react to water via hydrogen bonding. The percentages of absorption were 5.70 ± 1.45 , 8.97 ± 2.35 , 12.28 ± 3.42 and $14.12 \pm 5.36\%$ in 2% of HPMC compared to 5.30 ± 1.88 , 11.67 ± 4.27 , 14.27 ± 4.16 and $14.67 \pm 3.80\%$ in 12% of HPMC at 1, 60, 240 and 1,440 min. The percentages of absorption were 5.57 ± 0.97 , 8.31 ± 1.53 , 9.57 ± 1.41 and $11.68 \pm 1.90\%$ in 2% of CLMW compared to 5.16 ± 1.50 , 9.71 ± 1.95 ,

11.76±2.90 and 15.01±5.50 %g in 12% of CLMW at 1, 60, 240 and 1,440 min. The percentages of absorption were 5.13±0.97, 9.14±2.08, 11.22±3.10 and 13.31±2.49%g in 2% of Alg compared to 5.90±1.59, 8.94±2.41, 11.83±4.40 and 12.69±4.25%g in 12% of Alg at 1, 60, 240 and 1,440 min.

In the comparison of absorption profiles within each natural polyols group, the 6% and 8%HPMC showed the percentage of absorption higher than Bl foam at 240 and 480 min. ($p < 0.05$, ANOVA test) Although there was no significant between concentration, 6%, 8% and 10% HPMC showed high percent absorption in HPMC group. The absorption profiles of CLMW foam did not higher than Bl foam significantly ($p > 0.05$, ANOVA test), the 8%, 10% and 12% CLMW presented high percent values compare to other concentrations in CLMW group. The percentage of absorption in Alg group show high value in 6%Alg over Bl foam at 480 and 1440 min. ($p < 0.05$, ANOVA test) There was no significant difference between concentration, however, 4%, 6% and 8% Alg showed high percent absorption.

According to their structures, these three natural polymers had some different substituted groups; HPMC contains OH and OCH₃ group, Alg contains a COO-Na⁺ group whereas CHLW contains a NH₂ group. The foam with HPMC seemed had water sorption higher than Alg foam. However, Lewis S et al [161] stated the hydrophilicity of sodium alginate over HPMC. The mucoadhesive buccal tablet containing alginate could swell rapidly compare to HPMC. This might because of polymer solubilization of algae polymer. The alginate sponge might consist of part of the unreacted alginate which could swell and dissolve easily. This was a reason why it could not sustain much water within their network structure [162]. This effect was confirmed by percent weight loss and percent degradation of alginate foam over than other natural polyols.

The foam containing Alg could absorb water more than CLMW foam. The water sorption ability of foam dressings containing natural polyols might be explained by previous studies. The number of water molecules sorbed per repeating unit in the amorphous phase can be ranked as follows: alginate > chitosan [163]. The water sorption first occurred on polymer site. The chitosan could interact with two water

molecules per repeating unit at NH_2 group while four molecules are bound per repeating unit at COONa group in alginate. HPMC foam also presented good water sorption property comparable to Alg foams. This might be explained by functional groups contained in polymers. Fringant C et al [164] reported the cellulose could bound 2 water molecules per glucopyranosyl unit, however, the HPMC might interact with water higher than cellulose because it is a modified cellulose which contains methyl and hydroxypropyl ether groups .

Another factor related to water absorption is porosity. Because small pore size could prevent the water leak out from the surface before weighing. The BL foam had larger average pore size so it could absorb water less than natural polyols foams. The leaking out might be prevented by the handle the tea ball apparatus gently and reducing the suspending time. Among in natural polyols groups, the HPMC foam had the smallest pore size while CLMW showed the largest result. The water sorption of HPMC was the highest while CLMW was the lowest.

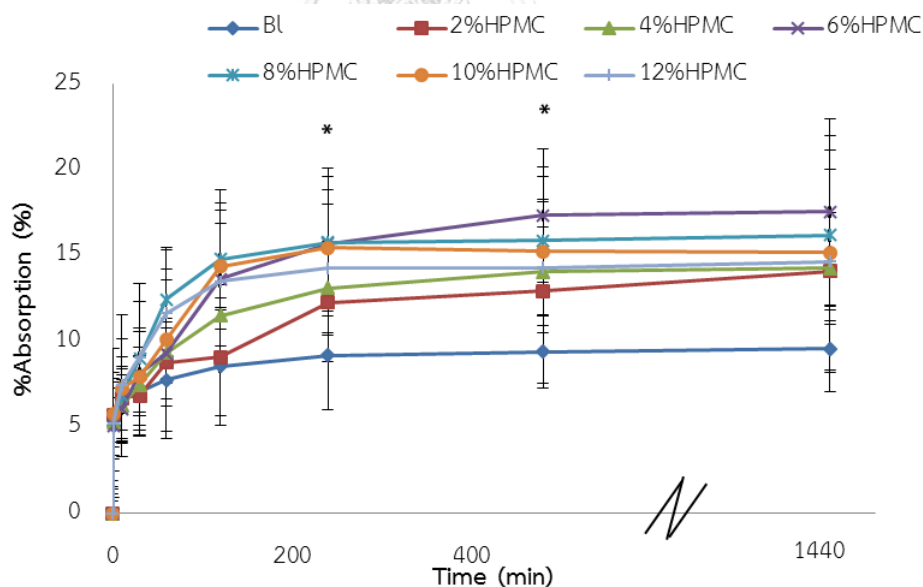


Figure IV-11. Absorption profiles of foam with a 2-12% concentration of HPMC. The 6% and 8% HPMC foam showed higher %absorption over BL foam at 240 and 480 min. ($P < 0.05$, ANOVA test)

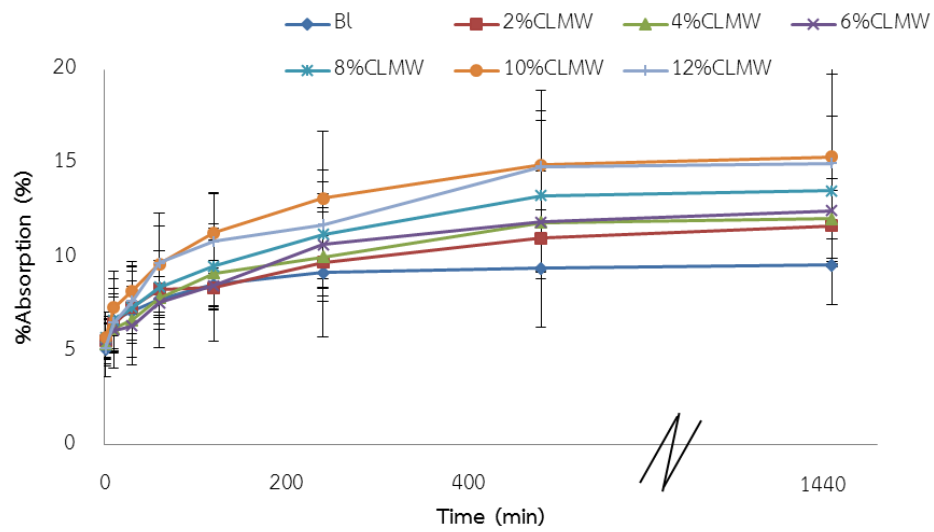


Figure IV- 12. Absorption profiles of foam with a 2-12% concentration of CLMW. There was no significant difference between BL foam and CLMW foam at any concentration.

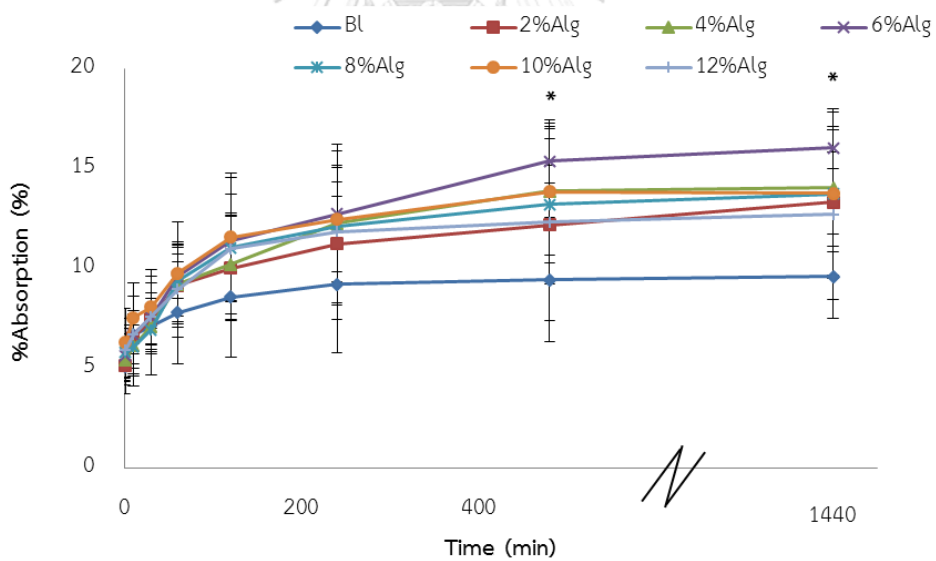


Figure IV-13. Absorption profiles of foam with a 2-12% concentration of Alg. The 6% Alg foam showed higher %absorption over BL foam at 480 and 1440 min. ($P < 0.05$, ANOVA test)

2.3) Desorption test

The percentages of desorption are shown in Figure IV-14 to Figure IV-16. All formulations dehydrated after 30 min (-3.27 ± 0.80 , -2.99 ± 1.37 , -3.25 ± 0.99 , -3.25 ± 0.99 , -4.60 ± 0.69 , $-4.20 \pm 1.12\%$ g in 2-12% of HPMC; -4.23 ± 1.01 , -3.77 ± 0.44 , -3.60 ± 1.89 , -4.38 ± 1.80 , -4.34 ± 1.19 , $-4.43 \pm 1.47\%$ g in 2-12% of CLMW; -3.63 ± 0.96 , -3.36 ± 1.05 , -3.48 ± 1.07 , -4.07 ± 0.87 , -4.33 ± 1.13 , $-4.29 \pm 0.82\%$ g in 2-12% of Alg). After that, the percentages for the desorption of the samples gradually increased over 48 hr. (-79.11 ± 3.53 , -79.25 ± 1.84 , -83.59 ± 2.62 , -83.59 ± 2.62 , -77.61 ± 4.62 , $-80.23 \pm 2.77\%$ g in 2-12% of HPMC; -81.22 ± 0.97 , -81.24 ± 3.41 , -80.34 ± 2.30 , -83.22 ± 2.81 , -82.97 ± 6.50 , $-78.63 \pm 5.18\%$ g in 2-12% of CLMW; -81.81 ± 1.88 , -81.85 ± 4.21 , -84.03 ± 3.79 , -81.11 ± 5.98 , -81.02 ± 5.73 , $-84.18 \pm 3.29\%$ g in 2-12% of Alg at 2,880 min). ($p < 0.05$, repeated measures ANOVA test) The natural polyols foam likely could retain the moisture at dressing more than Bl foam. The higher concentration of natural polyols could keep hydration by slowing the desorption rate. ($p > 0.05$, ANOVA test) However, it might not match for these results. At a higher concentration, especially 10% and 12%, the results were comparable to other concentrations. This could be explained for two reasons. First, these two concentrations absorbed a greater amount of water from an absorption test than other concentrations so the amount of water could lose more in the desorption test. Second, there were weight reductions in the natural polyols in the absorption-desorption processes. The weights after desorption were less than before absorption, which might have been caused by polymer solubilization. The loss of natural polyols, especially at a high concentration, might affect the retaining of water molecules in the foam sheet.

The larger pore size of the CLMW seemed to provide a higher rate of desorption compare to other natural polyols foam. (-8.81 ± 1.52 , $-8.19 \pm 2.81\%$ g in 4-6% of CLMW compared to -6.79 ± 1.99 , $-7.05 \pm 2.09\%$ g in 4-6% of the HPMC, and -7.86 ± 1.28 , $-7.32 \pm 0.78\%$ g in 4-6% of Alg, at 60 min).

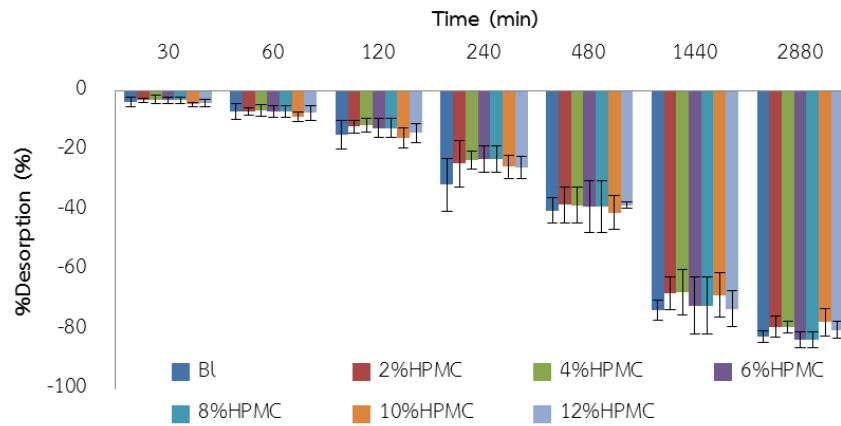


Figure IV-14. Desorption profiles of foam with a 2-12% concentration of HPMC. There were no significant differences between concentrations. ($p > 0.05$, ANOVA test)

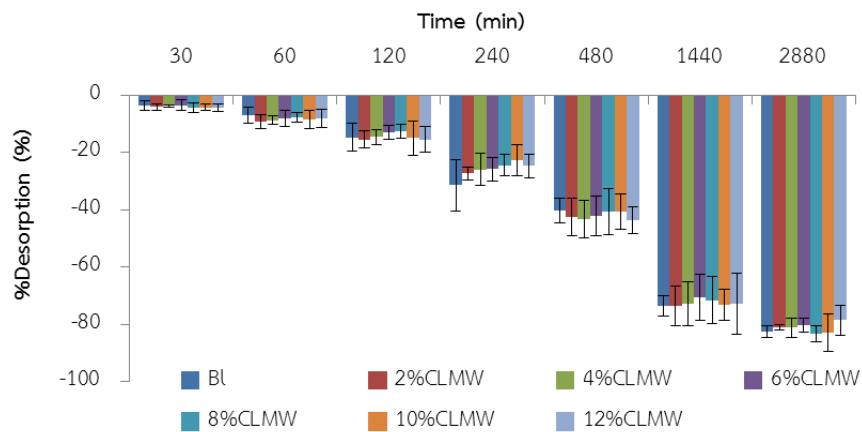


Figure IV-15. Desorption profiles of foam with a 2-12% concentration of CLMW. There were no significant differences between concentrations. ($p > 0.05$, ANOVA test)

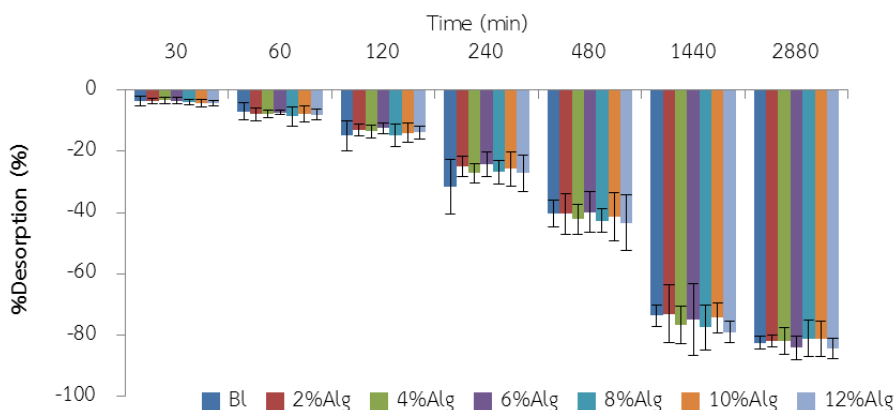


Figure IV-16. Desorption profiles of foam with a 2-12% concentration of Alg. There were no significant differences between concentrations. ($p > 0.05$, ANOVA test)

From the study of the absorption and desorption, the weight before absorption was compared to the weight after desorption. Table IV-5 presents the percentage of the weight loss of the foam sheet during those two studies. A higher concentration of natural polyols could increase the percentage loss, especially at 8-12% concentration. From the results, 8-12% HPMC and 6-12% Alg showed higher percentage loss higher than Bl foam significantly and also higher than CLMW at the same concentration. ($p < 0.05$, ANOVA test) CLMW had the lowest percentage of loss compared to other polyols. The weight loss of Alg group seemed higher than HPMC group but no significant results. ($p > 0.05$, ANOVA test)

The hydrophilic groups in natural polymers could interact with water and therefore polymer solubilization. The polarity of organic compounds could be ranked as following: acid > alcohol > amine groups [165]. This might be a reason that the percent weight loss of Alg > HPMC > CLMW foam. The hydrolysis effect of alginate foam seemed to be higher than other formulation because Alg contained the salt form of carboxyl groups (COONa) which presented the strongest hydrophilicity. The hydroxyl groups in HPMC could also interact with water molecules but showed lower percent compare to Alg. While CLMW had percent weight loss comparable to Bl, it might cause by pKa of CLMW. Although CLMW contained amine group which could form the hydrogen bond to water, the solubility might show few effects. This might because the

amino group of chitosan has a pKa value of about 6.5, the solubility of chitosan depends on the protonation of the free amino group. For this reason, it could soluble in acidic solution and also hardly solubilize in deionized water which had pH nearly 7.0.

Table IV-5. Percentage of the weight loss of foam dressing during an absorption and desorption test.

Formulation	%Loss	Formulation	%Loss	Formulation	%Loss
Bl	-0.15±0.34				
2%HPMC	-0.93±0.46	2%CLMW	-0.13±0.29	2%Alg	-1.18±0.48
4%HPMC	-1.68±0.41	4%CLMW	-0.06±0.34	4%Alg	-2.86±0.68
6%HPMC	-2.51±1.94	6%CLMW	-0.07±0.11	6%Alg	-3.70±0.45*
8%HPMC	-4.24±1.43*	8%CLMW	-0.27±0.84	8%Alg	-5.27±0.79*
10%HPMC	-5.17±0.88*	10%CLMW	-0.33±0.38	10%Alg	-5.25±2.64*
12%HPMC	-5.49±1.78*	12%CLMW	-0.37±0.66	12%Alg	-6.40±1.08*

* The natural polyols foam presented percentage weight loss higher than Bl foam ($p < 0.05$, ANOVA test)

2.4) Tensile strength test

Increasing the concentration of the polyols might decrease the strength, which can be observed in Figure IV-17. For the tensile strength, the lowest values were the concentrations of 10% and 12 percent (4.48 ± 0.68 , 4.53 ± 0.68 ($\times 10^{-2}$) MPa in the HPMC group; 4.26 ± 0.38 , 4.18 ± 0.38 ($\times 10^{-2}$) MPa in the CLMW group, and 4.95 ± 0.42 , 4.93 ± 0.41 ($\times 10^{-2}$) MPa in the Alg group. However, the tensile strength values were not significant among the same types of natural polyols. ($p > 0.05$, ANOVA test)

The concentrations affected the percentage of elongation. The 2% and 4% of HPMC were significantly higher than the 10% and 12% of HPMC (4.95 ± 0.59 , 5.28 ± 0.59 , 4.48 ± 0.68 and 4.53 ± 0.68 ($\times 10^{-2}$) MPa for 2, 4, 10 and 12% of HPMC, respectively, $p = 0.01$, ANOVA test). For other groups, they had a tendency but no statistical significance (179.09 ± 17.11 , $143.17 \pm 23.23\%$ in 2%, 12% of CLMW, $p = 0.909$ and 185.56 ± 15.04 ,

171.93±12.62% in 2%, 12% of Alg, $p = 1.00$, ANOVA test). The additive, especially large amounts of natural polyol powder which act as filler, could increase the prone of a tear or shred in the polyurethane foam. Latinwo et al [166] studied the effect of the fillers on the mechanical properties of flexible polyurethane foam and reported that it could increase foam hardness. Another reason was the cell walls had a low resistance to tearing. Normally, the foam contained billions of tiny cells, which were made of gas bubbles. These cells had very thin cell walls. The tear strength was the sum of the strength of the adjacent cell walls, so the foam had a low tear strength; this meant that each cell wall had a low resistance to tearing. The bulky structure of the excess natural polyols might hinder foam bonding result in decreases the strength of the foaming.

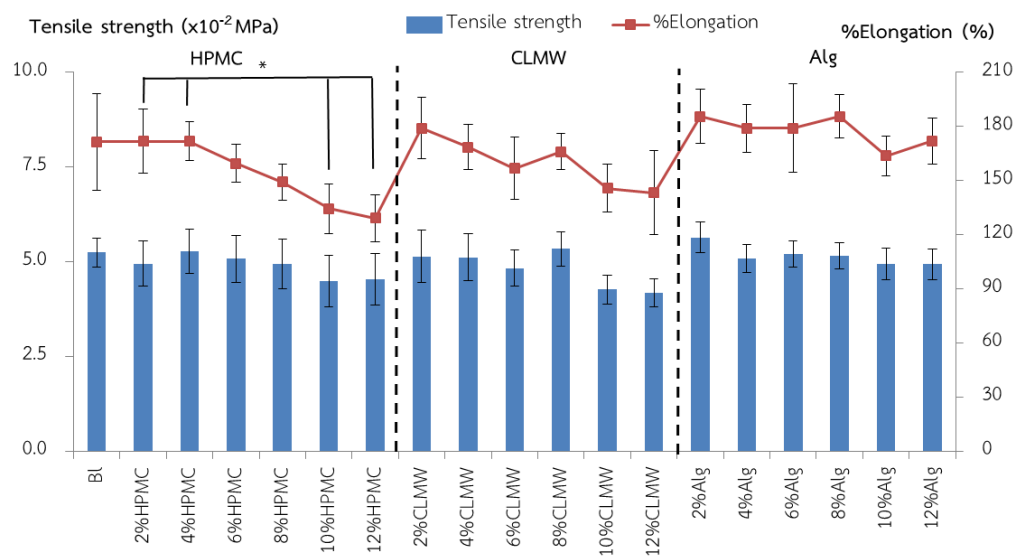


Figure IV-17. Tensile strength profiles of foam without / with natural polyols at a concentration of 2-12%. The natural polyols seemed to decrease foam strengths. There were significant between 2% and 4% HPMC over 10% and 12% HPMC. ($P < 0.05$, ANOVA test).

2.5) Compression test

The compressive strength could explain the response of the foam dressing while it experienced a compressive load. The force would decrease the pore sizes and be increasing the percentage of the strain would increase the compressive strength.

The compression strengths did not show any statistical differences ($p > 0.05$, ANOVA test) (Figure IV-18). It would be likely that the strength would increase when the concentration increased which clearly presented in HPMC and CLMW foams. This might be a result of the density of the foam in various concentrations. The compression strengths at a strain of 75% were higher than the 25% and 50% strains because the two grips were very close together, so it was under very stressed condition. As discussed above, the compressive strength was associated with foam density and/or viscosity. [153, 154, 167] The higher amount of powder in the mixture would lead to higher viscosity and also increase foam density which causes increasing the mechanical strength of the final foam. The concentration could increase the compressive strength between 2-6% of Alg foam however, it decreases at 8% concentration. The loose structure of high percent concentration of natural polyols foam which found the small pieces like flakes after preparation might be a reason for the strength reduction. This phenomenon confirmed the strength results of 10-12% Alg foam.

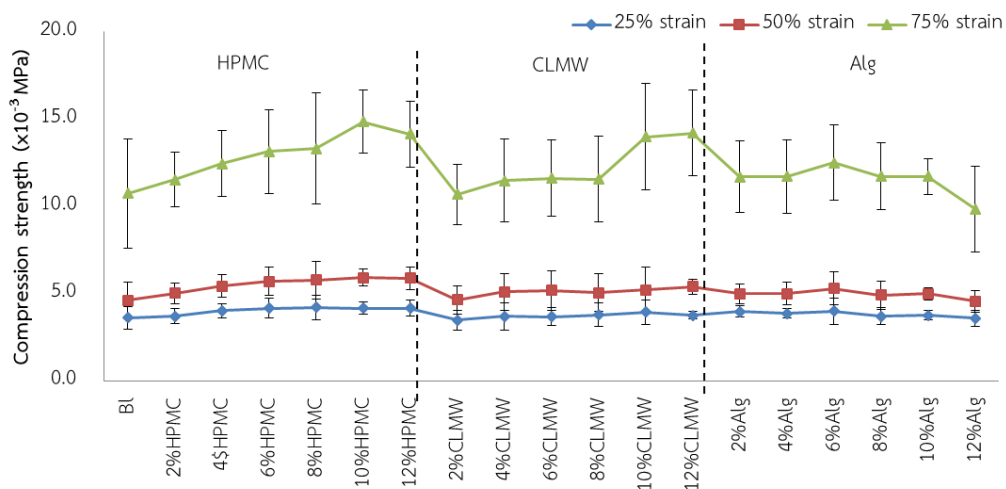


Figure IV-18. The compressive strength profiles of foam without / with natural polyols at a concentration of 2-12%. The strength would increase when the concentration increased which presented in HPMC and CLMW foams. However, there were no significant differences. ($p > 0.05$, ANOVA test)

2.6) Water vapor transmission test

The WVTR profiles of HPMC, CLMW, and Alg are shown in Figure IV-19 to Figure IV-21. The air could permeate at the time of 30 min (0.27 ± 0.14 , 0.26 ± 0.11 , 0.34 ± 0.15 , 0.36 ± 0.11 , 0.32 ± 0.09 , 0.31 ± 0.09 g/m².min in 2-12% of HPMC; 0.47 ± 0.21 , 0.38 ± 0.29 , 0.28 ± 0.05 , 0.28 ± 0.04 , 0.35 ± 0.15 , 0.26 ± 0.13 g/m².min in 2-12% of CLMW, and 0.37 ± 0.17 , 0.28 ± 0.07 , 0.28 ± 0.08 , 0.28 ± 0.10 , 0.36 ± 0.07 , 0.30 ± 0.08 g/m².min in 2-12% of Alg). Then, the rate gradually increased over time. ($p < 0.05$, repeated measures ANOVA test) The highest WVTR was presented in 8 hr compared to 30 min ($p < 0.05$, paired t-test) and higher than 24 hr. ($p > 0.05$, pair t-test)

Although the higher concentration of natural polyols had a smaller pore size and greater density, the WVTR results were not significantly different. ($p > 0.05$, ANOVA test) This might cause by the various pore sizes in foam structure. The WVTR of the CLMW seemed show the highest results compared to other polyols (27.86 ± 9.82 , 23.98 ± 11.99 , 24.45 ± 9.62 , 25.82 ± 11.43 , 24.69 ± 4.88 , 23.33 ± 10.05 g/m².min in 2-12% of CLMW; 23.26 ± 5.23 , 21.06 ± 6.75 , 19.77 ± 4.93 , 23.83 ± 4.78 , 23.46 ± 5.00 , 22.76 ± 5.54 g/m².min in 2-12% of HPMC, and 24.27 ± 10.16 , 22.30 ± 11.43 , 22.20 ± 4.67 , 23.28 ± 8.43 , 20.23 ± 7.31 , 23.18 ± 4.12 g/m².min in 2-12% of Alg, at 1,440 min). This might be because the larger porosity and hydrophilic functional group of CLMW foam compare to other natural polyols foams.

The WVTR at 24 hr of HPMC, CLMW, and Alg groups were in the range of 970.84-1060.50, 1063.86-1179.62 and 937.27-1106.37 g/m².24hr, respectively while Bl foam was 1309.56 ± 262.69 g/m².24hr. The commercial dressings exhibited a wide range of WVTR 76–9360 g/m².24hr. [116, 168] The lowest and highest value of WVTR might not good for wound healing condition. The very low WVTR might decrease the drainage of the exudate absorption by air permeability, however, the very high WVTR might permit water to vaporize extremely and lead to a dry wound bed condition. The dressing with mid-range of WVTR might be a good choice for wound management. [114]

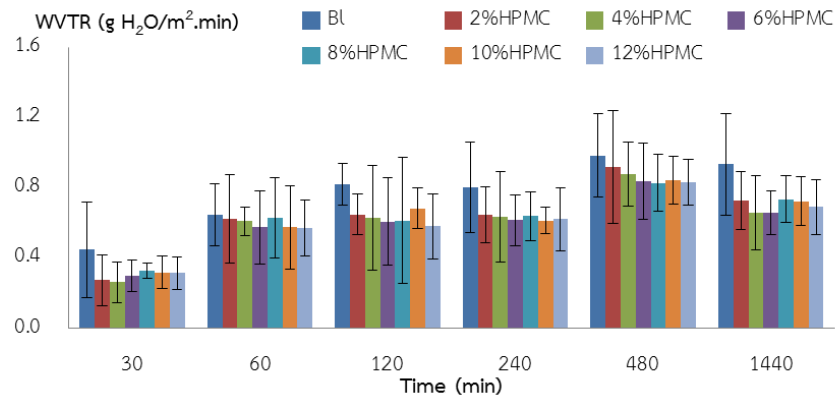


Figure IV-19. WVTR profiles of foam with a 2-12% concentration of HPMC. The natural polyols seem to decrease WVTR. ($p > 0.05$, ANOVA test)

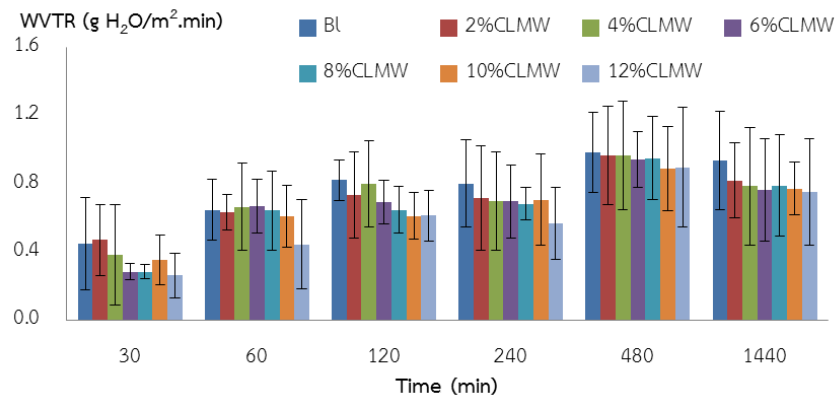


Figure IV-20. WVTR profiles of foam with a 2-12% concentration of CLMW. The natural polyols seem to decrease WVTR. ($p > 0.05$, ANOVA test)

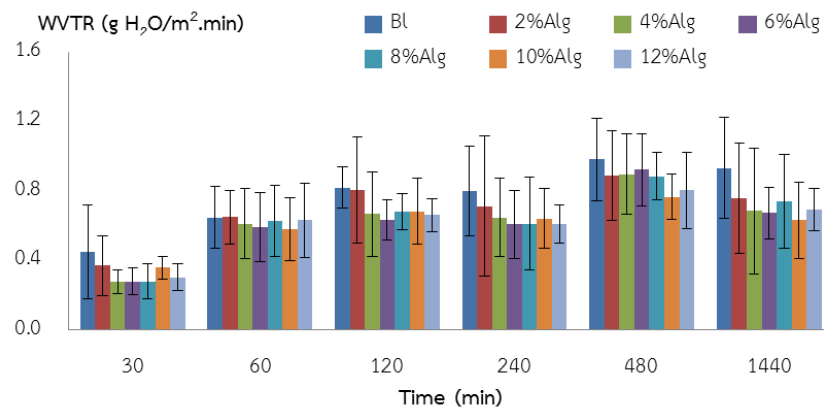


Figure IV-21. WVTR profiles of foam with a 2-12% concentration of Alg. The natural polyols seem to decrease WVTR. ($p > 0.05$, ANOVA test)

2.7) Foam integrity: enzyme degradation test

The concentrations of 4% and 6% were selected to do in this experiments because these two concentrations did not much effect from %weight loss of the absorption-desorption process. The percentage of the weight loss of variously prepared foam dressings in the lysozyme solution in which the enzyme was generally found in wounds and in the phosphate buffer pH 7.4 were compared at 48 hr, the results are shown in Table IV-6. It could be seen that adding natural polyols seemed to increase the percentage of the weight loss of the foam dressing except for those formulations with CLMW. The effect of the type of polyol was clearly shown, especially in formulation with Alg. Moreover, increasing the amount of the polyol increased the weight loss due to the polymer solubilization. In addition, there was no significant difference in the percentage of the weight loss in the lysozyme solution and in the phosphate buffer pH 7.4 ($p > 0.05$, paired t-test) indicating that the lysozyme had no effect on the dressing integrity. Minute residue could be found in the wound due to the degradation of the natural polyols, especially from 6% of the Alg formulation.

The percent weight loss from water sorption study less than weight loss from degradation test because of the method of tests. In water sorption study, the sample was filled in tea ball apparatus and sank in the water. After that, this gadget was taken out from the water and the sample was taken out and dried. The weight loss from this study might occur in polymer solubilization and the suspending step. In the degradation test, the sample freely floated in the water. Apart from solubilization of polymers, the weight loss from degradation test might occur from the rinsing step. The refreshment of the new solution every day and washing the sample with water for 3 times might be a physical force in increment of polymer solubilization.

Table IV-6. The enzyme degradation of foam without / with 4 and 6% of HPMC, CLMW, and Alg.

	4 hr		8 hr	
	Lysozyme	PBS	Lysozyme	PBS
Bl	0.19±0.80	0.20±1.03	0.31±1.24	0.17±1.13
4%HPMC	0.39±0.81	0.62±1.52	0.57±0.61	0.46±1.11
6%HPMC	1.17±1.35	1.02±0.96	2.05±0.73	1.13±1.35
4%CLMW	0.32±0.52	0.33±0.77	0.31±0.91	0.27±0.65
6%CLMW	0.02±0.52	0.18±0.63	0.23±1.23	0.27±0.62
4%Alg	0.82±1.59	1.18±1.86	2.51±1.26	2.29±0.88
6%Alg	3.68±0.41	3.75±0.52	4.14±0.51	3.64±0.29

	24 hr		48 hr	
	Lysozyme	PBS	Lysozyme	PBS
Bl	0.49±0.90	0.44±0.18	0.72±0.82	0.48±0.90
4%HPMC	1.11±0.64	0.72±0.99	1.28±0.91	1.22±1.43
6%HPMC	1.93±0.96	1.62±1.14	2.19±1.11	1.50±1.00
4%CLMW	0.31±1.05	0.38±0.87	0.49±0.67	0.29±0.87
6%CLMW	0.33±0.78	0.14±0.54	0.58±1.46	0.43±0.64
4%Alg	2.89±1.24	2.70±1.09	2.61±1.03	2.15±1.57
6%Alg	4.08±0.52	4.08±0.62	4.34±0.79	4.37±1.05

2.8) Fourier-transform infrared spectroscopy (FTIR) analysis

This technique is used to identify vibrations of the functional groups which infer to molecular structure characterization. Because the molecular bonds could vibrate at specific frequencies depending on the elements and the strength of bonds, the wavelength of light absorbed could determine the chemical bonding. The polyurethane foam without natural polyols and with a 6% concentration of HPMC, CLMW, and Alg were selected for this experiment. (Figure IV-22) In IR range, the

percentages of transmittance were those around 3100-3500 (Amine N-H stretch), 3200-3650 cm^{-1} (Alcohol/Phenol O-H stretch), 2850-2950 cm^{-1} (Alkyl C-H stretch), 1630-1750 cm^{-1} (Carboxylic acid C=O stretch), 1620-1680 cm^{-1} (C=C stretch), 1550-1640 cm^{-1} (N-H bending), 1340-1470 cm^{-1} (C-H stretch) and 1050-1150 cm^{-1} (C-O Stretch) [169, 170]. These peaks in those wavenumber ranges could be found in BL, 6% of HPMC, CLMW and Alg foam. The urethane or carbamate linkage consisted of carboxyl group (-COO-) and amine group (-NH-). It also contained alkane (-CH₂-CH₂-) and the hydroxyl group (-OH-) from polyols and aromatic ring from TDI. The hydrogen bonding in the polyurethane foam was caused by the N-H group acting as a proton donor of the urethane linkage while the carbonyl of the urethane group or other ester carbonyl or ester oxygen was the hydrogen bond acceptor. The degree of hydrogen bonding was observed in the N-H stretching region of the spectrum. Similar to Kim et al [171], the urethane linkage, the polymerization between the diisocyanates of 2,4-TDI and the hydroxyl groups of polyols, could be detected in peaks around 1735 cm^{-1} , which referred to the C=O of the urethane and 1550 cm^{-1} which referred to the N-H of the urethane.

The natural polyols added in the formulation could not affect the spectrum in both the position and intensity of each peak. The broad -OH- stretching which presented in 3252.19 to 3453.94 cm^{-1} in natural polyols powder could not find in PU foam. The small peak nearly 3300 cm^{-1} in PU foam might be N-H stretching. As a result of the low concentration (6% concentration) of natural polyols, the reaction or bonding in foam with natural polyols might not be able to detect.

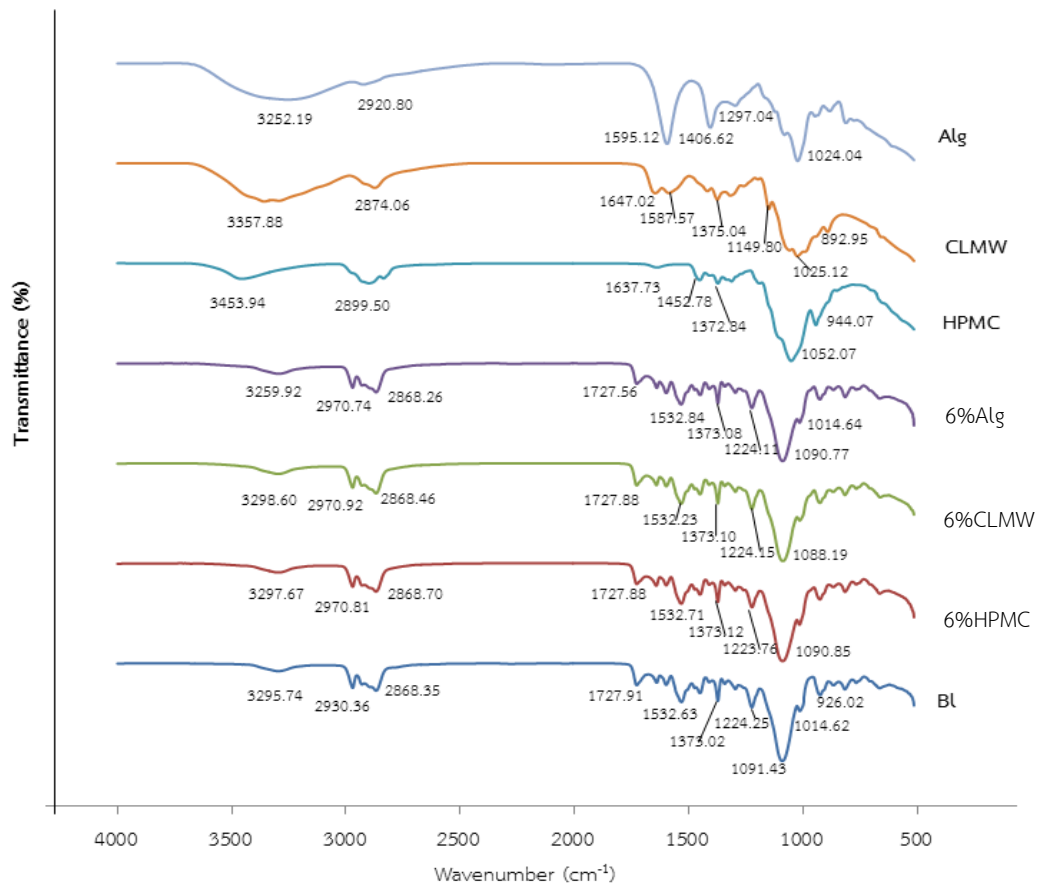


Figure IV-22. FTIR spectra of polyurethane foam without / with 6% of HPMC, CLMW, and Alg.

The comparison of IR spectrum of Bl, 6% and 10% of natural polyols were presented in Figure IV- 23 to Figure IV-25. The 10% of natural polyols provide the same peak with Bl and 6% natural polyols foam but larger intensity, especially in N-H stretching, C=C stretching and N-H bending. It might cause by some interaction could be detected in higher natural polyols concentration.

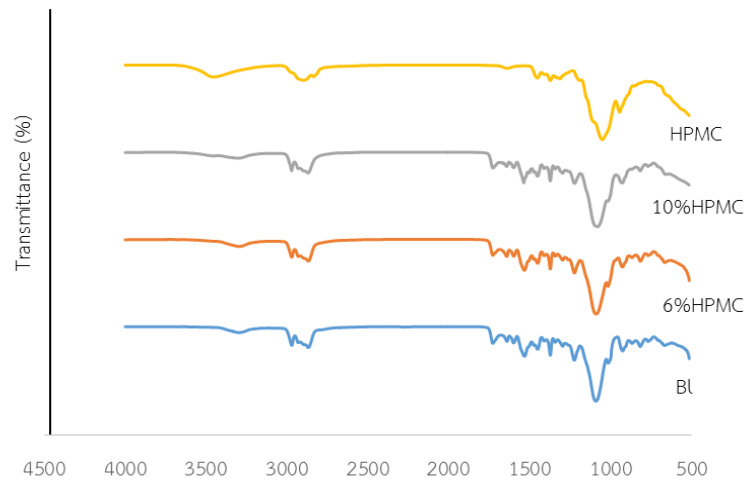


Figure IV- 23. FTIR spectra of polyurethane foam without natural polyols (BL), with 6 and 10% of HPMC and HPMC powder.

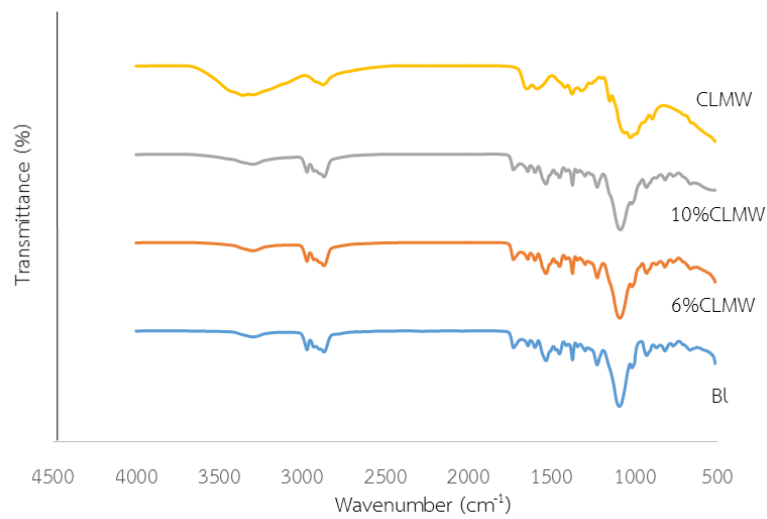


Figure IV- 24. FTIR spectra of polyurethane foam without natural polyols (BL), with 6 and 10% of CLMW and CLMW powder.

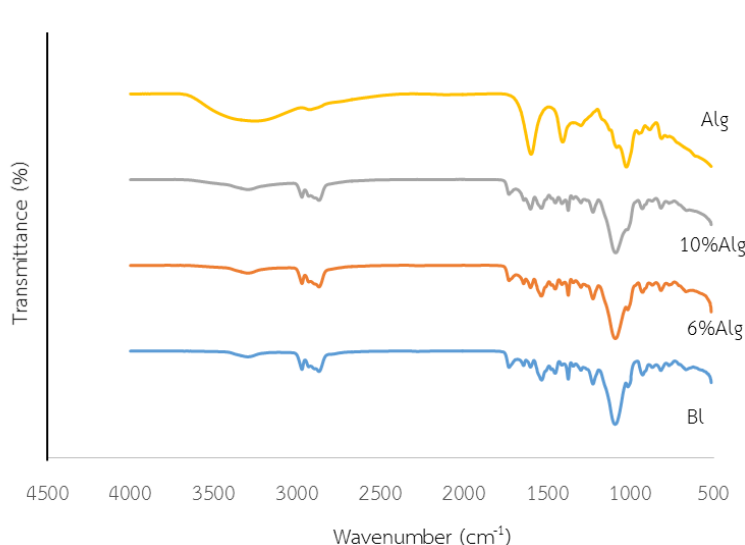
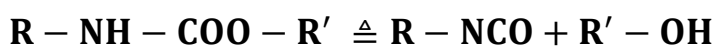


Figure IV- 25. FTIR spectra of polyurethane foam without natural polyols (Bl), with 6 and 10% of Alg and Alg powder.

2.9) Differential scanning calorimetry (DSC) analysis

This technique was used to determine the thermal transition of a sample under heating. The energy was required to maintain zero temperature difference between sample and references side and presented as an endothermic or exothermic peak. Figure IV-26 shows the differential scanning calorimetry (DSC) diagram of the Bl, 6%HPMC, 6%CLMW and 6%Alg dressing, and HPMC, CLMW, and Alg as a powder. The DSC thermograms of the polyurethanes showed a broad endotherm peak at a low temperature. This might have been caused by the loss of water in the samples. The second endothermic began around 260°C for Bl, 6%HPMC, and 6%CLMW while 6%Alg appeared at 250°C. This was caused by the decomposition of the urethane group which was formed by the reaction of isocyanate with water [172]. The o-acyl fission of polyurethane at the temperature below 300°C would produce isocyanate and alcohol [173]. The Equation IV-1 showed the possible equation of this thermal degradation.



...Equation IV-1

The Alg showed the sharp exothermic peak at around 241.49 °C. The enthalpy was 330.63 J/g. The 6% of sodium alginate powder mixed in the formulation resulted in a small peak at the same temperature. Its enthalpy was 12.92 J/g at 245.44 °C. This was the exothermic decomposition peak of sodium alginate which might be a partial decarboxylation reaction, oxidation reaction and opening of the pyranoid rings [137, 174]. The CLMW seemed to present the exothermic peak at around 280 °C. Sarmento et al [174] reported the high exothermic peak of alginate and chitosan at 247.8 and 311.0 °C, respectively. However, this could not be detected in C6 which the experiment had the maximum temperature of 300°C.

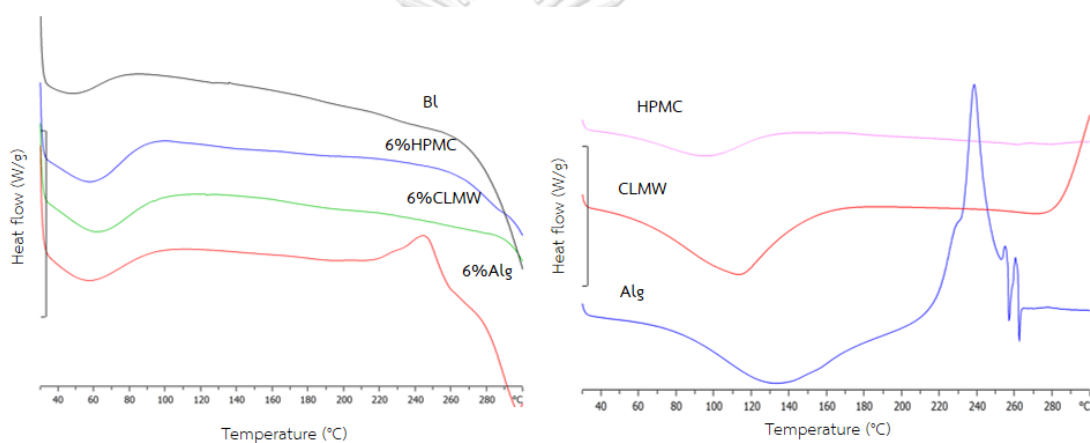


Figure IV-26. The DSC diagrams of the BL, 6% of HPMC, CLMW and Alg foam dressing and natural polyols powders.

From section two, the 4% and 6% concentration of HPMC, CLMW, and Alg were selected to do in the next study. These 2 concentrations presented moderate absorption-desorption properties. The lower percentage of weight loss, enzyme degradation, and higher tensile strength compared to foam with high percent concentration. Moreover, there were no flakes after preparation which occurred in high polyols concentration.

3. Preparation and determination of polyurethane foam dressing impregnated with silver nanoparticles and asiaticoside

3.1) Preparation of polyurethane foam dressing impregnated with silver nanoparticles

The foam without natural polyols of HPMC, CLMW and Alg were added with silver nanoparticles at 0.4, 0.6, 0.8 and 1.0 mg/cm² (0.4, 0.6, 0.8 and 1.0Ag) (Figure IV-27). After impregnation, the foam turned into a gray-black color. Foam with HPMC, CLMW, and Alg increased slightly in hardness compared to foam without natural polyols, which might have been caused by the drying process. The SEM photographs of the foam without natural polyols of 6% of HPMC (H6), 6% of CLMW (C6) and 6% of Alg (A6) were impregnated with 1.0 mg/cm² silver as shown in Figure IV-28. Some small dust which laid down on the surface of the pore structure were silver nanoparticles.

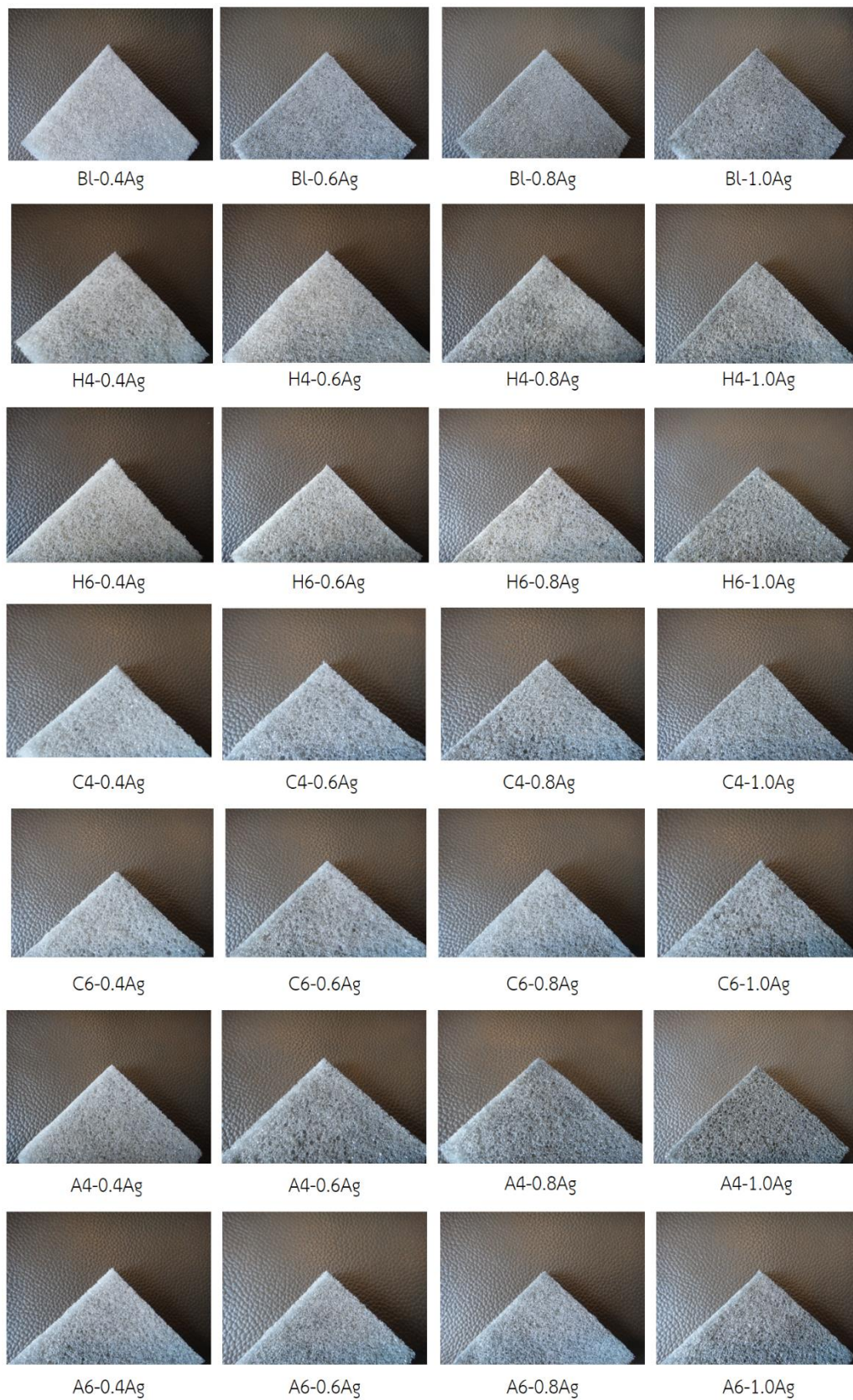


Figure IV-27. The appearance of foam dressing with different amounts of silver.

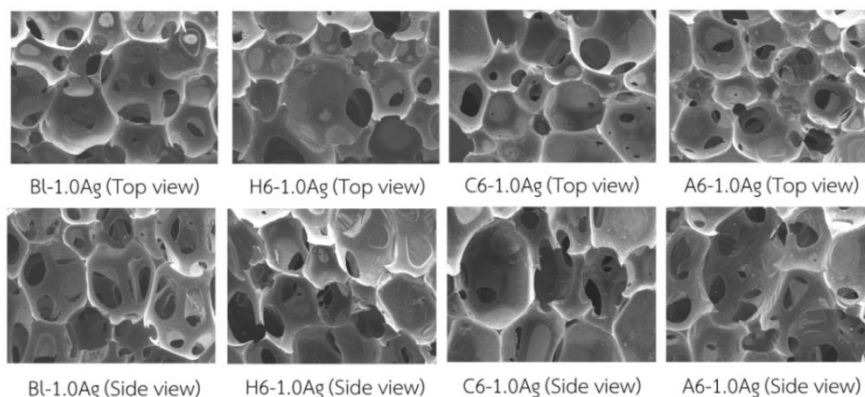


Figure IV-28. Foam without / with 6% of HPMC (H6), 6% of CLMW (C6) and 6% of Alg at 1.0 mg/cm² silver.

The silver nanoparticles could be confirmed by an Energy-dispersive X-ray (EDX) analysis, which consisted of spectra with peaks corresponding to all the different elements that were present in the sample. The sample was cross-sectional cut and placed on the stub. The quantitative analysis reported that the elements in the purple rectangle of the A6-1Ag-AS sample consisted of four main elements; 42.06% of carbon (C), 18.95% of oxygen (O), 1.9% of sodium (Na) and 37.09% of silver (Ag) (Figure IV-29).

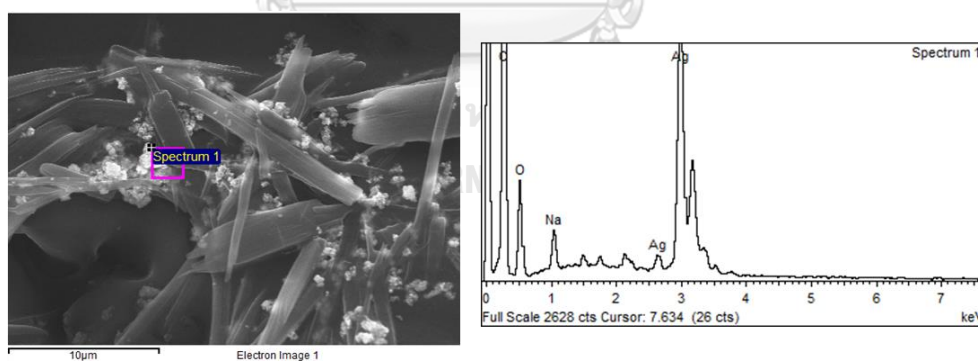


Figure IV-29. The spectra of the EDX analysis of the A6-1Ag-AS sample.

3.2) Active compound content

3.2.1) Silver content

The silver contents after preparation were in the range of 92.50-94.50 percent of the theoretical amount. Foam with alginate seemed to provide the highest silver content while foam with CLMW showed the lowest result (94.37±8.61 and 92.50±9.40

%, respectively). However, there were no significant differences between the silver amounts between 4 formulations ($p > 0.05$, ANOVA test). There also were some silver losses in all groups due to the preparation process. Even the used volume was less than maximum foam absorption volume; there was some residue in a tray after absorption. The rinsing the tray with water might be the excess volume which foam could not absorb. This might make the foam did not homogeneous dispersion. Another reason was silver content determination. The silver solution would be diluted after the acid foam degradation; this might be some agglomeration and precipitation. The centrifugation might increase sedimentation of precipitated silver. Yu et al [175] have reported that this aggregation could be accelerated by light because of the redox instability of silver.

3.2.2) Asiaticoside content

There was 94.0-96.0% of the asiaticoside content after impregnation and no statistical differences between groups. (95.47 ± 8.81 , 95.29 ± 8.35 , 95.21 ± 10.64 and 94.42 ± 10.90 for B1-1Ag-AS, H6-1Ag-AS, C6-1Ag-AS, and A6-1Ag-AS, respectively) ($p > 0.05$, ANOVA test). The main reason for percent loss was from preparing process which already discussed. Apart from the residue after foam absorption, asiaticoside might degrade from the drying process. Suwantong et al [176] found that there was percent loss of asiaticoside from fiber mat wound dressing vary from 5-15 percent under 40°C for 4 months. Because the asiaticoside in foam dressing was exposed to high temperature for 48 hr in the drying process so the high temperature might be the main reason in percent loss. Another possible reason was water. Because the asiaticoside powder was suspended in water before foam absorption. The asiaticoside was possibly hydrolyzed and produced an asiatic acid, two units of glucose and one unit of rhamnose (Figure IV-30). [177, 178]

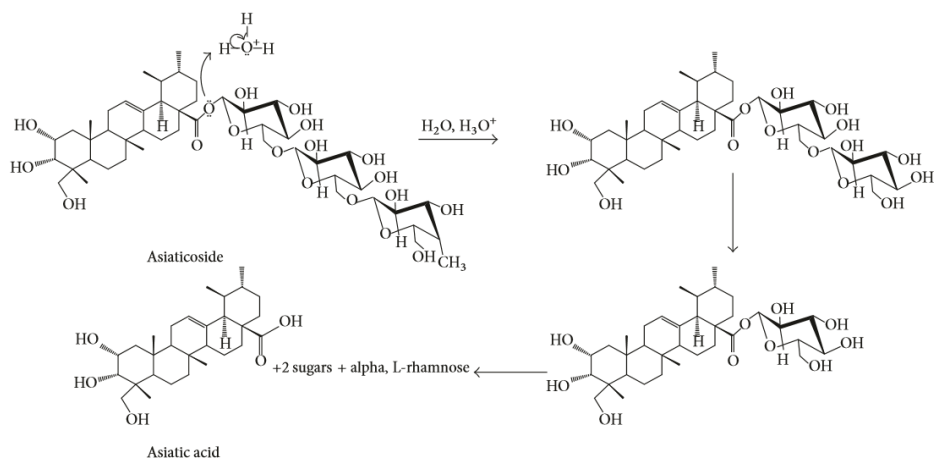


Figure IV-30. The possible mechanism of hydrolysis of asiaticoside [177]

3.3) Releasing of active compounds

3.3.1) Silver releasing profiles

The foam dressing would incorporate silver nanoparticles at 0.4, 0.6, 0.8 and 1.0 mg/cm² (0.4- 1.0Ag) according to the concentration of silver in the commercial dressing. The releasing profiles of the BL, HPMC (H4 and H6), CLMW (C4 and C6) and Alg (A4 and A6) are shown in Figure IV-31 to Figure IV-34. The silver stepped upward releasing over 24 hr. ($p < 0.05$, repeated measures ANOVA test) Both the concentrations of the silver and natural polyols affected the silver releasing profiles. The higher amount of Ag in dressing would effect to the higher amount of Ag release. The 1.0 mg/cm² silver concentration showed high releasing profiles in all groups. This concentration presented the releasing profiles significantly higher than 0.4 mg/cm² silver concentration throughout the study. ($p < 0.05$, repeated measures ANOVA test)

The silver rapidly released since 30 min from BL foam. The BL-1.0Ag foam showed higher releasing profiles over other concentrations while BL-0.8Ag foam showed the releasing profiles higher than BL-0.4Ag foam significantly. ($p < 0.05$, repeated measures ANOVA test) The silver might promptly release after the water contact because some of them just lay down on the surface which could be observed in BL formulation. Then, the releasing profiles were slow down after 4 hr. The agglomeration of small silver nanoparticles when releasing from foam dressing could

occur because the small particles prefer to accumulate together. The metal particles could become large particles to increase the stability. Holbrook et al [179] found that the size of released silver nanoparticles was shifted to a slightly larger population when compared to the pre-wetted surface. They also explained that the agglomeration of particles might reduce the surface area which led to decrease the amount and also prolong the rate of releasing profiles. In addition, the deionized water was used as the medium in this experiment in order to avoid the other ion signals interference. The silver nanoparticles might show low releasing profiles in deionized water. The property of the vehicle might be involved in active compound releasing. Hristovski et al and Peretyazhko et al [180, 181] found that the lower pH of the vehicle would provide the silver dissolution in water more than neutral pH.

The releasing manner of silver from natural polyols foam were quite differed from Bl foam. The rate of silver releases were slowly increased until 12 hr, then slightly decrease. The higher polyol concentration provided higher releasing profiles. The 6 percent concentration of natural polyols could facilitate silver release more than 4 percent. In the HPMC group, the H6-0.6Ag showed the releasing profiles higher than H4-0.6Ag and H6-0.8 Ag showed the releasing profiles higher than H4-0.8Ag. ($p < 0.05$, repeated measures ANOVA test) The H6-1.0Ag > H4-0.8Ag, H6-0.6Ag at 12 and 24 hr ($p < 0.05$, ANOVA test). In the CLMW group, the C6-1.0 Ag > C6-0.6Ag, C4-0.6Ag at 12 and 24 hr. ($p < 0.05$, ANOVA test) In the Alg group, the A6-1.0Ag showed the releasing profiles higher than A6-0.6Ag, A6-0.4Ag, A4-0.8Ag, A4-0.6Ag and A4-0.4Ag. ($p < 0.05$, repeated measures ANOVA test) The releasing profiles of A6- 1.0Ag > A6-0.6Ag, A4-0.6Ag, and A4-0.8Ag at 12 and 24 hr. ($p < 0.05$, ANOVA test)

In comparing between natural polyols groups, at 1.0 mg/cm^2 , A6 gave the highest results that were also higher than C4 and C6 throughout the study ($p = 0.013$ and 0.021 , respectively, repeated measures ANOVA test). The releasing profiles could be divided into two steps; the releasing from the surface and inner the porosity. When the water contacted the foam, the silver particles which were on the surface would release rapidly. For silver particles which incorporate inside the structure, the water

molecules would penetrate through the porous. The releasing profiles of silver from the Bl group were quite instant compared to the natural polyol group. It might be caused by the large pore size which permits the water and drug to pass through easily. Moreover, this might cause by the Bl foam had not hydrophilic functional groups like natural polyols foams. As the water sorption results which mentioned above, this was a reason that silver from natural polyols foams seems to be a delay and lower release. The natural polyols foam could absorb water more than Bl foam so the swelling effect could hinder the water molecules to penetrate and silver particles release. Among these three types, COONa of alginate foam was the strongest hydrophilic group. The erosion effect of Alg foam could increase the silver releasing profiles which could be confirmed from high percent weight loss compared to other natural polyols foam. The OH group also presented water-favorable property. The releasing profiles of HPMC foam were comparable to Alg foam. The CLMW foam seemed to provide low releasing profiles. This might cause by the polarity of amine group which was weaker than COONa and OH group. This experiment objects to determine the silver releasing manner, however, the wound exudate which included water, electrolytes, nutrients, inflammatory cells, enzyme and others debris tissue might increase silver release.

The higher silver and natural polyols concentrations provide higher silver release. The 1.0 mg/cm^2 concentration showed the releasing profiles higher than bacteria's minimum inhibit concentration (MIC) which would be discussed in the next study. From these results, Bl-1.0Ag and 6% of the natural polyols and 1 mg/cm^2 of the silver concentration (H6-1.0Ag, C6-1.0Ag, and A6-1.0Ag) would be selected for the next experiments.

Although the silver showed the low amount releasing profiles, there was a concept of silver containing in superabsorbent wound dressing. In order to avoid peri-wound maceration, the dressing should absorb excess exudate [182]. The dressing permits water vaporization and pull the exudate from the wound bed. In this step, some bacteria in exudate were absorbed and killed by silver inside the dressing. This

might be explained that this active compound could kill the bacteria via two steps; at the wound bed and inside the foam.

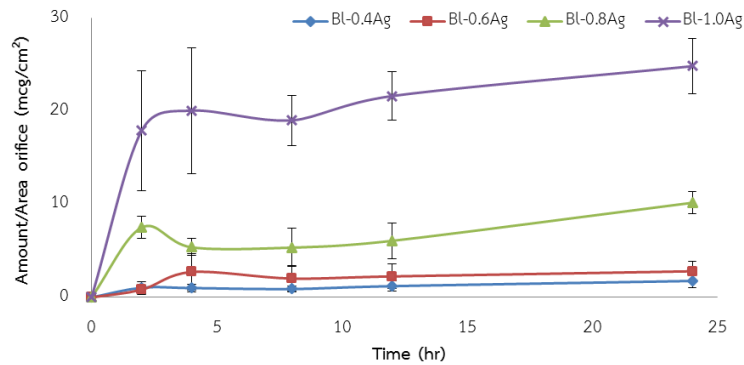


Figure IV-31. Silver releasing profiles of Bl foam impregnated with 0.4-1.0Ag. Bl-1.0Ag showed silver releasing profiles higher than other concentrations. ($p < 0.05$, repeated measures ANOVA test)

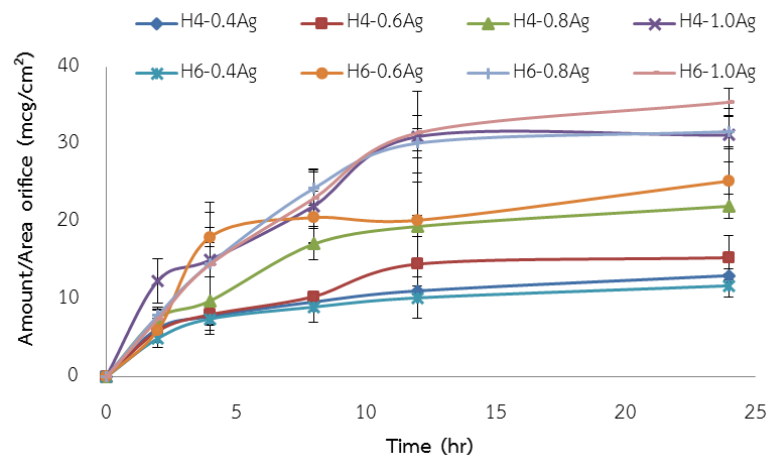


Figure IV- 32. Silver releasing profiles of foam dressings with H4 and H6 impregnated with 0.4-1.0Ag. The H6-0.6Ag showed the releasing profiles higher than H4-0.6Ag and H6-0.8 Ag showed the releasing profiles higher than H4-0.8Ag. ($p < 0.05$, repeated measures ANOVA test)

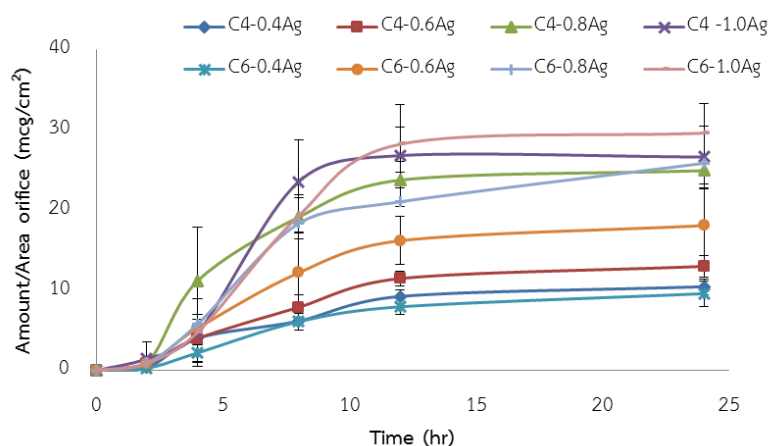


Figure IV-33. Silver releasing profiles of foam dressings with C4 and C6 impregnated with 0.4-1.0Ag. Higher silver and natural polyols concentration could increase silver release.

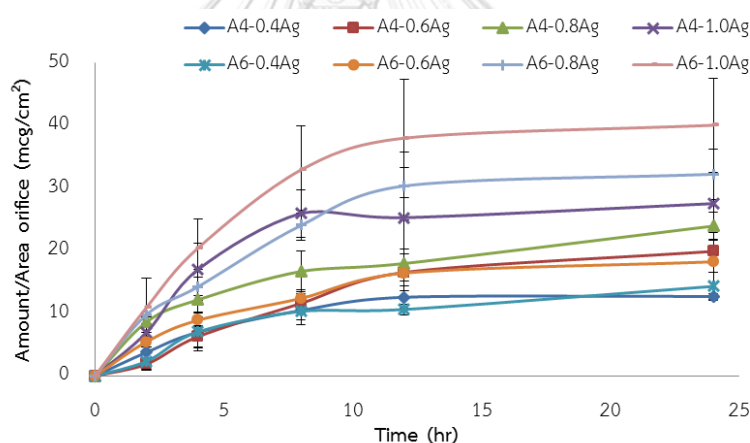


Figure IV-34. Silver releasing profiles of foam dressings with A4 and A6 impregnated with 0.4-1.0Ag. The A6-1.0Ag showed the releasing profiles higher than A6-0.6Ag, A6-0.4Ag, A4-0.8Ag, A4-0.6Ag, and A4-0.4Ag. ($p < 0.05$, repeated measures ANOVA test).

3.3.2) Asiaticoside releasing profiles

The asiaticoside from all formulations could release throughout the study. ($p < 0.05$, repeated measures ANOVA test) It was clearly shown that the foam sheets without natural polyol impregnated with silver at 1 mg/cm^2 and 5 % of asiaticoside (BL-1Ag-AS) rapidly released and gave the highest amount of asiaticoside, and the release was constant after 8 h ($p < 0.05$, repeated measures ANOVA test), followed by the

foam dressing with 6% of Alg (A6-1Ag-AS) while the C6-1Ag-AS was lowest. (Figure IV-35) The AS releasing profiles from A6-1Ag-AS was higher than C6-1Ag-AS. ($p = 0.01$, repeated measured ANOVA test)

Similar to the silver's release, asiaticoside from Bl-1Ag-AS was quite rapidly released then become gradually decrease. The releasing profiles manners of natural polyols foam were similar to foam without natural polyols. The asiaticoside which were on the outer surface could burst release while the actives which were inside the foam structure must be diffused through pores and channels. The larger porosity, the rate of asiaticoside releasing profiles from foam without natural polyols foam was faster than foam with natural polyols. The smaller pore size of foam with natural polyols might limits the water penetrates and also retards the drug release. Same as to silver releasing profiles, the hydrophilic functional groups might also effect AS releasing profiles. These natural polyols containing foam dressing could absorb water more than foam without natural polyols, they might swell when water contact. There were some swelling effect and gelling behavior of polymer which might retard the drug releasing in the hydration process [183, 184].

Apart from swelling behavior, the erosion effect could be found in hydration. The alginate might solubilize and lead to the erosion of the foam which could be confirmed by percent weight loss [123]. The percent weight loss of foam containing alginate seemed to be higher than other foams with natural polymers. This reason might increase the drug's releasing. Although the formulations with 6% of HPMC and 6% of CLMW (H6-1Ag-AS and C6-1Ag-AS) had a comparable release of asiaticoside ($p > 0.05$, repeated measures ANOVA test) The CLMW foam gradually released the active compound even after 48 hr while formulation with HPMC reached the plateau after 24 hr. This might because of the CLMW hydration manner. Although the CLMW present largest pore size over other natural polymers, the swelling effect and the solubility of chitosan in buffer solution could also delay the releasing profiles. This effect might relate to percent absorption which showed lower than other natural polymer foams.

Another possible factor was the electrostatic charge of functional groups of CLMW and Alg. The partial negative charge of asiaticoside might be attracted from the positive charge of the amine group of CLMW while it might be repulsed from the negative charge of the carboxylic group of Alg. The HPLC chromatograms of the asiaticoside releasing profiles from BL-1Ag-AS are shown in Figure IV-36 to Figure IV-41.

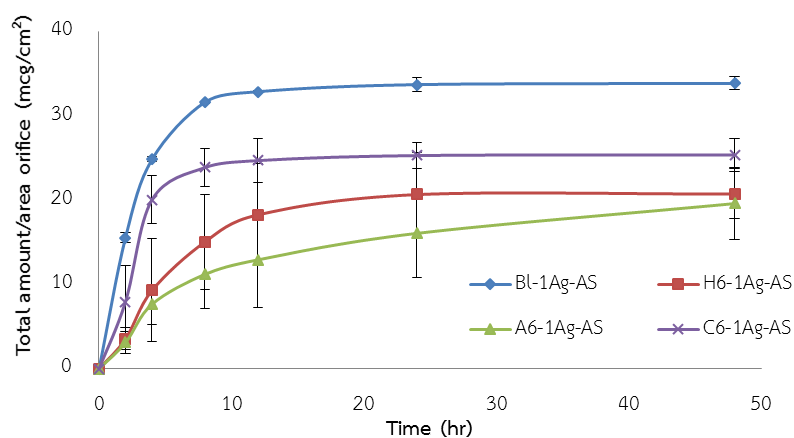


Figure IV-35. Asiaticoside releasing profiles

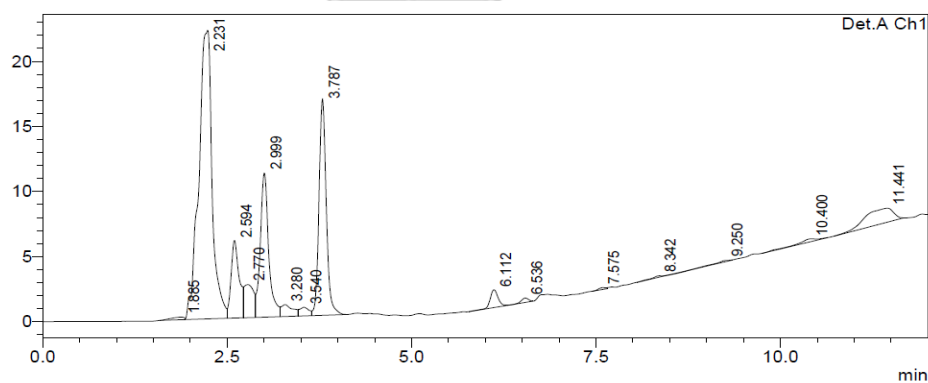


Figure IV-36. The HPLC chromatogram of the asiaticoside releasing profile from BL-1Ag-AS at 2 hr.

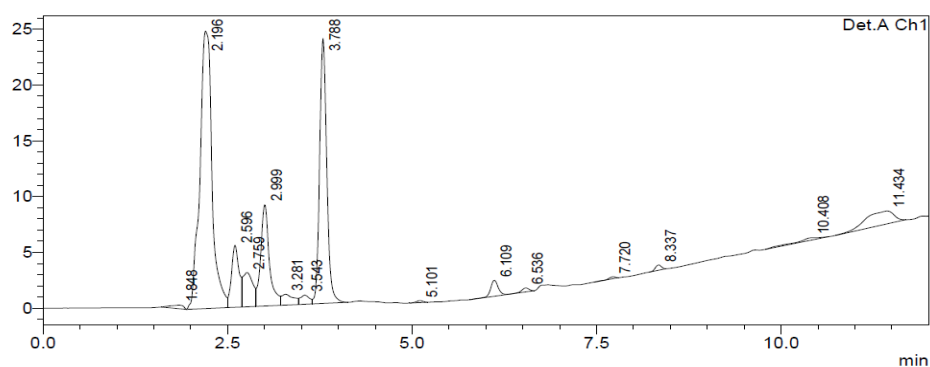


Figure IV-37. The HPLC chromatogram of the asiaticoside releasing profile from BL-1Ag-AS at 4 hr.

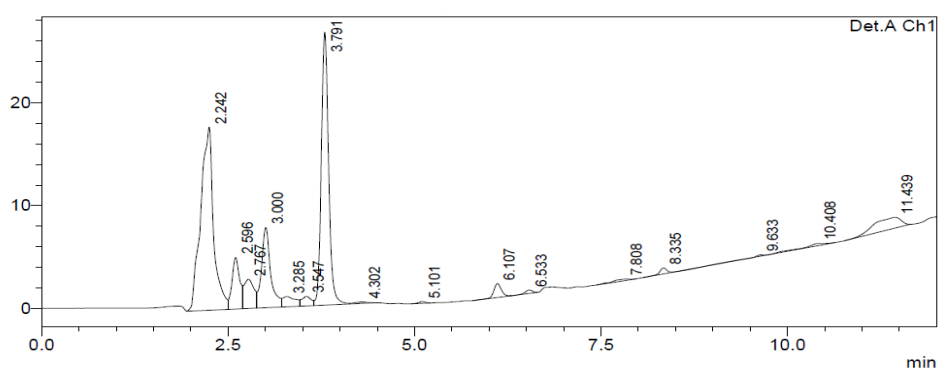


Figure IV-38. The HPLC chromatogram of the asiaticoside releasing profile from BL-1Ag-AS at 8 hr.

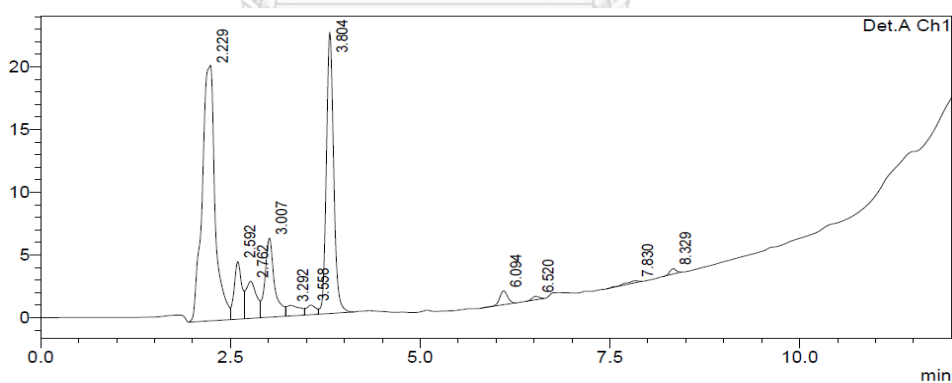


Figure IV-39. The HPLC chromatogram of the asiaticoside releasing profile from BL-1Ag-AS at 12 hr.

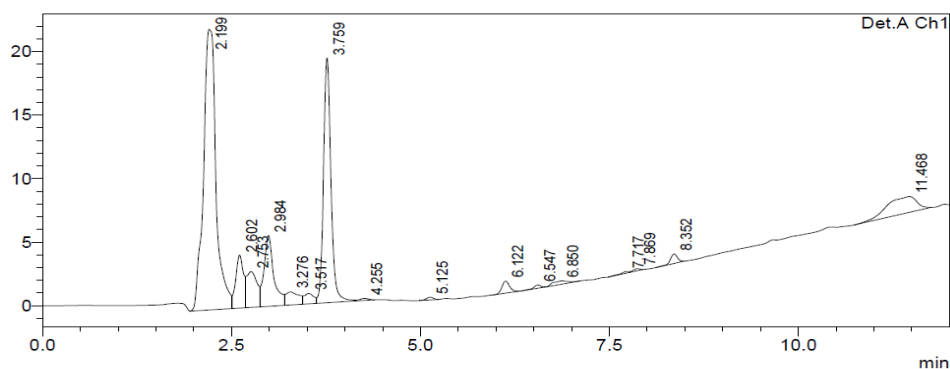


Figure IV-40. The HPLC chromatogram of the asiaticoside releasing profile from BL-1Ag-AS at 24 hr.

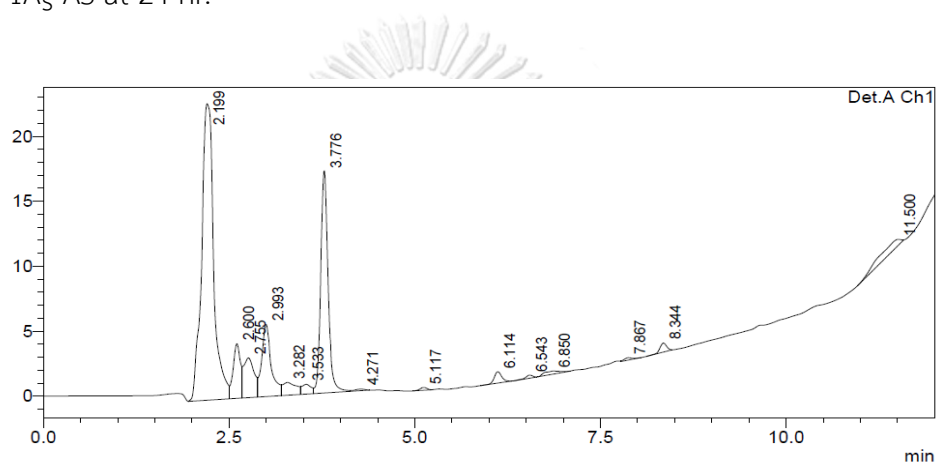


Figure IV-41. The HPLC chromatogram of the asiaticoside releasing profile from BL-1Ag-AS at 48 hr.

3.4) Active compound stabilities มหาวิทยาลัย

3.4.1) Silver stabilities SAKONGKORN UNIVERSITY

Silver nanoparticles impregnated in the foam dressing showed their stabilities upon the sterilization and accelerated storage condition. Although gamma rays could cause radiolysis and produce radical species; such as H^+ , OH^- in an aqueous solution [185] or it could grow into larger clusters [186], it might not be able to be observed in dry material. Figure IV- 42 shows the percent remaining of silver compare to the initial amount. There were %content about 94.12-96.90% after gamma radiation compares to after preparation. (96.90 ± 7.06 , 98.45 ± 8.43 , 94.12 ± 6.55 and 96.42 ± 7.25 % of BL-1Ag-AS, H6-1Ag-AS, C6-1Ag-AS, and A6-1Ag-AS, respectively) ($p > 0.05$, ANOVA test) After the storage under $40 \pm 2^\circ C$ and $75 \pm 5\%$ RH for 6 months, there were 94.77-102.96% remaining without significant difference. (94.77 ± 10.38 , 101.32 ± 7.41 , 102.96 ± 5.62 and

99.37±5.84% for BL-1Ag-AS, H6-1Ag-AS, C6-1Ag-AS and A6-1Ag-AS, respectively) ($p > 0.05$, ANOVA test) This might because of the fallen of silver powder. After opening the aluminum pouch, there could be observed some silver powder inside the aluminum pouch which detaches from the foam. These might affect percent silver loss.

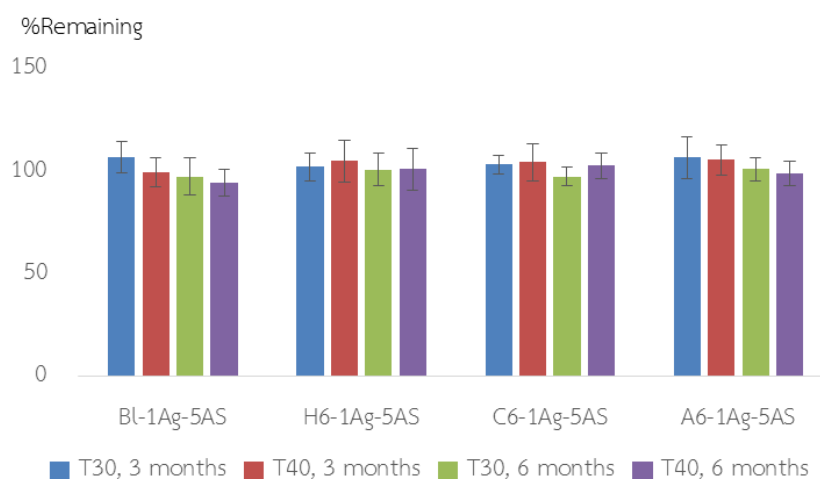


Figure IV- 42. The percent remaining of silver under an accelerated condition compare to amount at post-radiation. There were no significant differences in silver storage under accelerated condition.

3.4.2) Asiaticoside stabilities

The asiaticoside could be interfered with by radiation and heat. (Figure IV- 43). The %content of asiaticoside after gamma radiation compare to after preparation were in the range of 86.62-107.62%. (86.62±11.01, 100.28±21.73, 94.23±24.22 and 107.62±17.71% of BL-1Ag-AS, H6-1Ag-AS, C6-1Ag-AS, and A6-1Ag-AS, respectively) ($p > 0.05$, ANOVA test) The storage condition at a high temperature and humidity caused the asiaticoside degradation. The %remaining under 40±2°C and 75±5% RH condition for 6 months were in the range of 83.65-93.72%. (93.72±21.59, 83.65±18.92, 85.99±11.26 and 89.18±9.72 of BL-1Ag-AS, H6-1Ag-AS, C6-1Ag-AS, and A6-1Ag-AS, respectively) The asiaticoside content in BL-1Ag-AS was higher than those H6-1Ag-AS. ($p < 0.05$, ANOVA test)

All formulation shows the decreased %remaining after 6 months. These factors could be significantly detected between T30, 3 months compare to T40, 3 and 6 months condition of H6-1Ag-AS and T30, 3 months compare to T40, 6 months condition of A6-1Ag-AS. ($p < 0.05$, ANOVA test) The degradation of asiaticoside seemed to similar to previous studies. Puttarak et al [187] reported that the asiaticoside decomposed about 25-40 percent under 45°C, 75%RH for 16 weeks while another study reported that there was 15-20 percent degradation of asiaticoside which packing in light protect container at 40°C for 12 weeks [188]. The developed foam dressing was packed in completely sealed aluminum pouch so the degradation might not be affected from humidity. The oxidation might be the main reason of asiaticoside degradation. [131] The position which might occur the oxidation were the function groups such as alkene groups, hydroxyl groups, carboxylic groups and ester groups. The high temperatures also could lead to lower levels of asiaticoside content. [178] In addition, the packing and carrying which had a chance for light exposure might affect degradation.

The variation of sample size could affect the %remaining determination. The bigger size sample would present the larger amount of active ingredients. It was possible that the samples were tested in initial time were large size while the sample was test after storage was a small one. The percent remaining of under storage condition might be too low level compared to the initial time. This step should be carefully concerned.

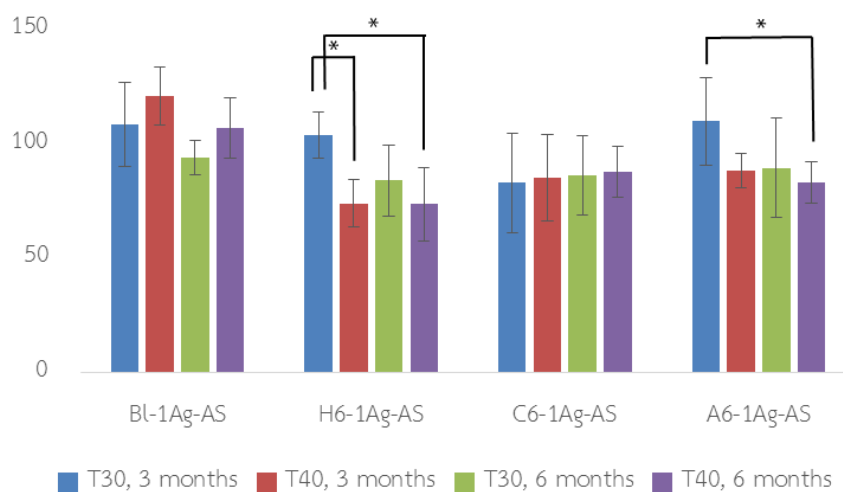


Figure IV- 43. The percent remaining of asiaticoside under an accelerated condition compare to amount at post-radiation. The %remaining of AS at T30, 3 months higher than T40, 3 and 6 months condition in the H6-1Ag-AS group and the %remaining of AS at T30, 3 months higher than T40, 6 months condition in the A6-1Ag-AS group. ($p < 0.05$, ANOVA test)

3.5) Antibacterial test

The results revealed that BL-1Ag-AS, H6-1Ag-AS, A6-1Ag-AS and C6-1Ag-AS exhibited the large clear inhibition zones, which were statistically non-significant ($p > 0.05$, ANOVA test) in every type of tested bacteria (Table IV-7, Figure IV-44 to Figure IV-47). The MIC of silver nanoparticles to *P. aeruginosa*, *S.aureus*, *E.coli*, and *B.subtilis* were in a range of 0.4-3.1 ppm [85, 189, 190] while the presented foam dressing was over 4-5 ppm on the releasing profiles. The inhibition zones of prepared foam dressing were larger than comparative foam dressing. ($p < 0.05$, ANOVA test) The silver in comparative group I was silver combined with alginate. The alginate polymer would delay the silver release when contact to the water. The comparative II dressing contained the coated silicone layer on the surface; this layer could hinder the silver release. The silver nanoparticles of developed foam dressing were in the porosity of foam structure, it could penetrate out the foam easier than comparative I dressing.

Table IV-7. Comparison of the inhibition zone of the prepared foam dressings on various bacteria types.

Formulations	Inhibition zone (mm) (mean \pm SD, n=3)			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Bl-1Ag-AS	31.9 \pm 3.5	25.5 \pm 3.4	31.6 \pm 4.8	28.7 \pm 2.6
H6-1Ag-AS	31.5 \pm 2.9	27.1 \pm 3.3	30.2 \pm 5.0	29.4 \pm 2.6
A6-1Ag-AS	31.3 \pm 3.4	27.7 \pm 4.8	34.0 \pm 4.7	30.0 \pm 4.0
C6-1Ag-AS	31.8 \pm 4.4	29.4 \pm 3.3	34.7 \pm 5.3	30.2 \pm 4.0
Bl	NZ	NZ	NZ	NZ
Bl-AS	NZ	NZ	NZ	NZ
Comparative I	15.7 \pm 0.8	14.8 \pm 1.4	16.3 \pm 2.0	18.6 \pm 1.7
Comparative II	10.5 \pm 8.4	7.8 \pm 6.1	9.9 \pm 8.5	8.3 \pm 6.5
Comparative III	NZ	NZ	NZ	NZ

NZ= no zone of inhibition

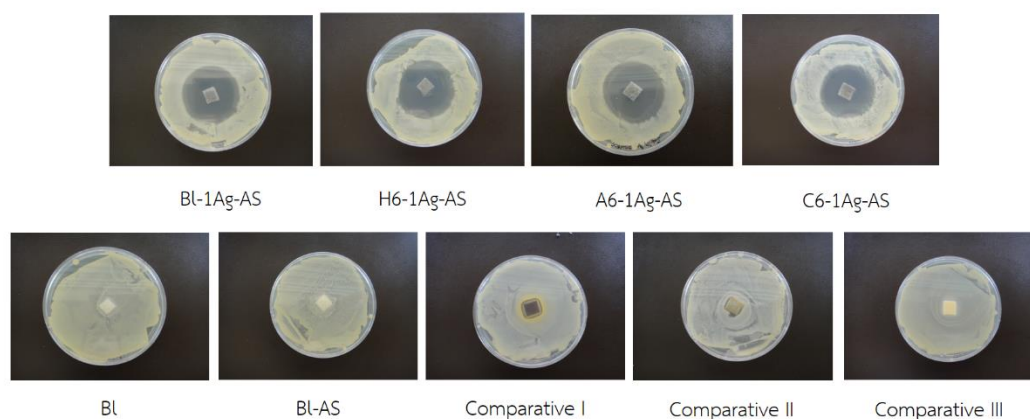


Figure IV-44. The inhibition zone of the prepared foam dressings on *S.aureus*.

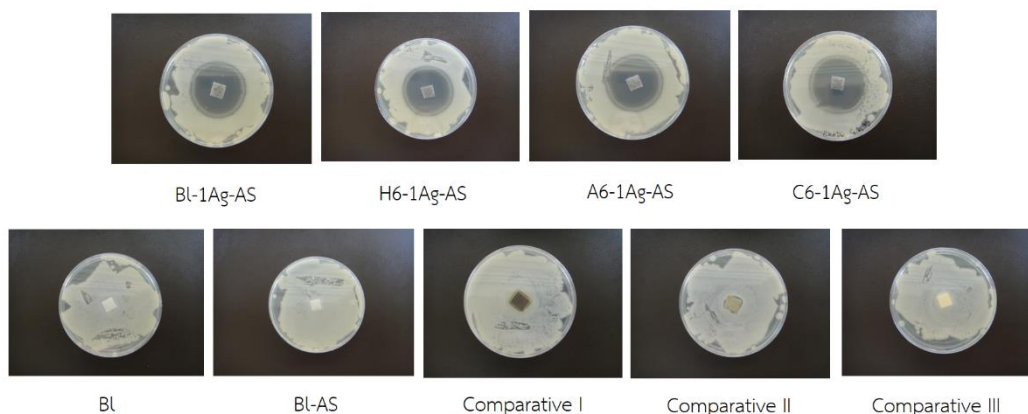


Figure IV-45. The inhibition zone of the prepared foam dressings on *B.subtilis*.

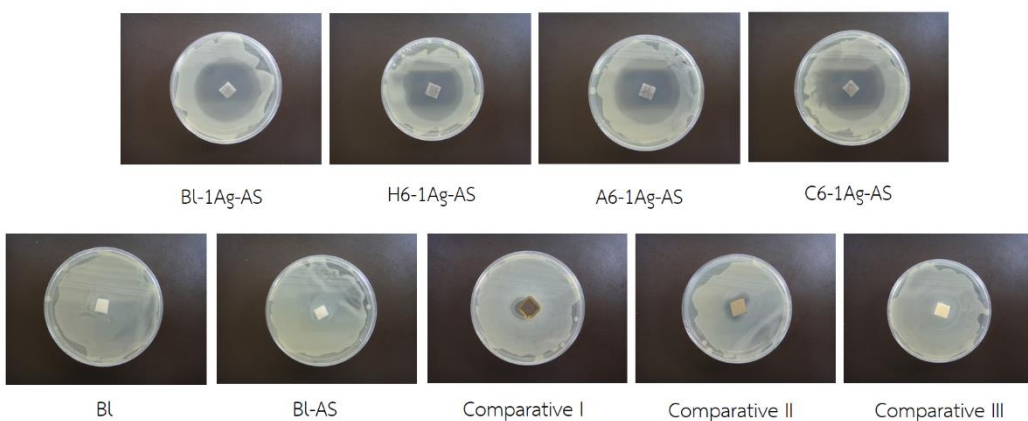


Figure IV-46. The inhibition zone of the prepared foam dressings on *E.coli*.

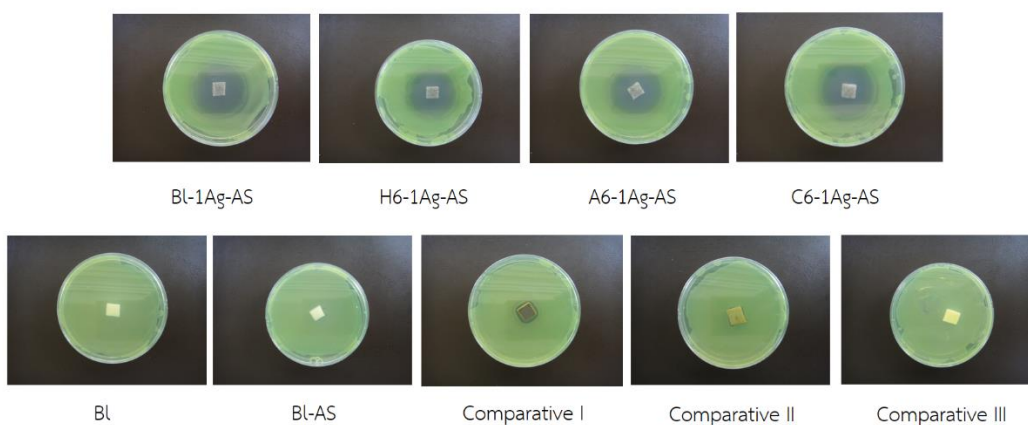


Figure IV-47. The inhibition zone of the prepared foam dressings on *P.aeruginosa*.

3.6) Cytotoxicity test

The developed foam sheets were aimed to be used for dermal wounds in which most cells found in this layer were fibroblasts. These cells generate an extracellular matrix and collagen by which the collagen fiber would intercalate into a matrix to fill up the damage and lost skin until the closing of the wound. Therefore, dressings should not have any toxicity to these cells.

The percent cell viability of Bl group was slightly less than the PBS group (101.16 ± 7.07 and 107.29 ± 9.71 %, respectively) ($p > 0.05$, ANOVA test). (Table IV-8) Because there was no foam added in PBS group while other groups contained foam in the well plate. The foam was added in each well plate might interfere with cell growth. The foam ingredient which was reported cytotoxicity was TDI [191, 192]. The 1 ppm of TDI could cause the cell membrane breakdown of human pulmonary epithelial cells then lead to cell death. The TEM confirmed the increasing pyknosis in TDI exposed pulmonary epithelial cells [192]. However, it could rapidly vapor under room temperature [193] so it was not likely that there was some TDI residue from post-production polyurethane foams. Moreover, there were reports that the polyurethane foams did not cause cytotoxicity [194, 195]. The natural polyols used in this study were biocompatibility [196-198] and presented comparable results which infer that these polymers did not cause cytotoxicity. The foam with 5% asiaticoside (Bl-AS) showed high percent cell viability ($113.34 \pm 9.97\%$). The asiaticoside concentration which determined in the cytotoxicity test was varied from 1-1000 μM and showed that AS at 1000 μM could decrease cell viability. [98, 101] The AS releasing profiles were in the range of 15-35 μg which much less than previous studies so it was non-cytotoxicity. Moreover, it was also added in various topical formulations because it was well known in wound healing properties including cell proliferation, migration, and collagen synthesis.

In silver toxicity, there were some evidence that silver might cause cell death [199, 200]. The silver concentrations used in commercial wound dressings were range from 0.08-1.50 mg/cm^2 [125, 126] while the silver which added in this foam formulation

was in the range of commercials. This might be assured that silver concentration would not be harmful. From the test, the result of foam with silver and asiaticoside might be from the balance between effects of two active ingredients. They all seem to be high level compare to the aforementioned groups which could infer that silver plus asiaticoside did not affect cell proliferation. Moreover, the cytotoxicity in vitro might not confirm the toxicity in vivo. Concentration exposure, route, and duration are the key factors for silver toxicity [201]. Supp et al [199] found that a wound dressing with nanocrystalline silver caused cytotoxicity in cell cultures; however, this effect could not be observed in mice. It might conclude that foam dressing, natural polymers and two active compounds did not cause toxicity to human fibroblasts.

Table IV-8. Percentage of the cell viability of various foam dressings.

Group	PBS	Bl	6%HPMC	6%CLMW	6%Alg
Cell Viability (%)	107.29±9.71	101.16±7.07	104.41±8.18	102.35±5.42	100.01±5.77
Group	Bl-AS	Bl-1Ag-AS	H6-1Ag-AS	C6-1Ag-AS	A6-1Ag-AS
Cell Viability (%)	113.34±9.97	100.22±8.37	100.56±10.84	101.02±9.12	100.88±10.19

3.7) Sterility test

A, B, C, and D were labeled on the tube with Bl-1Ag-AS, H6-1Ag-AS, A6-1Ag-AS and control, respectively (Figure IV- 48). The first tubes of the study group showed the turbidity after 14 days (Bl-1Ag-AS, H6-1Ag-AS, A6-1Ag-AS) compared to the control group. However, the second tubes showed all clear solutions, which might have been caused by the active compounds solubilization. The results were confirmed by the second tube: no turbidity. From this study, these products complied with the test for sterility. Gamma radiation could be used for foam dressing sterilization.

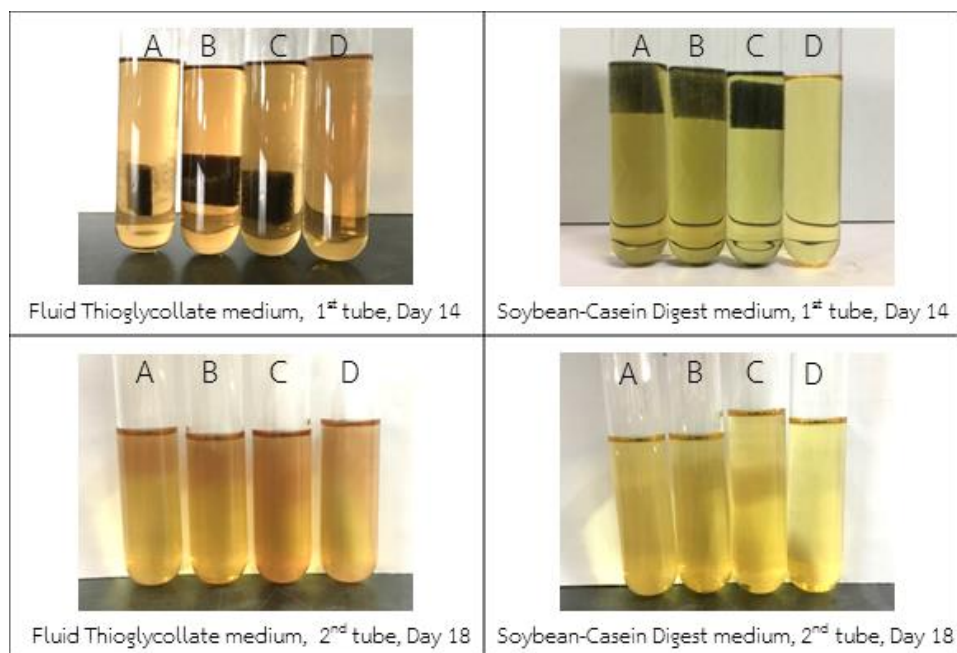


Figure IV- 48. The turbidity of the sterility test in a fluid thioglycollate medium and soybean-casein digest medium.

From the studies of Part I, the foam dressing with 6% of Alg and 1 mg/cm² of silver plus asiaticoside (A6-1Ag-AS) was chosen to be used in the study for Part II due to the appropriate absorption-desorption property, stability, and non-cytotoxicity. It could prove the high silver and asiaticoside releasing profiles compare to other natural polyols. Bl-1Ag-AS was chosen to compare the efficacy of wound healing in a porcine model.

Part II: Efficacy and safety in animal model

1. Skin irritation of selected foam dressing on rabbits

There was no redness and swelling on the tested area for a period of over 72 hr. (Table IV-9) They were also no dermatologic effects after dressing the application compared to the control side. The total evaluation score was zero on both sides. This result was the data to assure in which the polyurethane foam dressing with alginate and silver nanoparticles plus asiaticoside (A6-1Ag-AS) was safe to apply on the skin.

Table IV-9. The redness and swelling score in the study and control group over 72 hr.

Group	1 hr		24 hr		48 hr		72 hr	
	Redness	Swelling	Redness	Swelling	Redness	Swelling	Redness	Swelling
Rabbit 1 - A6-1Ag-AS	0	0	0	0	0	0	0	0
Rabbit 1 - Control	0	0	0	0	0	0	0	0
Rabbit 2 - A6-1Ag-AS	0	0	0	0	0	0	0	0
Rabbit 2 - Control	0	0	0	0	0	0	0	0
Rabbit 3 - A6-1Ag-AS	0	0	0	0	0	0	0	0
Rabbit 3 - Control	0	0	0	0	0	0	0	0

2. Efficacy and safety of prepared foam sheets in pigs

Each pig received ten square deep partial thickness excisional wounds. (Figure IV- 49) The average initial wound areas were 2.02 ± 0.05 , 2.04 ± 0.17 , 2.05 ± 0.15 , 2.03 ± 0.23 , 2.02 ± 0.18 cm² in the comparative group I-II, study group I-III which included BL-1Ag-AS, A6-1Ag-AS, and A6-1Ag groups, respectively with no significant difference. ($p = 0.995$, ANOVA test). The wounds were randomly assigned to be treated with five types of dressing. (Figure IV-50) All wounds were deep partial thickness wounds, which had pathology in the dermis layer.

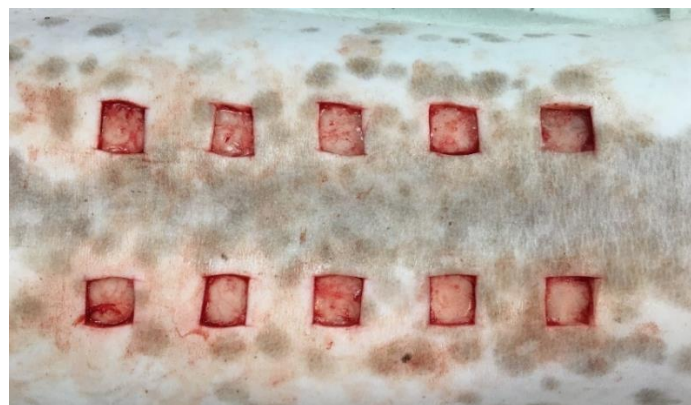


Figure IV- 49. Ten dermal wounds on the dorsal area of the pig

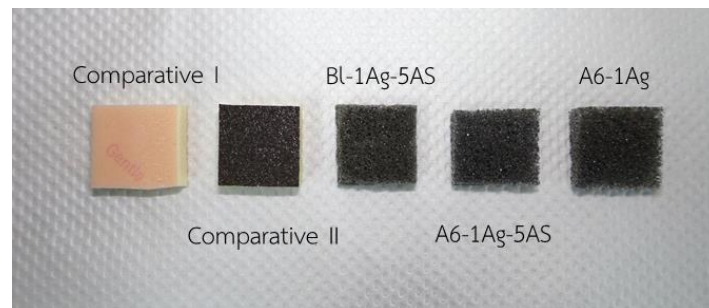


Figure IV-50. Five types of wound dressing used in the pig study

Figure IV-51 shows the appearance of the wounds which received different treatments on 0, 4, 7, 14 and 21 days. These photographs revealed a significant acceleration of the time of wound closure observed in comparative group II and A6-1Ag-AS group over A6-1Ag group. At day 7, the granulation tissue was fully grown in A6-1Ag-AS group which demonstrated the red connective tissue compared to the white and dry wound appearance in A6-1Ag group. The healthy red color in the new generative tissues over the wound bed could refer to blood supplies that were forming to deliver nutrients to the tissues. The cells in this area formed the extracellular matrix. The white and dry wound bed appearance demonstrated to show the slower rate of wound healing. At day 14 and 21, all groups except A6-1Ag group show almost completely granulated tissues and epithelialization especially in comparative group II and A6-1Ag-AS group. It confirmed with the results that the wounds in these two groups were significant healed faster than the wound in the A6-1Ag group.

The percentage of the wound closure of comparative groups and study groups are shown in Figure IV-52. The average percentage of epithelialization was 84.59 ± 8.09 on Day 21. From other studies, the superficial partial thickness wound could completely heal within 5 days while the deep partial thickness wound might take 27 days. [202, 203] Because this experiment was performed in deep partial thickness wound which deeper than superficial partial thickness wound but not full thickness wound, the healing time might take longer than superficial partial thickness wounds. There were no significant differences between the group at Days 4, 7 and 14. ($p > 0.05$, ANOVA test) However, at the 21st day after the creation of the wound, the percentage

mean of the wound's closure was significantly faster in the A6-1Ag-AS group and comparative group II than in A6-1Ag group. ($p=0.04$ in both pairs, ANOVA test) Bl-1Ag-AS group showed a smaller closure in size than the A6-1Ag group, but there was no statistical significance. ($p > 0.05$, ANOVA test) It might have been caused by the asiaticoside in the dressing (Bl-1Ag-AS and A6-1Ag-AS groups) that could promote the healing process. Lee et al [98] reported that this compound could stimulate the migration of epithelial cells. Moreover, the comparative groups I and II had smaller pore sizes than the study groups. This might retain more hydration than the study groups. Moreover, the comparative group I also contained a thin film as a backing of the foam dressing. The moist wound could heal faster than a dry wound because the epithelial cells could migrate easily [32]. The comparative group II had an alginate content which was the hydrophilic polymer and also combined with small porosity property. These might facilitate moisture at the wound bed and could detect the difference between comparative group II and A6-1Ag group.

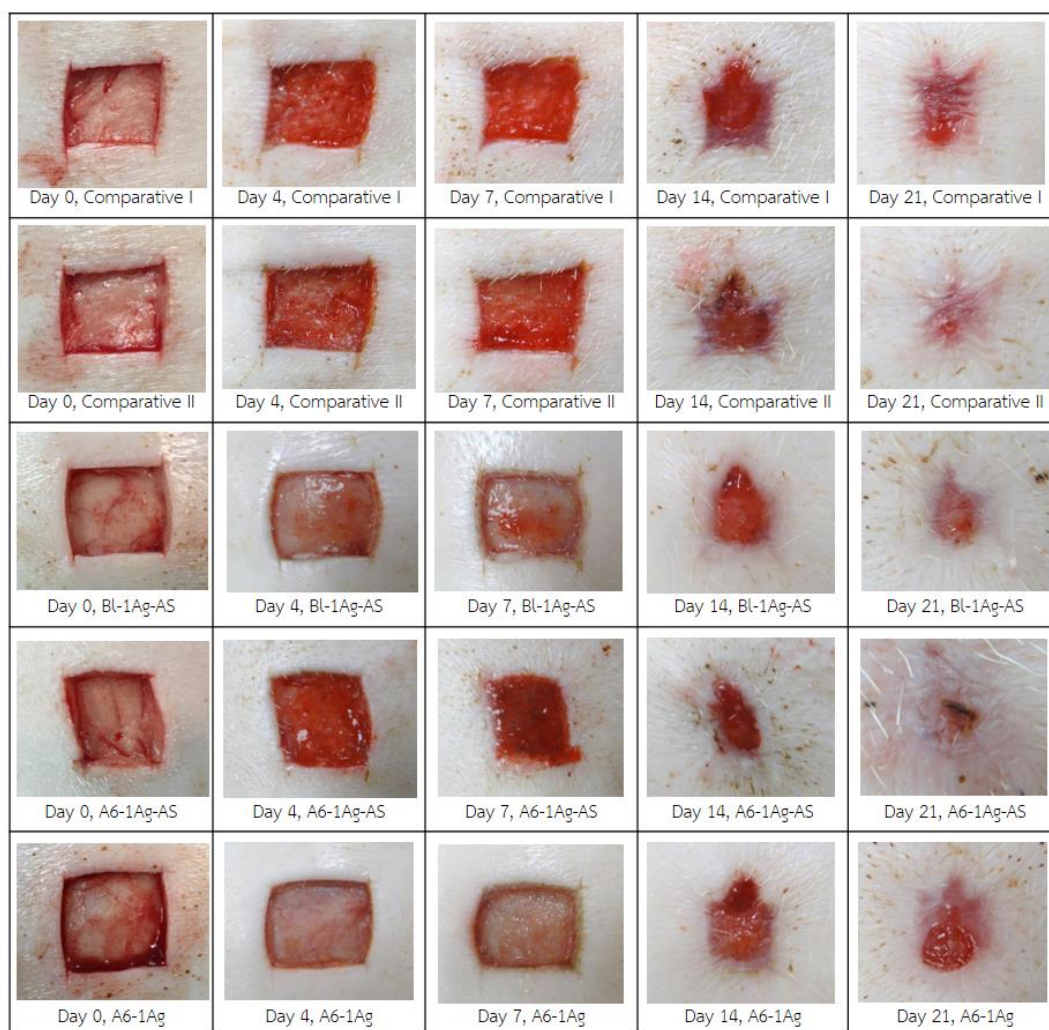


Figure IV-51. Wound appearance at Days 0, 4, 7, 14 and 21. The comparative group II and A6-1Ag-AS presented wound healing faster than A6-1Ag. ($p < 0.05$, ANOVA test)

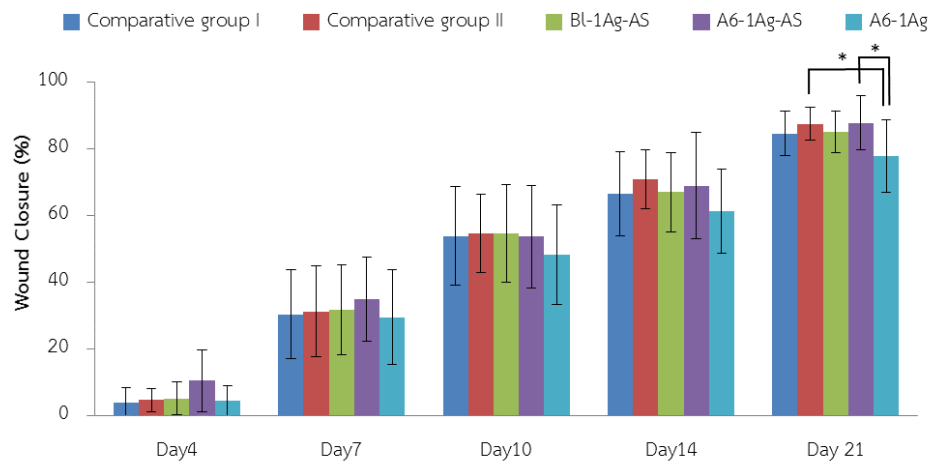


Figure IV-52. Percentage of wound closure of five treatments. The comparative group II and A6-1Ag-AS presented wound healing faster than A6-1Ag. ($p < 0.05$, ANOVA test)

The positive effects of asiaticoside on wound healing, especially in reepithelialization and reparation were found in the histological analysis of the wounds, which were evaluated in four parameters: the epithelial cell layer, number of the inflammatory cells, number of fibroblasts and number of capillaries. Comparing the wound lesions between treatment groups, the epithelial cell layers, extracellular matrix and the trichrome stained of extracellular matrix were shown in Figure IV-54 to Figure IV-56. The normal skin was taken from an area, which had not been wounded including the epithelial cells, collagen bundles, fibroblasts and some of the inflammatory cells and new capillaries. (Figure IV-53) The three skin layers were epidermis, which is the outer layer, dermis which is the second thick layer and hypodermis, which mainly consists of adipose tissue. The histologic evaluations on day 7 and 14 including epithelium cell layer, amounts of the inflammatory cells and fibroblasts were reported in Table IV-10.

Table IV-10. The histologic evaluation of the wounds

Parameters	Histological grading score				
	comparative I	comparative II	Bl-1Ag-AS	A6-1Ag-AS	A6-1Ag
Epithelium cell layer (Day 7)	1.103±0.995	1.029±0.797	1.477±0.731	1.205±0.904	1.462±0.884
Epithelium cell layer (Day 14)	2.279±0.826	2.238±0.850	2.128±0.850	2.652±0.482*	2.440±0.675
Amount of inflammatory cell (Day 7)	1.940±0.740	2.120±0.627	2.000±0.782	2.140±0.670	2.200±0.571
Amount of inflammatory cell (Day 14)	0.915±0.351	0.880±0.397	0.860±0.337	0.880±0.397	0.800±0.508
Amount of fibroblast (Day 7)	0.860±0.572	0.820±0.720	0.760±0.687	1.120±0.746	0.920±0.695
Amount of fibroblast (Day 14)	1.851±0.551	1.920±0.444	2.100±0.463	2.140±0.572*	1.780±0.648
Amount of new capillary (Day 7)	1.280±0.607	1.260±0.600	1.280±0.497	1.380±0.567	1.271±0.574
Amount of new capillary (Day 14)	0.809±0.537	0.800±0.398	0.860±0.416	0.900±0.521	0.820±0.524

* Significance consider, $p < 0.05$. The epithelial cell layer of wounds treated with the A6-1Ag-AS group was higher than the Bl-1Ag-AS group. The amount of fibroblasts of wounds treated with the A6-1Ag-AS group was higher than the A6-1Ag group at 14 days. ($p < 0.05$, ANOVA test)

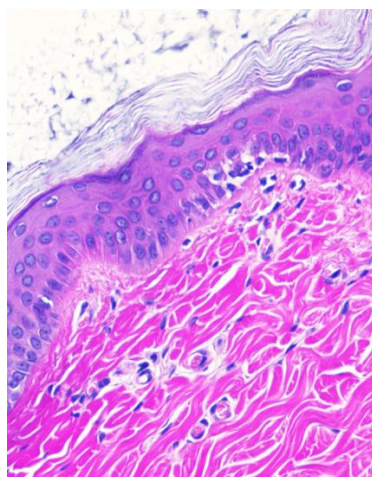


Figure IV-53. Histologic of normal skin

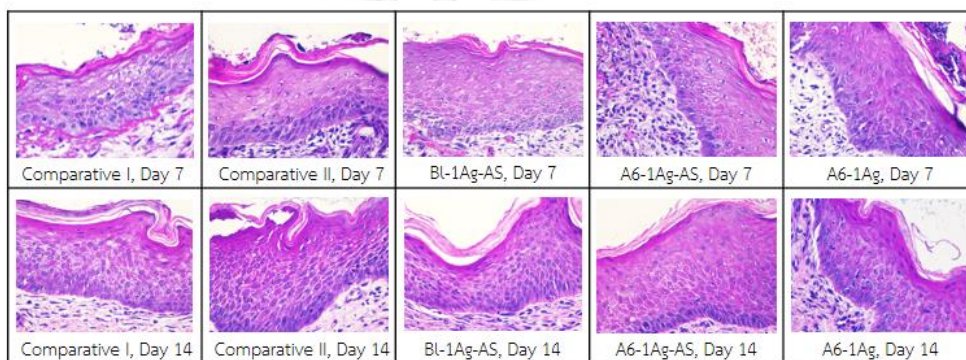


Figure IV-54. The histologic cross-section of the epithelial cells layer. The epithelial cell layer of wounds treated with the A6-1Ag-AS group was higher than BL-1Ag-AS group. ($p < 0.05$, ANOVA test)

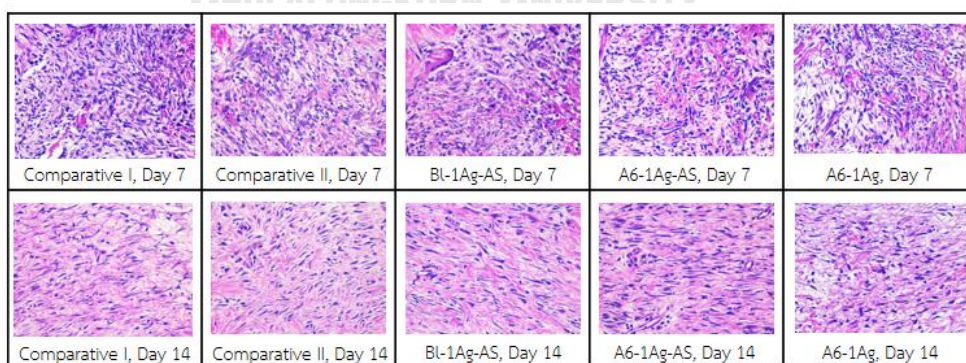


Figure IV-55. The histologic cross-section of ECM of the wound. The inflammatory cells and new capillaries increase at 7 days then decreased at 14 days.

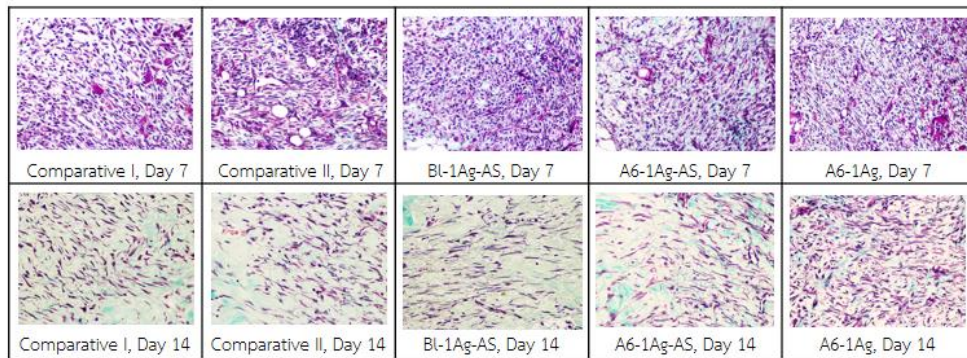


Figure IV-56. Histological cross-section of a healing skin wound (trichrome stained). The amount of fibroblasts of wounds treated with the A6-1Ag-AS group was higher than the A6-1Ag group at 14 days. ($p < 0.05$, ANOVA test)

The reepithelialization occurred for seven days (Figure IV-54). There were some epithelial cells from the neighboring epidermis that began to replicate and migrate into the wound bed. In all groups, the average epithelial cell layer notably increased at Day 14 compared to Day 7 ($p > 0.05$, ANOVA test). They might have been caused by the epithelial cell growth covering the wound bed, which would prevent dehydration and protect the wound externally. At Day 14, Bl-1Ag-AS group had an epithelial cell layer score significantly less than A6-1Ag-AS group ($p < 0.05$, ANOVA test). The alginate in the A6-1Ag-AS group might be a reason to keep hydration and facilitate the proliferation of the epithelial cell. In addition, asiaticoside might be involved in this process. Cheng et al [204] reported that this compound could activate intestinal epithelial cell growth.

Lots of inflammatory cells were observed on Day 7 and dramatically decreased in Day 14 (Figure IV-55). ($p < 0.05$, paired t-test) The inflammatory phase normally occurred within the first week after injury. Macrophage and neutrophil chemotaxis would remove debris cells and bacteria. After that, the extracellular matrix was produced in the proliferative phase in which the inflammatory cells would have less importance. Although there were some evidence about the *Centella asiatica* extract reducing inflammation [205, 206], there was no significant difference in the inflammatory cells score between the groups at each point of time ($p > 0.05$, ANOVA

test). This might be because these wounds were deep partial thickness wounds, and the inflammation might be greater than a superficial partial thickness wound. Moreover, itching of the wound might occur during the healing process, so the animal might scratch and let inflammatory cells be released in all the wounds.

At Day 7, all the wounds had some granulation tissue. The collagen which was exhibited in pink fiber mixed with the fibroblasts showed as purple satellite-shape cells. The amount of the fibroblasts increased in Day 14 compared to Day 7, especially in comparative group II and A6-1Ag-AS group. ($p=0.02$ in both pairs, paired t-test) At Day 14, there were more fibroblasts found in the A6-1Ag-AS group than those in the A6-1Ag group. ($p < 0.05$, ANOVA test) The collagen fiber became denser, which was the signs of regeneration of the dermis. This result confirmed the findings of previous studies [103, 207] in which asiaticoside activates fibroblast proliferation. The increasing of the fibroblasts led to an increase of the collagen fibers and wound's strength. The collagen fiber should be confirmed by the photos from the histologic stained with Masson's trichrome in which the collagen is stained in green-blue color (Figure IV-56). The wound tissue from A6-1Ag group had loose collagen fiber in both points of time when compared to other groups.

The new capillaries were found on Day 14 less than on Day 7 (Figure IV-55). ($p < 0.05$, paired t-test). As a result of the nearly completed healing, and the nutrients and oxygen were a lesser necessity. Even though some data showed the asiaticoside activated angiogenesis [100], there was no difference in these study groups. ($p > 0.05$, ANOVA test) Also, other factors might be involved in the wound healing; such as animal genetics, food and water consumption, and self-traumatized site from the animal. There was a dermatologic effect in comparative group I. (Figure IV- 57) There were some rashes on the skin surface around the wound. This may have been caused by the adhesive layer that recovered after discontinuing the dressing. Therefore, there was no dermatologic effect found in the study groups.



Figure IV- 57. The dermatologic effect in comparative group I

This study was performed in deep surgical wounds which were clean wounds. However, they could be infected due to animal behavior. The pigs usually scratched the wounds on the wall and the floor. The microbe might infect easily. The silver in PU dressing prevented the infection which might occur. Without infection, the wound could heal continuously without dermatologic reaction. Combination of silver and asiaticoside in PU foam would present satisfied results. The percentages of the wound's closure and histological data supported that the PU foam dressing with alginate and silver plus asiaticoside (A6-1Ag-AS) could accelerate wound healing through the migration of the epithelial cells and the proliferation of the fibroblast in a deep partial thickness wound of a porcine model. This formulation (A6-1Ag-AS) was selected to perform in the clinical study.

Part III: Irritation test in human volunteers

A total of 30 healthy volunteers (male 11, 36.67% and female 19, 63.33%) with an average age of 36.57 ± 9.24 years participated in the study. Initially, the erythema level showed no difference between the developed dressing group (A6-1Ag-AS) and comparative dressing group (256.97 ± 81.02 and 254.59 ± 71.64 , respectively, $p = 0.901$, paired t-test). The results from both groups also did not show any significant differences at each processing point of time of up to 14 days (Table IV-11 and Figure IV-58 to Figure IV-59). ($p > 0.05$, paired t-test) For the comparison within each dressing, the mean of the erythema level in Days 3, 7 and 14 was slightly higher than Day 0 but they were not significant differences. ($p > 0.05$, ANOVA test) The healthy volunteers

normally did not apply anything on the upper arm, the skin might feel uncomfortable after dressing application. Moreover, the short sleeves might irritate the adhesive tape. For the pain and itching score, there were no significant differences between the two dressing groups in both parameters. (pain score: 0.37 ± 1.07 , 0.30 ± 0.88 units; $p = 0.326$, paired t-test, and itching score: 1.73 ± 1.95 , 1.23 ± 1.36 units; $p = 0.134$, paired t-test for the developed and comparative dressing). This might be because of the soft and flexible properties of foam dressing.

This study was compared to the commercial dressing which was a positive control. However, the negative control such as the skin which was applied only adhesive tape did not perform in this study. One volunteer presented some rash around the dressing on both arm sides which caused by adhesive tape. The testing areas which applied with the dressing were still being normal skin. The application technique should be concerned. The tight and compressive adhesive tape might lead to skin redness. The gentle and soft attachment was an important recommendation in order to prevent skin irritation. The hot climate also involved being a factor which might lead to skin uncomfortably. However, this experiment compares between two dressings within one volunteer, the variation between subjects would be eliminated. Moreover, the concentrations of active compounds were in the range of commercial products and also confirmed by the animal study. Thus, it could be concluded that the polyurethane foam dressing with Alg impregnated with silver nanoparticles and asiaticoside did not cause any irritation on healthy skin and was further studied in patients.

Table IV-11. Erythema level of foam dressings

Erythema Level (Unit)	Group	Day 0	Day 3	Day 7	Day 14
	Developed dressing	256.97 ± 81.02	284.81 ± 80.33	294.29 ± 79.51	285.57 ± 80.13
	Comparative dressing	254.59 ± 71.64	274.53 ± 69.80	284.58 ± 72.19	275.45 ± 75.34
	p Value	0.901	0.584	0.617	0.798

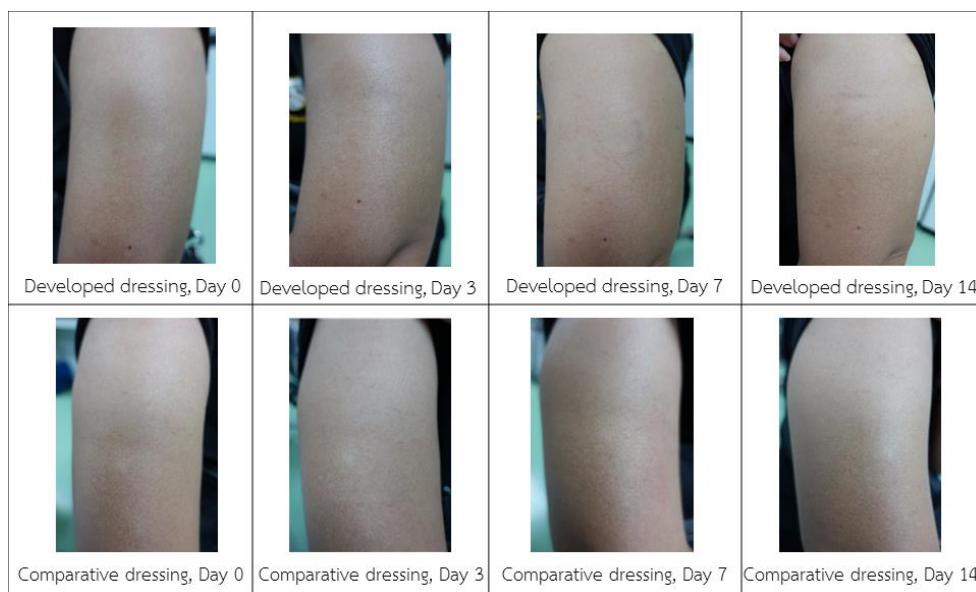


Figure IV-58. Irritation signs from the developed foam dressing compared to comparative dressing. (Case I)



Figure IV-59. Irritation signs from the developed foam dressing compared to comparative dressing. (Case II)

Part IV: Clinical efficacy test

The demographic data are summarized in Table IV-12. Twenty-eight wounds of 14 subjects were selected for the purpose of this study with nine males (64.29%) and five females (35.71%). None of the participants dropped from the study. The average

age was 31.14 ± 12.58 years with BMI 22.79 ± 2.30 kg/m². The most commonly affected wounds were the right arms (35.71% in both groups). The average wound areas were 12.75 ± 6.32 cm². The baseline characteristics were not significantly different ($p > 0.05$, paired t-test).

Table IV-12. Demographic data

Characteristics	Value	
Age (years)	31.14 ± 12.58	
Sex		
Male	9 (64.29%)	
Female	5 (35.71%)	
BMI (kg/m ²)	22.79 ± 2.30	
Wound characteristics	A6-1Ag-AS group	Comparative group
Wound area (cm ²)	12.03 ± 4.73	13.48 ± 7.71
Wound position		
Right arm	5 (35.71%)	5 (35.71%)
Left arm	1 (7.14%)	4 (28.57%)
Right leg	4 (28.57%)	2 (14.29%)
Left leg	4 (28.57%)	3 (21.43%)

The comparison of the healing effect was performed within the same patient. The one wound would be applied with the developed alginate polyurethane foam dressing impregnated with silver and asiaticoside (A6-1Ag-AS group) while another wound would be applied with gauze dressing with 0.5% of chlorhexidine acetate (comparative group). Wound closure in the A6-1Ag-AS group was faster than the comparative group (7.71 ± 1.33 vs 9.00 ± 2.45 days, $p = 0.03$, paired t-test). The percentage of reepithelialization and pain score were followed up for 12 days, and all patients did not experience any infections and skin adverse reactions. The percentage of reepithelialization of the A6-1Ag-AS group was higher than the comparative group on Days 6 and 8 (77.06 ± 16.70 vs $60.73 \pm 27.13\%$ on day 6, $p = 0.04$, paired t-test and

93.51±8.91 vs 85.84±15.46% on day 8, $p = 0.01$, paired t-test, respectively) (Figure IV-60). The asiaticoside is the main compound that accelerates wound healing. The mechanism included the induction of collagen type I synthesis through the phosphorylation of Smad pathways [102]. It might also increase the antioxidants level in the initial time of the healing process. These results confirmed previous animal and clinical studies [107, 108, 208]. The patients' pain score assessment in the A6-1Ag-AS group was lower than the comparative group on Days 4 and 6 (4.29±1.2 vs 5.14±0.86 units on Day 4, $p = 0.03$, paired t-test and 3.00±0.96 vs 3.50±1.16 units on day 6, $p = 0.03$, paired t-test, respectively) (Figure IV-61). This could be because the pore size of the foam dressing was smaller when compared to gauze dressing. (0.253-0.327 and 1.320-1.485 mm² in foam dressing and gauze dressing, respectively) It could prevent hair follicle growth and migration into the pore size. The dressing could be easily removed and did not stick to the wound. The efficacy of wound dressing in trauma patients was shown in Figure IV-62 to Figure IV-65.

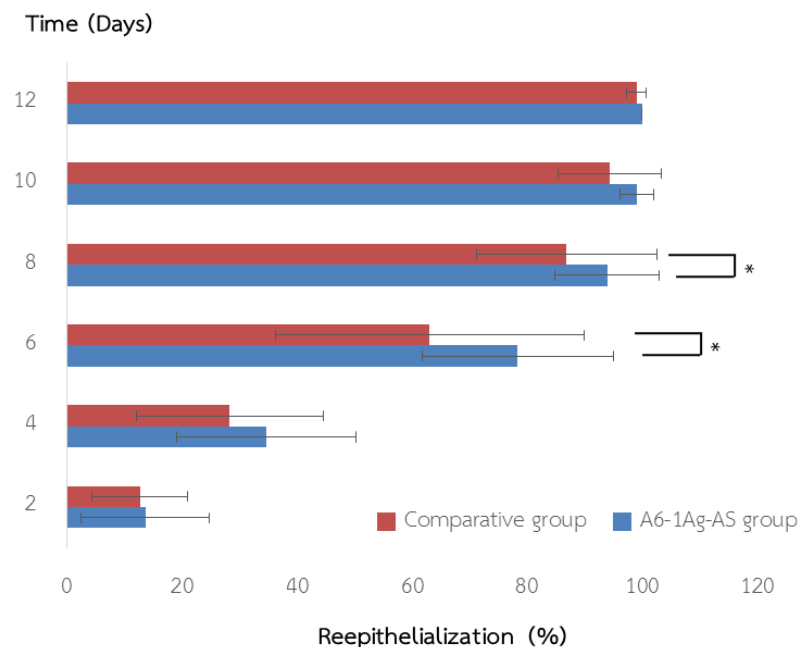


Figure IV-60. Percentage of reepithelialization in A6-1Ag-AS and comparative groups. The percentage of reepithelialization of the A6-1Ag-AS group was higher than the comparative group on Days 6 and 8. ($p < 0.05$, paired t-test)

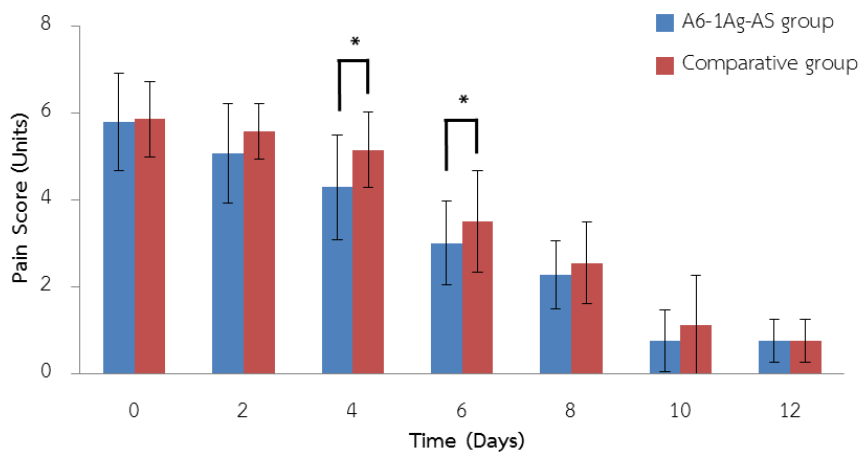


Figure IV-61. Pain score assessment in A6-1Ag-AS and comparative groups. The patients' pain score assessment in the A6-1Ag-AS group was lower than the comparative group on Days 4 and 6. ($p < 0.05$, paired t-test)



Figure IV-62. Wounds treated with polyurethane foam dressing (A6-1Ag-AS group) and gauze dressing (comparative group) (case I)



Figure IV-63. Wounds treated with polyurethane foam dressing (A6-1Ag-AS group) and gauze dressing (comparative group) (case II)



Figure IV-64. Wounds treated with polyurethane foam dressing (A6-1Ag-AS group) and gauze dressing (comparative group) (case III)



Figure IV- 65. Wounds treated with polyurethane foam dressing (A6-1Ag-AS group) and gauze dressing (comparative group) (case IV)

The traumatic wounds were classified as contaminated wounds, which were prone to be infected. The silver nanoparticles would prevent wound infection. The developed foam dressing could absorb the exudate greater than gauze dressing. Asiaticoside played an important role by accelerating wound healing, so the treated wound demonstrated faster-wound closure. The foam dressing might be left for more than 48 hr although it was changed every two days in this study. These results confirmed the efficacy of wound dressing in pig study that the polyurethane foam dressing with silver nanoparticles plus asiaticoside enhanced wound closure. The properties of foam dressing such as high water absorption, air permeability, non-cytotoxicity and the releasing profiles could support the wound environment and also accelerate the healing mechanism. The softness of the foam dressing which was confirmed by irritation test would protect further trauma. There were many studies which determined the healing effect of the formulation containing *Centella asiatica* extract in animal studies. Moreover, the silver dressings were already proved in clinical studies. However, the wound dressing combined with silver nanoparticles and asiaticoside has not been investigated yet. This study is the first evidence which

performed in both animal and human wounds. The efficacy of developed wound dressing in this clinical study presented as the faster wound closure without complications which could decrease the outpatient hospital visits, cost of treatment and also improve patients' convenience and quality of life.



CHAPTER V

CONCLUSION

In the preliminary study, foam was made from polypropylene glycol and 2% of natural polyols including pregelatinized starch (PGS), corn starch (CS), gelatin (Ge), sodium carboxymethylcellulose (CMC), sodium alginate (Alg), hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), chitosan low MW (CLMW) and chitosan high MW (CHMW) mixed with isocyanate and appropriate additives. Foam without natural polyols (Bl) was also produced. The loaf of foam was sliced to get foam sheet. The prepared foam was white, soft and flexible foam.

The formulation, which had the large pore sizes were Ge and CHMW while the Alg and HPMC showed the average low results. The natural polyol foams, which seemed to provide a higher percentage of absorption compared to Bl were HPMC, CMC, Alg, and CLMW. In the desorption test, foams with CMC, Alg, HPMC, MC and CLMW formulation were likely to show a lower percentage of desorption than other natural polyols. The tensile strength and compressive strength did not find any significant results. The Bl seemed to have a higher WVTR rate over natural polyol groups while foam with Ge, CMC, and Alg seemed to show low WVTR. In the degradation, the foam degraded in an enzyme solution did not differ from foam degrading in a buffer solution. The percentage of degradation of foam with natural polyols slightly increased compared to foam without natural polyols. Although there were no significant differences in the preliminary study, foam with HPMC, CLMW and Alg were selected according to the high absorption capacity, low desorption rate, good mechanical strength and less degradation. These three natural polyols would be conducted in the next study.

In section two, foam with each type of natural polyols: HPMC, CLMW, and Alg, varied from 2% to 12% in their concentrations. The foam had some small pieces like flakes at a high concentration, especially 8-12%. The foam with a high concentration of natural polyols seemed to produce smaller pore sizes; furthermore, the higher concentration could increase the capacity of absorption. The smaller pore size of the HPMC foam might prevent water from dropping out when taking the gadget out of the water before weighing, so the water absorption profiles seemed to be superior to other natural polyols. All formulations dehydrated after 30 min then they were gradually increased with time. The larger pore size of the CLMW might affect the higher rate of desorption. The formulations, which contained a higher concentration of natural polyols commonly retained hydration, but they might lose weight from the natural polyols in the absorption-desorption processes, which might be caused by polymer solubilization. The loss of natural polyols, especially at a high concentration, might affect retaining the water molecules in the foam sheet.

Increasing the concentration might decrease the strength and percentage of elongation. In contrast, the compression strengths were likely to increase when the concentration increased. In the WVTR, the foam permitted the air to pass through for 30 min then the rate was gradually increased with time. Although the higher concentration had a smaller pore size and more density, the WVTR results were not significantly different. In addition, there was no significant difference in the percentage of the weight loss in the lysozyme solution and in the phosphate buffer. From the FTIR analysis, the spectra of the polyurethane foam with 6% of HPMC, CLMW, and Alg were not different from foam without any natural polyols. This might have been caused by the low percentage of natural polyols that could not be detected by this method. The confirmation results were performed with 10% concentration. There were some larger intensities in N-H stretching, C=C stretching and N-H bending peak areas. There were also no differences found between the groups in the DSC analysis.

From the section two, foam without any natural polyols (Bl), two concentrations (4% and 6%) of three types of natural polyols (HPMC, CLMW, and Alg)

were selected to be impregnated with silver nanoparticles in a range of 0.4-1.0 mg/cm². The exact amount of silver nanoparticles was suspended in water and then impregnated in the foam by absorption and drying processes. Both the silver and natural polyols concentrations affected the silver releasing profiles. Higher silver concentration affected greater silver release by simple diffusion. This result was consistent with the natural polyol groups. The higher concentration of polyols provided higher releasing profiles. From the silver releasing profiles, foam without natural polyols (Bl), 6% of natural polyols HPMC (H6), CLMW (C6) and Alg (A6) with 1 mg/cm² of silver nanoparticles were chosen to add a 5% concentration of asiaticoside.

The foam sheets without natural polyols impregnated with silver at 1 mg/cm² and 5% of asiaticoside (Bl-1Ag-AS) rapidly released and gave the highest amount of asiaticoside then the release was constant after 8 hr followed by a foam dressing with 6% of Alg (A6-1Ag-AS). A foam dressing with 6% of CLMW (C6-1Ag-AS) presented the lowest rate, but it still released over 48 hr.

The silver contents after preparation were in the range of 92.50-94.50% of the theoretical amount. The percent remaining of silver were not changed from the initial amount which infers the silver did not degrade in storage under accelerated condition. The asiaticoside amount was 94.0-96.0% of the theoretical amount. This compound could be degraded by gamma radiation, humidity, light and high temperature.

All formulations showed large clear inhibition zones, which were statistically non-significant in every type of tested bacteria. The MTT assay reported the preliminary safety result of the foam dressing with silver and asiaticoside.

In accordance with A6-1Ag-AS, which presented high silver and asiaticoside releases over other polyols, it was selected to be used in animal studies. There was no redness and swelling on the tested area over 72 hr in the rabbit model. There were also no dermatological effects after the foam dressing application was compared to the control side. The efficacy in pig models, Bl-1Ag-AS was also chosen in order to compare the efficacy of A6-1Ag-AS. The positive effects of asiaticoside on the wound's

healing, especially in the reepithelialization and reparation, were found in the histological analysis. The percentage of the wound's closure and histological data supported that A6-1Ag-AS could accelerate wound healing by the migration of the epithelial cells and the proliferation of fibroblast in a deep partial thickness wound of a porcine model.

From studies of pigs and rabbits, it might be confirmed that A6-1Ag-AS could facilitate wound healing without any dermatological adverse effects. This formulation was performed in clinical studies. The results showed that A6-1Ag-AS did not cause irritation on healthy skin volunteers and was further studied in patients. Data from trauma patients found that the day of the wound closure in the wound treated with A6-1Ag-AS was lower than the wound treated with standard commercial dressing. The percentage of the reepithelialization of the study group was higher than the comparative group on Days 6 and 8. The pain assessment score from the study group was less than the comparative group. All patients did not experience any infections and skin adverse reactions. It could infer that the polyurethane foam dressing with silver nanoparticles plus asiaticoside could promote wound closure.

This research was performed since the formulation, development, characterization of the properties, animal studies and clinical studies. The polyurethane foam dressing with silver nanoparticles and asiaticoside showed satisfactory results; thus, this could be a candidate wound dressing in the future.

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APPENDIX A - ETHIC APPROVAL FOR ANIMAL STUDY

Protocol No. <u>MUVS-2016-09-34</u> (Assigned by FVS-ACUC)	
	
Documentary Proof of Ethical Clearance The Faculty of Veterinary Science - Animal Care and Use Committee (FVS-ACUC), Mahidol University	
.....	
Protocol Title:	Development of polyurethane based foam dressing impregnated with silver nanoparticles and asiaticoside for dermal wound treatment: animal study
Principal Investigator:	Yada Akkhawattanangkul
Name of Institution:	Faculty of Veterinary Science, Mahidol University
E-mail:	yada.akk@mahidol.edu
Approved by the Faculty of Veterinary Science- Animal Care and Use Committee	
FVS-ACUC Review	
Signature of Chairman:	<i>Withawat Wiriyarat</i>
	(Assistant Professor Dr. Withawat Wiriyarat, Chairman of FVS-ACUC)
Signature of Dean:	<i>P. Ratanakorn</i>
	(Associate Professor Parntep Ratanakorn) (Dean of the Faculty of Veterinary Science, Mahidol University)
Date of Approval:	November 1 st , 2016
Date of Expiration:	February 28 th , 2017

APPENDIX B - ETHIC APPROVAL FOR HEALTHY VOLUNTEERS STUDY

AF 02-12



The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
 Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
 Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 091/2017



Certificate of Approval

Study Title No.051.1/60 : DEVELOPMENT OF POLYURETHANE-BASED FOAM DRESSING IMPREGNATED WITH INORGANIC BACTERIACILDAL AGENT AND HERBAL EXTRACTS FOR DERMAL WOUND TREATMENT: DERMATOLOGIC EFFECT IN HEALTH VOLUNTEER STUDY

Principal Investigator : MISS NANTAPORN NAMVIRIYACHOTE

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences,
Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature:  Signature: 
 (Associate Professor Prida Tasanapradit, M.D.) (Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.)
 Chairman Secretary

Date of Approval : 8 May 2017 **Approval Expire date** : 7 May 2018

The approval documents including

- 1) Research proposal
- 2) Patient/Participant Information Sheet and Informed Consent Form
- 3) Researcher  Project No. 051-1/60
Date of Approval - 8 MAY 2017
- 4) Questionnaire Approval Expire Date - 7 MAY 2018

The approved investigator must comply with the following conditions:

1. The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.
2. Strictly conduct the research/project activities as written in the proposal.
3. Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).
4. Report to the RECCU for any serious adverse events within 5 working days
5. Report to the RECCU for any change of the research/project activities prior to conduct the activities.
6. Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.
7. Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.

APPENDIX C - ETHIC APPROVAL FOR PATIENTS STUDY

2 WANGLANG Rd. BANGKOKNOI
BANGKOK 10700



Tel. +66 2419 2667-72

Fax. +66 2411 0162

Siriraj Institutional Review Board

Certificate of Approval

COA no. Si 355/2017

Protocol Title(English) : The Efficacy of Polyurethane Foam Dressing Impregnated with Nanoparticles Silver and *Centella asiatica* Extract in Treatment of Dermal Wound

Protocol Title(Thai) : ประสิทธิภาพของแผ่นปิดแผลชนิดโฟมพอลิยูรีเทนที่มีส่วนผสมของอนุภาคนาโนซิลเวอร์และสารสกัดใบบัวบกในการรักษาบาดแผลที่มีความลึกระดับที่สอง

Protocol number : 224/2560(EC1)

Principal Investigator/Affiliation : Prof. Pornprom Muangman, M.D. / Department of Surgery
Faculty of Medicine Siriraj Hospital, Mahidol University

Research site : Faculty of Medicine Siriraj Hospital


Approval includes :

1. SIRB Submission form
2. Participant information sheet
3. Informed consent form
4. Case Record Form
5. Patient card
6. Pain score
7. Curriculum vitae


Approval date : June 23, 2017

Expired date : June 22, 2018

This is to certify that Siriraj Institutional Review Board is in full compliance with international guidelines for human research protection such as the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP)


 (Prof. Chairat Shayakul, M.D.)
 Chairperson

29 JUN 2017
 date


 (Prof. Dr. Prasit Watanapa, M.D., Ph.D.)
 Dean of Faculty of Medicine Siriraj Hospital

30 JUN 2017
 date

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APPENDIX D - PUBLICATION IN ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES
(AJPS)

ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 11 (2016) 114-115



Available online at www.sciencedirect.com

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Physico-mechanical characterization of polyurethane foam dressings containing natural polyols



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ARTICLE INFO

Article history:

Available online 25 November 2015

Keywords:

Polyurethane foam
Natural polyols
Starch
Physico-mechanical properties

Advanced wound dressings are replaced traditional dressings in order to provide appropriate environment for healing and also to promote cell migration [1]. The key characteristics of wound dressings are non-toxic, highly absorbed, air permeable, biocompatible and have good mechanical properties [2]. Due to providing moist environment and also protecting maceration at the wound edge area, foam dressings are used in various clinical applications [3]. They are mostly prepared from polyurethane between polyols and isocyanate polymerization. In this study, natural polymers were added to improve hydrophilicity and absorption activity of polyurethane foam formulations. Their morphology, absorbing and mechanical profiles were investigated.

Polyurethane foam formulations consisting of 100 percent of polypropylene glycol (PPG, molecular weight 3000), toluene diisocyanate (TDI), deionized water, silicone surfactant, amine catalyst and tin catalyst were prepared with and without 2 percent of starch polymers, starch 1500 and corn starch as

natural polymers with abundant polyols by high-speed stirring until a foam reaction occurred and air drying for 24 hours. The SEM appearance (Fig. 1A) revealed larger pore size of foams containing starches compared to that without natural polymer especially at the side view. Large molecular size with scattering hydroxyl groups of the investigated starches might cause pore enlargement during chemical reaction. Initially, all prepared foams had high rate of water absorption but the rate gradually and continuously decreased after 10 minutes (Fig. 1B). However, foams with corn starch seemed to dehydrate faster than other foams. The moisture vapor transmission rate of that without natural polymer was higher than those with starches at all-time points. The tensile strength, percentage of elongation and elastic modulus results of the latter formulations were higher than those from the former. Moreover, in compression profiles, the stress at 25, 50 and 75 percent strain of starch 1500 foam was lower than those from foams with corn starch and without starch. From our preliminary study, starch 1500

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Peer review under responsibility of Shenyang Pharmaceutical University.

<http://dx.doi.org/10.1016/j.ajps.2015.11.100>

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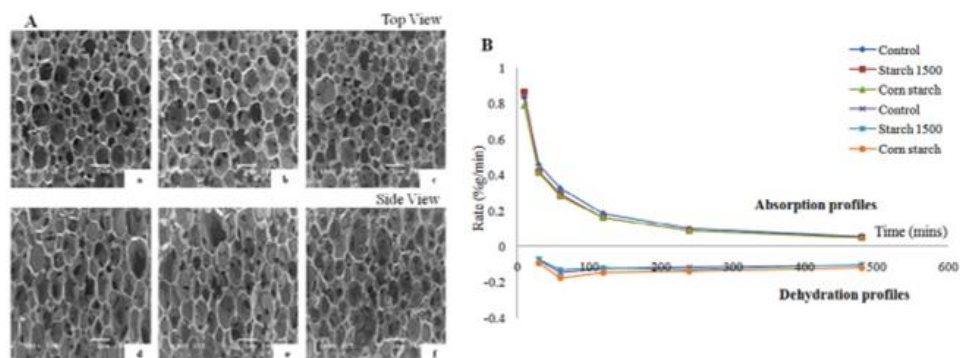


Fig. 1 – SEM photographs of without natural polymer, starch 1500 and corn starch foams (a, b and c, top view; d, e and f, side view, respectively) (A) and Absorption and dehydration profiles of without natural polymer (control), starch 1500 and corn starch foams (n = 9) (B).

may be good alternative to improve polyurethane foam characteristics due to initial high absorption rate and low dehydration rate. The appropriate concentration of natural polyols and their properties should be further investigated.


Acknowledgements

The authors gratefully acknowledge the financial support from The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship and from Agricultural Research Development Agency (ARDA), Thailand.

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APPENDIX E - SAFETY DETERMINATION OF FOAM DRESSING ON RABBIT: TISTR
REPORT



วว-TISTR

รายงาน

เรื่อง	:	การทดสอบการก่อความระคายเคืองเบื้องต้นต่อผิวหนังของ "AAgAS" ในกระต่าย
ชื่อตัวอย่างทดสอบ	:	-
ลักษณะตัวอย่างทดสอบ	:	สีเหลืองครีมแบบบางสีดำ มีรพูน
เลขที่คำขอบริการ	:	ฝกผ. 32/60, รหัส 09-12-59
ผู้ขอบริการ	:	ศ.ดร.กาญจน์ทิพย์ กุทธิเดช คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
วันที่ได้รับตัวอย่างทดสอบ	:	9 ธันวาคม 2559
วันที่ทำการทดสอบ	:	30 มกราคม - 14 กุมภาพันธ์ 2560
การทดสอบเลขที่	:	P 0275/2017
วิธีการทดสอบ	:	วิธีทดสอบหมายเลข 404 : Acute Dermal Irritation / Corrosion ของ OECD Guidelines for Testing of Chemicals (2015)

สรุปผลการทดสอบ

การทดสอบการก่อความระคายเคืองเบื้องต้นต่อผิวหนังของ "AAgAS" ในกระต่าย ดำเนินการตามวิธีทดสอบหมายเลข 404 : Acute Dermal Irritation / Corrosion ของ OECD Guidelines for Testing of Chemicals (2015) โดยใช้ตัวอย่างทดสอบปริมาณ 0.5 กรัม ที่ถูกทำให้ชื้นด้วยน้ำกลั่น ปิดลงบนผิวหนังกระต่ายนาน 4 ชั่วโมงแล้วล้างออก จากนั้นตรวจผลการแดงและอาการบวมของผิวหนังที่เวลา 1, 24, 48 และ 72 ชั่วโมง

ผลการทดสอบ ตรวจไม่พบอาการแดงและบวมของผิวหนังกระต่าย

ผลการทดสอบ/วิเคราะห์รับรองผลเฉพาะตัวอย่างที่ได้ทำการสอบเทียบเท่านั้น
นำมาผลการทดสอบ/วิเคราะห์ไปโฆษณาโดยมิได้รับอนุญาตเป็นลายลักษณ์อักษรจาก วว.

1 ของ 6

สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย (วว.)
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 E-mail : tistr@tistr.or.th Website : www.tistr.or.th

วิสัยทัศน์ : เป็นองค์กรชั้นนำระดับอาเซียนในด้านวิจัย พัฒนา และบริการด้านวิทยาศาสตร์ เทคโนโลยีและนวัตกรรม

APPENDIX F - RAW DATA

Porosity

Bl

0.776	0.077	1.247	0.382	0.071	0.079	0.123	0.292
0.381	0.169	0.802	0.371	0.153	0.112	0.086	0.189
0.078	0.230	0.061	1.489	0.669	0.141	0.094	0.173
0.122	0.084	3.521	0.287	0.148	0.324	0.126	0.196
0.081	0.074	0.307	0.235	0.098	0.062	0.923	0.209
0.079	0.096	0.173	0.105	0.437	0.084	0.255	0.189
0.180	0.056	1.917	2.108	0.151	0.066	0.110	0.526
0.068	1.013	0.246	1.156	0.076	0.062	0.323	0.386
0.070	0.355	0.226	0.117	0.148	0.074	0.445	0.083
0.054	0.575	0.366	0.347	0.305	0.138	0.051	0.073
0.122	0.105	0.148	0.324	0.127	0.198	0.050	0.215
0.185	0.764	0.182	0.326	0.063	0.150	0.258	0.059
0.576	0.050	0.073	0.066	0.247	0.211	0.092	0.505
0.071	0.893	1.900	0.125	0.060	0.061	0.737	0.130
0.136	0.203	0.436	0.156	0.850	0.254	0.060	0.103
0.266	0.107	0.084	0.431	0.077	0.130	0.319	0.078
0.373	0.121	0.147	0.569	0.384	0.099	0.311	0.065
0.169	1.113	0.061	0.155	0.141	0.207	0.132	0.167
0.313	2.706	0.052	0.144	0.056	0.090	0.150	0.053
0.052	0.085	0.069	0.258	0.929	0.182	0.131	0.083
0.255	0.136	0.051	0.112	0.137	0.290	0.136	0.081
0.490	0.338	0.054	0.102	0.516	0.152	0.380	0.175
0.129	0.196	0.052	0.179	0.317	0.082	0.213	0.109
0.319	0.364	0.175	0.165	0.623	0.988	0.159	0.053
0.246	0.156	0.279	0.021	0.154	0.141	0.360	0.302
0.241	0.744	0.113	0.076	0.242	1.872	0.081	0.307
0.177	0.098	0.732	0.051	0.059	0.076	0.075	0.150
0.165	0.108	0.619	0.081	1.157	0.160	0.098	0.167
1.061	0.299	0.089	0.218	0.362	0.136	0.125	0.116
0.087	0.078	0.120	0.289	0.188	0.137	0.195	0.072
0.304	0.131	0.168	0.125	0.088	0.099	0.088	0.147
0.421	0.064	0.283	0.294	0.065	1.125	0.052	0.418
0.139	0.059	0.108	0.286	0.980	0.130	0.337	2.296
0.080	0.058	0.161	0.096	0.333	0.578	0.223	

0.329	1.871	0.358	0.349	0.489	0.939	0.111	
4.379	0.055	0.075	0.056	0.380	0.138	0.053	
0.154	0.984	0.078	0.069	0.314	0.344	0.192	
0.492	0.934	0.091	0.054	0.753	0.688	0.829	
0.090	0.104	0.361	0.208	0.065	0.063	0.089	
0.107	0.058	0.465	0.138	0.307	0.058	0.095	
0.062	0.106	0.022	0.060	0.618	0.225	0.259	
0.213	0.289	0.887	0.123	0.156	0.121	0.741	
0.087	7.743	0.231	0.112	1.130	0.056	0.065	
0.106	0.153	0.066	0.103	0.069	0.080	0.425	
0.057	0.098	0.120	0.052	0.189	0.346	0.087	
0.127	0.412	0.125	0.056	0.177	0.087	0.115	
0.093	0.540	0.273	0.214	0.241	0.134	0.184	
0.076	0.328	0.087	0.104	0.992	1.023	0.120	
0.056	0.212	0.283	0.067	0.175	0.127	0.114	
3.023	0.335	0.059	0.262	0.565	0.107	0.219	

H2

0.083	3.806	0.151	0.074	0.067	0.068	0.103	0.153	0.136
0.205	0.111	0.077	0.378	0.176	0.077	0.113	0.059	0.068
0.065	0.178	0.347	0.226	0.284	0.163	0.070	0.055	0.078
0.075	0.097	0.843	0.184	0.104	0.138	0.209	0.133	0.063
0.062	0.389	1.612	0.265	0.065	0.116	0.090	0.222	3.281
0.076	0.166	0.177	0.154	0.773	0.062	0.287	0.230	0.054
0.077	0.152	0.758	0.626	0.337	0.059	0.100	0.071	0.061
0.079	0.614	0.056	0.053	0.105	0.067	0.103	0.159	0.122
0.089	0.090	0.086	0.142	0.734	0.119	0.623	0.496	0.105
0.178	0.455	0.074	0.191	0.056	0.143	0.180	0.093	0.121
0.150	0.083	0.079	0.325	0.071	0.060	0.444	0.140	0.084
0.065	0.076	0.050	0.245	0.074	0.286	0.813	0.246	0.068
0.076	0.130	1.009	0.186	7.195	0.478	0.596	0.065	0.161
0.070	0.065	0.117	0.124	0.079	0.053	0.051	0.067	0.106
0.143	0.130	0.196	1.810	0.229	0.061	0.055	0.082	0.078
0.073	0.150	0.097	0.648	0.284	0.187	0.057	0.107	0.238
2.135	0.464	0.132	0.268	0.228	0.378	0.539	0.062	0.106
0.240	0.080	0.062	0.539	0.135	0.359	0.219	0.053	0.052
0.304	0.059	0.316	0.754	0.110	0.343	0.358	0.244	0.062
0.078	0.457	0.090	0.138	0.445	0.087	0.092	0.289	0.075
0.116	0.119	0.059	0.321	0.079	0.070	0.377	0.151	0.399

0.429	0.371	0.134	0.075	0.203	0.186	0.112	0.085	0.227
0.192	1.181	1.223	0.199	0.523	0.051	0.118	0.426	
0.328	0.108	0.167	0.222	0.267	0.204	0.146	0.126	
0.143	0.276	0.113	0.443	0.213	0.279	0.127	0.122	
0.020	0.183	0.698	0.054	0.065	0.222	1.800	0.078	
0.538	0.229	0.603	0.063	2.933	0.242	0.124	0.179	
0.143	0.122	0.273	0.291	0.549	0.104	2.348	0.053	
0.291	0.090	0.379	0.407	0.159	0.088	0.704	0.185	
3.014	0.328	0.147	0.099	0.644	0.222	0.103	0.063	
0.076	0.198	0.080	0.255	0.928	0.070	0.426	0.295	
1.052	0.253	0.506	0.379	0.909	0.054	0.631	0.341	
0.131	0.088	0.671	0.074	0.604	0.112	0.834	0.098	
0.138	0.473	0.022	0.079	0.187	0.179	0.540	0.321	
0.287	0.193	0.052	0.074	0.178	0.306	0.129	0.103	
0.107	0.149	0.051	0.072	0.128	0.068	0.366	0.217	
0.556	0.070	0.412	0.165	0.136	0.828	1.491	0.546	
0.200	0.271	0.286	0.806	0.348	0.069	0.181	0.122	
0.053	0.075	0.138	0.337	0.502	0.386	0.320	0.121	
0.161	0.274	0.068	0.120	0.497	0.097	0.060	0.135	
0.753	0.176	0.074	0.275	0.099	0.415	0.325	2.421	
0.076	0.147	0.149	0.203	0.243	0.052	0.056	0.055	
0.192	0.064	0.050	0.177	0.054	0.365	0.104	0.069	
0.163	0.286	0.290	1.331	0.167	0.068	0.054	0.054	
0.254	0.122	0.065	0.408	0.093	0.285	0.202	0.133	
0.226	0.067	0.066	0.155	0.280	0.183	0.529	0.109	
0.074	0.165	0.061	0.274	0.071	0.175	0.254	0.270	
0.099	0.069	0.811	0.051	0.193	0.094	0.498	0.174	
0.057	0.067	0.135	0.122	0.251	0.185	0.407	0.117	
0.146	0.074	0.078	0.077	0.073	0.057	0.386	0.107	

H4

0.257	0.028	0.075	0.030	0.458	0.139	0.266	0.082	0.164
0.070	0.125	0.036	0.187	0.048	0.132	0.067	0.131	0.337
0.080	0.123	0.028	0.021	0.202	0.381	0.050	0.516	0.138
0.220	0.086	0.084	0.026	0.262	0.054	0.063	0.045	0.045
0.188	0.223	0.113	0.025	0.139	0.115	0.323	0.029	0.023
0.021	0.595	1.825	0.078	0.094	0.068	0.164	0.045	
0.141	0.026	0.023	0.206	0.030	0.244	0.123	1.240	
0.164	0.023	0.037	0.051	0.531	0.192	0.029	0.248	
2.804	0.042	0.291	0.717	0.541	0.155	0.020	0.020	

0.332	0.412	0.026	0.086	0.071	0.158	0.077	0.269	
0.042	0.221	0.382	0.054	0.025	0.048	0.037	0.169	
0.041	0.087	0.131	0.044	0.028	0.224	0.021	0.034	
0.021	0.069	0.321	0.071	0.048	0.112	0.030	0.047	
1.106	0.586	0.050	0.116	0.259	0.207	0.033	0.062	
0.170	0.030	0.206	0.037	0.031	0.137	0.039	0.066	
0.170	0.161	0.040	0.454	0.909	0.166	0.274	0.063	
0.181	0.029	0.025	0.029	0.392	0.027	1.114	0.057	
0.177	0.859	2.798	0.826	0.048	0.159	0.022	0.026	
0.633	0.068	0.116	0.108	0.036	0.456	0.250	0.077	
0.160	0.024	0.082	0.059	2.738	0.627	0.043	0.128	
0.021	0.466	0.023	0.069	0.332	2.583	0.064	0.053	
0.024	0.608	0.094	0.040	0.236	0.166	0.103	0.486	
2.670	0.796	0.034	0.146	0.027	0.645	0.055	0.047	
0.766	0.023	0.477	0.065	0.088	0.163	0.057	0.052	
0.308	0.020	0.259	0.090	0.788	0.110	0.145	0.207	
0.508	0.072	0.288	0.399	0.037	0.438	0.033	0.030	
0.393	0.544	5.047	0.109	0.086	0.223	0.020	0.035	
0.601	0.133	0.735	0.046	0.039	0.331	0.043	0.139	
0.171	0.455	0.150	2.214	0.299	0.187	0.038	0.249	
0.043	0.084	0.288	0.139	0.175	0.103	0.048	0.096	
0.264	0.709	0.026	0.119	0.568	0.691	0.036	0.248	
0.022	0.707	0.731	0.028	0.023	0.051	0.024	0.078	
0.190	0.087	0.239	0.278	3.071	0.113	0.042	0.508	
0.203	0.235	0.073	0.332	0.183	0.030	0.023	0.026	
0.040	0.516	0.037	0.022	0.078	1.525	0.197	0.319	
0.345	0.655	0.755	0.155	0.110	0.491	0.119	0.408	
0.124	0.342	0.060	0.498	0.729	0.026	0.509	0.078	
0.045	0.380	0.169	0.205	1.772	0.025	0.350	0.207	
0.061	0.180	0.515	0.041	0.065	0.093	0.030	0.089	
0.542	0.035	0.249	0.275	0.099	0.074	0.115	0.045	
0.308	0.492	0.228	0.315	0.325	0.849	0.024	0.074	
0.280	0.325	0.595	0.025	0.438	0.214	0.160	0.302	
0.552	0.243	0.172	0.356	0.037	0.172	0.365	0.096	
0.061	0.561	0.192	0.124	0.025	0.426	0.040	0.136	
0.030	0.293	0.021	0.046	0.521	0.127	0.025	0.031	
0.160	0.092	0.134	0.150	0.165	0.034	0.232	0.021	
0.322	0.031	0.436	0.501	0.435	0.788	0.024	0.226	
0.161	0.452	0.087	0.059	0.379	0.037	0.131	0.045	
0.082	0.089	0.066	0.417	0.147	0.101	0.109	0.021	

0.027	0.194	0.083	0.403	0.219	0.070	0.385	0.329	
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H6

0.045	1.853	0.290	0.929	0.081	0.024	0.496	0.599	0.298	0.310
0.095	0.130	0.022	0.032	0.075	0.040	0.071	0.020	0.095	0.058
0.052	0.669	0.099	0.027	0.059	0.047	0.137	0.118	0.044	0.403
0.117	0.102	0.048	0.233	0.200	0.083	0.196	0.342	0.124	0.222
0.141	1.446	0.027	0.082	0.708	0.025	0.102	0.099	0.083	0.023
3.408	0.265	0.071	0.073	0.528	0.514	0.025	0.026	0.047	1.110
0.141	0.082	0.130	1.689	0.046	0.099	0.044	0.371	0.023	0.032
0.041	0.097	0.829	0.791	0.042	0.049	0.100	0.023	0.185	0.056
1.573	5.059	0.221	0.184	0.024	0.038	0.317	0.211	0.026	0.329
0.021	0.062	0.072	0.120	0.096	0.020	0.040	0.022	0.031	0.033
0.205	0.820	0.050	0.154	0.020	0.037	0.078	0.029	0.112	0.174
2.011	0.067	0.050	0.521	0.046	0.239	0.322	0.198	0.023	0.493
0.371	0.031	2.158	0.028	0.137	0.746	0.315	0.327	0.027	0.421
0.177	0.298	0.107	0.056	0.177	0.245	0.258	0.024	0.408	0.443
0.095	0.137	0.103	0.331	0.291	0.038	0.044	0.165	0.070	0.034
0.044	2.819	0.050	0.732	0.359	0.183	0.340	0.203	0.507	0.074
0.075	0.516	0.025	0.030	0.059	0.029	0.062	0.101	0.067	0.059
0.045	0.106	0.037	0.470	0.041	0.021	0.073	0.039	0.029	0.034
1.068	0.025	0.033	0.028	0.035	0.090	0.022	0.076	0.020	0.204
0.034	0.900	0.103	0.066	0.157	0.183	0.040	0.045	0.040	0.371
0.118	0.047	0.131	0.025	0.551	0.052	0.068	0.033	0.051	0.436
0.071	1.874	0.265	0.023	0.791	0.146	0.164	0.035	0.026	0.043
2.989	0.130	0.055	0.835	0.096	0.036	0.055	0.068	0.134	0.112
0.422	0.054	1.434	0.101	0.486	0.146	0.023	0.074	0.051	0.108
0.088	0.078	0.685	0.048	0.099	0.065	0.408	0.024	0.030	2.786
0.274	0.344	0.170	0.026	3.925	0.028	0.024	0.064	0.031	0.528
0.175	0.053	0.052	0.076	0.021	0.482	0.455	0.050	0.037	0.029
0.031	0.411	0.255	0.020	0.062	0.047	0.051	0.028	0.042	0.033
0.102	0.059	1.635	0.075	0.271	0.030	1.439	0.174	0.036	0.088
0.021	0.069	0.279	0.047	0.086	3.365	0.093	0.236	0.032	0.021
0.105	0.111	0.036	0.250	0.532	0.222	0.022	0.038	0.073	0.098
0.097	0.119	0.324	0.151	0.040	0.074	0.486	0.030	0.026	0.027
0.022	0.485	0.739	0.084	0.667	0.111	0.108	0.023	0.035	0.049
0.029	0.043	0.171	0.126	0.086	0.172	0.029	0.024	0.159	0.049
0.778	0.287	0.786	0.114	0.433	0.084	0.049	0.036	0.035	0.037
0.598	0.120	0.208	0.032	0.689	0.065	0.164	0.159	0.085	0.032

1.554	0.087	1.185	0.391	0.152	0.264	0.193	0.092	0.024	
2.018	0.042	0.530	0.028	0.318	0.188	2.019	0.565	0.020	
0.481	0.036	0.368	0.318	0.393	0.027	0.974	0.024	0.137	
0.233	0.044	0.722	0.075	0.160	0.021	0.431	0.067	0.338	
0.068	0.117	0.021	0.028	0.031	0.419	0.493	0.033	0.400	
0.508	0.054	0.375	0.534	0.178	1.182	0.225	0.172	0.077	
0.106	0.152	0.184	0.095	0.112	0.026	0.458	0.048	0.074	
0.231	0.026	0.125	0.086	0.301	0.029	0.032	0.088	0.492	
0.695	0.034	0.561	0.282	0.038	0.094	0.225	0.097	0.627	
0.056	0.053	0.034	0.036	0.258	0.075	0.088	0.034	0.036	
0.029	0.055	0.194	0.983	0.049	0.729	0.364	0.119	0.039	
0.040	0.329	0.274	0.164	0.032	0.638	0.029	0.108	0.044	
0.030	0.032	0.864	0.065	0.474	0.047	0.111	0.025	0.094	
0.077	0.054	0.781	0.195	1.368	0.056	0.030	0.354	0.022	

H8

0.071	0.071	0.046	0.055	0.06	0.157	0.262	0.09	0.053	
0.022	0.036	0.072	0.047	0.085	0.04	0.417	0.036	0.123	
0.129	0.074	0.042	0.081	3.807	0.101	0.355	0.036	0.859	
0.131	0.029	0.051	0.049	0.08	1.65	0.403	0.024	0.022	
0.032	0.063	0.156	0.162	0.792	0.12	0.383	0.056	0.053	
0.445	0.123	0.089	0.055	0.024	0.213	0.17	0.028	0.742	
0.108	0.088	0.225	0.876	0.237	0.133	0.027	0.02	0.159	
0.081	0.036	0.028	0.023	0.045	0.347	0.132	0.027	0.055	
0.045	0.058	0.028	0.164	0.531	0.126	0.025	0.036	0.029	
0.081	0.204	0.048	0.085	0.254	0.656	0.022	0.087	0.044	
0.028	0.024	0.198	0.123	0.117	0.217	0.025	0.332	0.349	
0.456	0.083	0.245	0.096	0.472	0.049	0.235	0.224	0.043	
0.077	0.08	0.033	0.451	0.174	0.843	0.054	0.199	0.063	
0.146	0.021	0.241	0.074	0.06	0.805	0.024	0.671	0.051	
0.047	0.076	0.075	2.565	0.033	0.024	0.08	0.028	0.061	
0.034	0.214	0.222	0.136	0.027	0.057	0.071	0.944	0.076	
0.697	0.061	0.038	0.185	0.221	0.509	0.085	0.64	0.021	
0.251	0.154	0.19	0.039	0.027	0.031	0.03	0.276	0.061	
0.093	0.04	0.245	0.156	0.589	0.08	0.04	0.572	0.252	
0.614	0.029	0.4	0.095	0.034	0.067	0.027	0.048	0.052	
3.121	0.14	0.606	0.107	0.055	0.245	0.029	0.393	0.075	
0.194	0.321	0.85	2.793	4.669	0.813	0.021	0.367	0.047	
0.02	0.425	0.091	0.25	0.301	0.043	0.022	0.032	0.197	

0.216	0.105	0.053	0.086	0.429	0.046	0.258	0.04	0.288
0.171	0.179	0.027	0.17	0.335	0.041	0.044	0.051	0.033
0.102	0.023	0.028	0.028	1.73	1.347	0.0131	0.235	0.103
0.021	0.262	0.316	4.469	0.276	0.149	0.476	0.025	0.021
0.044	0.317	2.382	0.047	0.053	0.856	0.279	0.085	0.047
0.127	0.031	0.025	0.022	0.294	0.219	0.241	0.039	0.035
0.21	0.064	0.038	0.297	0.125	0.355	0.541	0.035	0.026
0.22	0.03	0.171	0.028	0.056	0.216	0.758	0.279	
0.102	4.972	0.065	0.0186	0.052	0.175	0.178	0.201	
0.229	0.083	0.082	0.124	1.009	0.208	0.156	0.278	
0.265	0.021	0.43	0.421	0.399	0.271	0.298	0.084	
0.034	0.135	0.036	0.272	0.072	0.139	0.106	0.517	
0.676	0.167	0.727	0.02	0.194	0.374	0.302	0.03	
0.135	0.042	1.231	0.182	0.563	0.077	0.021	0.073	
0.164	0.255	0.076	0.504	0.198	0.065	0.301	0.28	
0.338	0.32	0.132	0.061	0.303	0.028	0.061	0.377	
0.486	0.536	0.052	0.363	0.041	0.112	0.278	0.058	
0.238	0.064	0.031	0.04	0.172	0.184	0.298	0.076	
0.318	0.028	0.66	0.277	0.41	0.068	0.253	0.028	
0.222	0.036	0.151	0.42	0.034	0.19	0.042	0.243	
0.33	0.385	0.857	0.672	0.938	0.084	0.063	0.021	
0.078	0.118	0.071	0.625	0.133	0.074	0.026	0.072	
0.198	0.026	0.206	0.052	0.483	0.053	0.021	0.273	
0.414	0.509	0.918	0.141	0.063	0.068	0.024	0.097	
0.882	0.669	0.124	0.119	0.11	0.248	0.028	0.159	
0.046	0.503	0.129	0.036	0.248	0.021	0.023	0.101	
0.47	0.069	0.097	0.208	0.167	0.174	0.153	0.039	

H10

0.062	0.054	0.021	0.032	0.044	0.022	0.054	1.144	0.347	0.025
0.033	0.043	0.020	0.021	0.355	0.034	0.963	0.629	1.823	0.414
0.183	0.036	1.027	0.250	0.377	0.205	0.048	0.024	0.039	0.104
0.205	0.473	0.845	0.192	0.099	0.186	0.022	0.430	0.096	0.529
0.140	0.080	0.230	0.030	2.602	0.036	0.421	0.048	0.046	0.028
0.039	0.046	0.029	0.030	0.148	0.118	0.330	0.025	0.102	0.021
0.046	0.028	0.072	0.029	0.128	0.237	0.035	0.516	0.124	0.079
0.041	0.133	0.034	0.063	0.037	0.028	0.197	0.519	0.162	0.131
0.283	0.050	0.083	0.200	0.043	0.170	0.224	0.694	0.232	0.086
0.091	0.034	0.474	0.326	0.049	0.200	0.331	1.132	0.140	0.098

0.028	0.345	0.175	0.516	0.025	0.343	0.134	0.098	1.293	0.366
0.445	0.022	0.131	0.149	0.037	0.035	0.360	0.348	0.334	0.035
0.059	0.033	0.053	0.444	0.400	0.030	0.156	0.034	0.083	0.136
0.066	0.048	0.025	0.111	0.489	0.083	0.189	0.406	0.678	0.102
0.027	0.914	0.080	0.143	0.030	0.025	0.629	0.027	0.128	0.029
0.037	0.154	0.077	0.161	0.122	2.457	0.501	0.061	0.031	0.111
0.054	0.021	0.021	0.020	0.022	0.476	0.207	0.082	0.110	0.035
0.120	0.453	0.346	0.027	0.060	0.101	0.484	0.072	0.084	0.062
0.049	0.075	0.020	0.239	0.072	0.535	0.144	0.057	0.342	0.026
0.974	0.500	0.087	0.039	0.302	0.031	0.052	0.093	0.200	0.070
0.065	0.031	0.074	0.043	0.127	0.157	0.060	0.034	0.043	0.022
0.177	0.070	1.102	0.023	0.021	0.059	1.006	0.024	0.362	0.124
0.061	0.207	0.140	0.025	0.036	0.086	0.027	0.059	0.433	0.057
0.042	0.075	0.087	0.066	0.489	0.031	0.268	0.432	0.552	0.345
0.081	0.553	0.042	2.068	0.074	0.175	0.167	0.038	0.033	0.023
0.315	0.023	0.363	0.113	0.086	0.158	0.026	0.025	0.054	0.258
0.046	0.150	0.439	0.021	0.987	0.195	0.051	0.102	0.117	0.086
0.080	0.264	0.026	2.056	0.046	0.121	0.169	0.038	0.028	0.027
2.108	0.040	0.192	0.024	0.141	0.314	0.028	0.112	0.034	0.169
0.052	0.083	0.302	0.021	0.224	0.226	0.130	0.184	0.026	0.023
0.095	0.053	0.064	0.023	0.151	0.098	0.021	0.069	0.118	0.142
0.581	0.597	3.927	0.166	0.035	0.192	0.304	0.495	0.028	0.037
0.533	0.202	0.048	0.171	0.317	0.032	0.210	0.377	0.033	0.076
0.025	0.584	0.190	0.027	0.811	0.789	0.077	0.316	0.086	0.171
2.021	0.090	0.032	0.102	0.602	0.048	0.204	0.133	0.033	0.035
0.055	5.350	0.072	0.095	0.858	0.053	0.184	0.042	0.100	0.187
0.038	0.030	0.090	0.029	4.228	1.190	0.089	0.030	0.065	0.048
0.054	0.032	0.114	0.546	0.165	0.029	0.051	0.061	0.030	0.023
0.026	0.064	0.161	0.712	0.129	3.506	0.034	0.045	0.361	0.022
0.044	0.119	0.175	0.295	0.503	0.071	0.061	0.040	0.225	0.021
0.186	0.217	0.032	0.035	0.216	0.078	0.070	0.079	0.092	0.077
0.514	1.394	1.805	0.756	0.077	0.117	0.020	0.058	0.022	0.359
0.030	0.033	0.044	0.048	0.571	0.039	0.035	0.039	0.291	0.051
0.030	0.024	0.021	0.936	0.111	0.920	0.023	0.085	0.115	0.260
0.116	0.748	0.037	0.922	0.179	2.649	0.113	0.036	0.075	0.172
0.557	0.031	0.041	2.248	0.115	0.446	0.026	0.050	0.079	0.048
0.098	0.474	0.096	0.026	0.065	0.238	0.025	0.021	0.121	0.824
0.210	0.021	0.636	0.051	0.412	0.026	0.023	0.036	0.082	0.623
2.214	0.047	0.027	0.285	0.302	0.140	0.023	0.168	0.037	0.172
0.874	0.269	0.025	0.080	1.445	0.305	0.033	0.212	0.347	0.212

0.837	0.806	0.030	0.031	0.085	0.033	0.032	0.063	0.162	0.048
0.088	0.021	0.077	0.091	0.053	0.030	0.096	0.037	0.029	0.044
0.068	0.036	0.289	0.079	0.039	0.052	0.053	0.026	0.059	0.022
0.291	0.030	0.077	0.063	0.025	0.077	0.129	0.108	0.032	0.024
0.040	0.029								

H12

0.049	0.069	0.090	0.107	0.055	0.034	0.083	0.034	0.287	0.712
0.026	0.132	0.044	0.023	0.038	0.239	0.042	0.928	0.077	0.117
0.025	0.141	0.553	0.527	0.058	0.085	0.329	0.038	0.420	0.102
0.040	0.184	0.063	0.383	0.059	0.034	1.068	0.282	0.022	0.131
0.152	0.365	0.040	0.071	1.427	0.942	0.111	0.298	0.480	0.228
0.537	0.041	0.102	0.030	0.049	0.080	0.104	0.436	0.540	0.341
0.632	0.061	0.031	0.330	0.166	0.023	0.323	0.646	0.482	0.109
0.068	0.047	0.103	0.049	0.166	0.620	0.429	0.020	0.043	0.106
0.160	0.210	0.078	0.023	0.079	0.034	0.138	0.211	0.116	0.110
0.028	0.523	0.038	0.104	0.051	0.418	0.170	0.132	0.032	0.143
0.114	0.416	0.087	1.098	0.076	0.090	0.259	0.098	0.152	0.138
0.207	1.018	0.045	0.026	0.043	0.065	0.067	0.124	0.092	0.448
0.492	0.083	0.077	0.046	0.099	0.093	0.033	0.060	0.097	0.279
0.185	0.066	0.049	0.225	0.158	0.063	0.059	0.155	0.189	0.095
0.151	0.555	0.059	0.063	0.112	0.027	0.067	0.023	0.059	0.557
0.312	0.106	0.075	0.219	0.058	0.024	0.088	0.308	0.167	0.106
0.351	0.022	0.505	0.233	0.044	0.074	0.029	0.031	0.064	0.426
0.034	0.040	0.031	0.254	0.934	0.757	0.040	0.136	0.032	0.094
0.020	0.038	0.138	0.024	0.676	0.223	0.034	0.020	0.039	0.048
0.055	0.131	0.221	0.035	0.144	0.056	0.072	0.029	0.440	0.120
0.321	2.059	0.404	4.199	0.197	0.037	0.025	0.022	0.446	0.090
0.320	0.053	0.093	0.728	4.934	0.663	0.075	0.330	0.318	0.460
1.349	0.521	0.045	0.062	0.154	0.021	0.026	0.273	0.229	0.035
0.721	0.059	0.041	0.034	0.519	0.151	0.241	0.042	0.228	0.100
0.038	0.070	0.055	0.159	0.151	0.953	0.316	0.068	0.145	0.756
0.320	0.465	0.024	0.037	0.141	0.146	0.286	0.104	0.177	0.407
0.422	1.399	1.110	1.736	0.120	0.072	0.196	0.213	0.228	0.032
0.041	0.138	0.804	0.125	0.054	0.025	0.165	0.186	0.312	0.215
0.862	0.077	1.231	0.056	0.411	1.019	0.146	0.160	0.177	0.546
0.868	1.276	0.144	0.022	0.024	0.026	0.182	0.199	0.222	0.047
0.093	0.138	1.793	0.516	0.861	0.074	0.178	0.196	0.209	0.213
0.056	0.391	0.104	0.139	0.036	0.053	0.173	0.109	0.172	0.332
0.047	0.026	0.160	0.072	0.224	0.066	0.459	0.057	0.072	0.056

0.158	0.225	0.050	0.026	0.023	3.975	0.938	0.070	0.022	0.103
0.677	0.188	0.028	1.219	0.021	0.078	0.179	0.055	0.033	0.071
0.021	0.079	0.126	0.209	0.042	0.211	0.358	0.026	0.022	0.026
0.062	0.274	0.457	2.260	0.409	1.121	0.132	0.403	0.061	0.022
0.081	0.049	1.178	0.114	0.034	0.471	0.433	0.217	0.042	0.292
0.355	0.182	0.189	0.329	0.626	0.147	0.274	0.060	0.044	0.027
1.051	0.826	0.055	0.066	0.060	0.086	0.157	0.071	0.089	0.024
0.290	0.409	0.416	0.025	0.508	2.792	1.681	0.082	0.022	0.063
0.095	0.230	0.094	0.985	0.242	0.039	0.221	0.082	0.088	0.049
2.699	0.048	1.984	0.206	0.180	0.034	0.250	0.095	0.107	0.029
0.893	0.142	0.560	0.110	0.745	0.040	0.149	0.027	0.073	
1.428	2.266	0.400	0.074	0.421	0.267	0.096	0.048	0.076	
0.107	0.044	0.097	1.156	1.077	0.492	0.157	0.042	0.032	
0.115	0.817	0.063	0.404	0.336	0.383	0.167	0.023	0.028	
0.146	0.161	0.021	0.290	0.109	0.470	0.107	0.082	0.022	
0.111	0.054	0.639	0.146	0.025	0.286	0.483	0.021	0.029	
1.027	0.039	0.367	0.494	0.264	0.067	0.243	0.074	0.445	

C2

0.330	0.082	0.128	0.104	0.166	0.197	0.871	0.168
0.090	0.219	0.063	0.309	0.469	0.101	0.484	3.745
0.139	0.081	0.345	0.367	0.087	0.051	0.169	0.076
0.059	0.115	0.060	0.377	0.097	0.150	0.065	0.145
0.498	0.174	0.087	0.104	0.066	0.123	0.624	0.147
2.071	0.119	0.076	0.062	0.140	0.078	0.106	0.056
0.202	0.084	0.174	0.140	5.643	0.300	0.280	0.063
0.183	0.075	0.195	0.267	0.053	0.284	0.053	0.367
0.055	0.078	0.061	0.120	0.079	0.064	0.160	0.056
0.315	0.051	0.123	0.198	0.052	0.988	0.998	0.052
0.063	0.061	0.084	0.051	0.221	0.100	0.088	0.131
0.359	0.086	0.054	0.167	0.078	0.069	0.161	0.241
0.444	0.452	0.074	0.177	0.175	0.109	0.071	0.205
0.073	0.132	0.099	0.124	0.680	0.804	0.182	0.088
0.064	0.062	0.064	0.065	0.322	0.733	0.348	1.826
4.661	0.061	0.062	0.413	0.085	0.109	0.059	0.332
0.540	0.601	0.084	0.484	1.056	0.248	0.316	0.052
0.050	0.069	0.802	0.053	0.492	0.147	0.331	0.052
0.346	0.239	0.094	0.229	0.213	0.076	3.665	0.087
0.063	0.374	0.125	0.162	0.106	0.216	0.068	0.156

0.162	0.606	0.189	0.160	0.108	0.076	0.505	0.284
0.952	0.417	0.143	0.805	0.284	0.218	0.093	0.088
1.799	0.061	0.061	0.295	0.178	0.225	0.204	0.080
0.085	0.063	0.112	0.330	0.315	0.074	0.263	1.289
0.058	0.104	0.068	3.383	0.270	0.340	0.800	0.071
0.391	0.654	0.114	0.143	0.103	0.223	0.188	0.080
0.161	0.090	0.159	0.362	0.287	0.204	0.061	
0.055	0.050	0.288	0.079	0.063	0.150	0.210	
0.123	0.961	0.550	0.651	0.142	2.360	0.157	
0.461	0.101	0.054	0.359	0.184	0.228	0.160	
0.357	0.067	0.076	0.060	0.410	0.818	0.165	
0.059	0.295	0.072	0.317	0.344	0.237	0.158	
0.098	0.060	0.072	0.054	0.064	0.411	0.170	
0.108	0.413	0.175	1.038	0.115	0.744	0.160	
0.288	0.176	0.381	0.064	0.056	0.817	0.601	
0.051	0.156	0.113	0.107	0.147	0.253	0.082	
0.065	0.133	0.149	0.337	0.282	0.198	0.166	
0.114	0.142	0.145	0.846	0.125	0.273	0.174	
0.180	0.057	0.054	0.455	0.090	0.067	0.717	
0.161	0.336	0.148	0.097	0.171	0.938	0.123	
0.111	0.167	0.086	0.089	0.158	0.355	0.054	
0.946	0.905	0.144	0.111	0.216	0.733	0.070	
0.149	0.356	0.359	0.055	0.062	3.437	0.434	
0.205	0.066	0.052	0.260	0.158	0.464	0.079	
0.173	0.094	0.063	0.069	0.171	0.136	0.999	
0.665	0.279	0.096	0.484	0.098	0.337	0.066	
0.260	0.308	0.292	0.178	0.163	0.053	0.887	
0.520	0.064	0.411	0.104	0.168	0.069	0.429	
0.067	0.079	0.099	0.121	0.022	0.071	0.071	
0.201	0.660	0.086	0.052	0.084	0.052	0.255	

C4

0.171	0.213	0.117	0.080	0.036	0.031	0.789	0.106
0.072	0.230	0.051	0.557	6.178	0.123	0.378	0.112
0.031	0.062	0.079	0.065	0.095	0.081	0.183	0.225
0.027	0.028	0.095	0.221	0.037	0.101	0.325	0.144
0.137	0.070	0.064	0.035	0.052	0.142	0.782	0.027
0.867	0.805	0.029	0.118	0.212	0.899	0.021	0.030
0.107	0.098	0.363	0.152	0.060	0.393	0.976	0.035

0.050	0.050	0.613	0.022	0.021	0.441	2.332	0.307
0.024	0.539	0.025	0.565	0.331	0.031	0.027	0.030
0.082	0.535	0.241	0.066	0.147	0.202	1.033	0.097
0.080	0.399	0.048	0.115	0.130	0.129	0.327	0.025
0.025	0.194	0.050	0.145	0.167	0.034	0.071	0.078
0.036	0.405	0.366	0.425	0.132	0.170	0.089	0.031
0.383	0.204	0.021	0.053	6.623	0.267	0.514	0.038
0.275	0.208	0.086	0.361	0.313	0.245	0.625	0.043
0.152	0.085	2.385	1.784	0.153	0.460	0.132	0.036
0.066	0.089	0.072	0.636	0.336	0.121	0.028	0.023
0.025	0.149	0.053	0.049	0.150	1.253	0.087	0.515
0.032	5.211	0.424	0.300	0.022	2.020	0.192	0.563
0.022	0.023	0.030	0.042	0.045	0.031	0.082	0.213
0.027	0.053	0.021	0.594	0.216	0.029	0.604	0.068
0.149	0.178	0.056	0.205	0.041	0.140	0.317	0.127
0.035	0.117	0.319	2.977	0.105	0.633	0.444	0.080
0.243	0.349	0.110	0.406	0.237	5.002	0.086	0.199
0.656	0.353	0.030	0.785	0.991	1.888	0.179	0.336
0.047	1.127	0.043	0.125	0.700	0.256	0.233	0.278
0.112	0.193	0.162	0.425	0.238	0.123	0.316	0.527
0.416	0.168	0.261	0.861	0.047	0.032	0.110	0.067
0.022	1.174	0.106	1.884	0.843	2.242	0.465	0.135
0.131	0.208	0.809	0.591	0.724	0.192	0.317	0.024
0.047	0.137	0.454	0.032	0.540	0.079	0.069	0.057
0.171	0.046	0.456	0.160	0.030	0.545	0.080	0.036
0.255	0.409	1.239	1.087	0.081	0.154	0.093	0.243
0.033	0.229	0.144	0.954	0.382	0.437	0.638	0.073
0.291	1.117	0.076	0.050	0.469	0.042	0.043	0.094
0.170	1.212	0.102	0.510	0.305	0.311	0.237	0.062
0.071	0.452	0.552	0.268	0.376	0.088	0.181	0.247
0.074	0.090	0.760	0.385	0.275	0.021	0.324	0.103
0.054	0.501	1.393	0.358	0.026	0.905	0.085	0.021
0.233	0.296	0.058	0.037	0.232	0.311	0.137	0.087
0.496	0.140	0.719	0.284	0.228	0.393	0.032	0.041
0.064	0.327	0.148	0.170	0.318	0.064	0.027	0.063
0.516	1.055	0.109	0.059	0.173	0.165	0.042	0.057
0.385	0.511	1.058	0.198	0.125	0.260	0.035	0.028
0.373	0.178	0.035	0.098	0.032	0.131	0.022	0.033
0.066	0.103	0.162	0.029	0.085	0.115	0.044	0.068
0.059	0.353	0.368	0.225	0.108	0.107	0.078	0.026

0.215	0.217	0.147	0.283	0.060	0.090	0.101	0.091
0.187	0.174	0.128	0.169	0.120	0.347	0.026	0.048
0.383	0.368	0.367	0.030	0.037	0.323	0.043	0.058
0.272	0.059	0.103	0.088	0.030	0.231	0.166	

C6

0.193	0.288	0.452	0.389	0.098	0.072	0.057	0.403
0.223	0.771	0.323	0.203	0.031	0.221	0.560	0.626
0.100	0.092	0.068	5.436	0.048	0.022	0.092	0.047
0.028	0.315	0.072	0.023	0.023	0.133	0.154	0.355
0.056	0.068	0.215	1.200	2.566	0.024	0.109	0.152
0.072	0.052	0.456	0.072	0.387	0.075	0.137	0.252
0.023	0.130	0.655	0.269	0.396	0.039	0.035	0.140
0.033	0.040	0.059	0.044	0.151	4.936	0.179	0.027
0.342	0.284	0.029	0.681	0.065	0.073	0.030	0.046
0.719	0.176	0.391	0.041	0.380	0.146	0.026	0.619
0.035	0.214	0.022	0.635	0.537	0.052	0.207	0.231
0.187	0.056	0.300	0.337	2.101	0.033	0.211	0.223
0.534	0.026	1.600	0.276	0.301	0.151	0.093	0.148
0.247	1.383	0.131	0.027	0.034	0.086	0.067	0.055
0.559	0.605	0.821	0.165	0.189	0.027	0.273	0.044
0.175	0.052	0.043	0.297	0.169	5.507	0.028	0.233
0.051	0.036	0.054	0.032	0.704	0.130	0.045	0.055
0.100	0.023	0.269	0.056	0.034	0.144	0.173	0.096
1.595	0.031	0.005	0.172	0.021	0.261	0.038	0.202
2.218	0.024	0.050	0.038	0.476	0.026	0.081	0.032
0.082	0.085	0.069	0.130	1.189	0.048	0.044	0.055
2.229	0.017	0.099	0.389	0.278	0.212	0.059	0.163
1.284	0.165	0.032	0.710	0.094	0.046	0.029	0.033
0.022	0.008	0.549	0.049	0.432	0.076	1.548	0.094
0.725	0.314	0.025	0.127	0.072	0.707	0.273	0.043
0.065	0.052	0.035	0.527	0.214	0.032	0.084	0.103
0.021	0.016	0.117	0.334	1.463	0.111	0.223	0.023
0.052	0.110	0.020	0.106	1.195	0.999	0.086	0.184
0.021	0.023	0.360	0.159	0.118	0.114	0.456	0.061
0.289	0.053	0.409	0.190	0.119	2.084	0.050	0.023
0.144	0.140	0.081	0.414	0.111	0.042	0.178	0.166
0.104	0.377	0.115	0.336	0.035	0.314	0.280	0.033
1.102	0.141	0.307	0.212	0.032	0.264	0.281	0.380

0.420	0.054	0.461	0.258	0.154	0.095	0.119	0.053
1.093	0.281	0.926	0.124	0.088	0.725	0.184	0.027
0.735	0.162	0.026	0.463	1.172	0.106	0.109	0.167
0.588	0.303	0.121	0.280	0.117	0.309	0.623	0.260
1.228	0.051	0.127	0.568	0.530	0.239	0.083	0.062
0.031	0.213	0.253	0.085	0.971	0.697	0.064	0.068
0.194	0.894	0.320	0.535	0.122	0.181	0.891	0.046
0.037	0.028	0.040	0.392	0.386	0.372	0.056	0.179
0.105	0.244	0.605	0.324	0.198	0.356	0.048	0.075
0.191	0.109	0.108	0.294	0.358	0.270	0.020	0.099
0.311	0.020	0.168	0.061	0.278	0.031	0.023	0.245
0.078	0.032	0.417	0.034	0.061	0.030	0.061	0.024
0.032							

C8

0.043	0.035	0.034	0.287	0.119	0.188	0.027	0.264
0.338	0.086	0.056	0.134	0.776	0.038	0.061	0.045
1.078	0.052	1.469	0.048	0.218	0.059	0.026	0.061
0.045	0.022	0.954	0.090	1.472	0.181	0.064	0.130
0.037	0.027	0.183	0.271	0.024	0.178	0.114	0.090
0.125	0.053	0.150	0.100	0.051	5.511	0.109	0.112
0.040	0.091	0.196	0.070	0.049	2.154	0.220	0.181
0.160	0.041	0.114	0.041	0.027	0.051	0.312	0.052
0.053	0.158	0.099	0.170	0.029	0.029	0.175	0.062
0.379	0.040	0.025	1.555	3.084	0.274	0.228	0.026
0.043	0.337	0.168	0.298	0.039	0.156	0.129	0.037
0.763	0.048	0.031	0.399	0.020	0.387	0.301	0.024
0.045	0.212	0.234	5.580	0.049	0.029	0.550	0.229
0.264	0.026	0.043	0.196	0.271	0.964	0.042	0.255
0.031	0.051	0.300	0.101	0.391	0.230	0.093	0.022
0.635	0.020	0.118	0.239	0.218	0.568	0.102	0.125
0.353	0.076	0.302	1.077	1.370	0.754	0.126	0.022
0.026	1.725	0.408	0.160	0.039	0.619	0.518	0.026
0.069	0.042	0.047	0.317	0.230	0.052	0.469	0.082
0.039	0.764	0.240	0.162	0.025	0.515	0.084	0.034
0.398	0.042	0.193	0.054	0.335	0.295	0.071	0.318
1.049	0.058	0.118	0.572	0.234	0.023	0.025	0.103
0.249	0.266	0.546	0.217	0.023	1.580	0.109	0.064
0.162	0.044	0.338	0.724	0.095	0.195	0.126	0.056

0.102	0.127	0.180	0.213	0.061	0.031	0.039	0.099
0.158	0.029	0.147	0.026	0.051	0.970	0.105	0.161
0.490	0.713	1.068	0.501	0.180	0.082	0.104	0.025
1.004	0.313	0.028	0.146	0.048	0.257	0.071	0.031
1.184	0.061	0.377	0.054	0.214	1.965	0.037	0.040
0.479	0.034	0.050	0.488	1.190	0.020	0.221	0.228
0.090	0.025	0.468	0.090	1.069	0.034	0.198	0.198
0.158	0.047	0.468	0.990	0.123	0.466	0.225	
0.074	1.868	0.321	0.258	0.797	0.222	0.301	
0.338	0.023	2.284	0.391	0.507	0.063	0.441	
0.086	0.090	0.142	0.718	0.851	1.322	0.186	
0.056	0.101	0.250	0.681	0.529	0.423	0.120	
1.147	0.623	0.036	0.221	0.222	0.519	0.104	
0.065	0.114	0.215	0.477	0.306	0.319	0.755	
0.029	0.025	0.196	0.052	0.025	0.420	0.516	
0.735	0.030	0.033	0.175	0.286	0.081	0.228	
0.113	0.107	0.116	0.478	0.301	0.185	0.240	
0.116	0.551	0.218	0.322	0.231	0.175	0.024	
0.639	0.549	0.331	0.043	0.033	0.371	0.235	
0.497	0.735	0.065	0.220	0.147	0.093	0.128	
0.177	0.854	0.109	0.025	0.229	0.326	0.028	
0.128	0.175	0.024	0.122	0.427	0.086	0.054	
0.427	0.253	0.032	0.205	1.312	0.290	0.102	
0.039	0.088	0.137	0.056	0.049	0.301	0.177	
0.062	0.036	0.087	0.075	0.120	0.024	0.024	
0.023	0.431	0.117	0.331	0.024	0.055	0.022	

C10

0.079	0.165	0.056	0.158	0.133	0.182	0.197	
0.021	0.424	0.024	0.121	0.023	0.086	0.297	0.360
0.718	0.156	0.035	0.051	0.049	0.275	0.086	0.022
0.089	0.290	0.032	0.031	0.049	0.058	0.205	0.489
0.137	0.039	0.032	0.055	0.089	0.186	0.157	0.073
0.028	0.022	0.081	0.037	0.144	0.027	0.028	1.345
0.047	0.068	0.098	0.023	0.039	0.039	0.066	0.036
0.520	0.245	0.042	0.066	0.036	0.051	0.029	0.579
0.029	0.076	0.065	0.035	0.224	1.712	0.027	0.386
0.026	0.037	0.087	0.183	0.117	0.037	1.066	0.067
0.024	0.022	0.066	0.026	0.053	0.029	0.050	0.032

0.021	0.021	0.024	0.029	0.028	0.813	0.492	0.500
0.172	0.179	0.112	0.022	0.062	1.457	0.186	0.085
0.183	0.035	0.039	0.116	0.051	0.080	0.525	0.051
0.372	0.216	0.061	0.105	0.050	0.028	0.056	0.080
0.175	0.040	0.064	0.050	0.020	0.025	0.020	0.464
0.151	0.024	0.028	0.039	0.039	0.070	0.026	0.026
0.042	0.029	0.101	1.192	0.043	0.308	0.155	0.089
0.526	0.036	0.121	0.237	0.022	0.021	0.026	0.092
3.368	0.034	0.043	0.038	0.917	0.022	0.663	0.144
0.063	0.089	0.569	0.123	0.095	0.050	0.196	0.037
0.072	0.036	0.040	0.206	3.901	0.049	0.141	0.024
0.445	0.222	0.026	0.207	0.075	0.054	0.347	0.239
0.053	0.884	0.767	0.201	0.236	0.514	0.024	0.021
0.020	0.043	0.116	0.300	0.069	0.035	0.033	0.094
0.093	0.046	0.060	0.200	0.153	2.229	0.131	0.224
0.486	0.045	0.215	0.030	0.245	0.043	0.243	0.075
0.032	0.053	0.129	0.046	0.043	0.537	1.565	0.139
0.199	0.079	0.096	0.053	0.031	0.251	0.228	0.114
0.042	3.728	0.124	0.113	0.119	0.296	0.645	0.155
0.300	0.182	0.067	1.022	0.078	0.041	0.136	0.107
0.424	0.077	0.171	0.033	0.045	0.209	0.355	0.124
0.236	0.295	0.028	0.064	0.084	0.022	0.021	0.189
0.766	0.060	0.034	0.246	0.187	0.097	0.358	0.715
0.031	0.021	0.390	0.040	0.233	0.052	0.177	0.057
0.027	0.091	0.538	0.103	0.095	0.284	0.073	0.269
0.205	0.021	0.070	0.066	0.023	0.098	0.024	1.030
0.135	0.025	0.126	0.025	0.134	0.136	0.142	0.136
0.064	0.137	0.058	0.159	0.227	0.894	0.056	0.725
0.354	0.134	0.126	0.048	0.075	0.263	0.041	0.197
0.212	1.080	0.391	2.210	0.055	0.847	0.023	0.071
0.249	1.045	0.237	0.038	0.388	0.045	0.065	0.130
0.034	0.185	0.061	0.026	0.023	0.031	0.029	0.084
2.912	0.157	0.026	0.118	0.616	0.964	1.611	0.191
0.602	0.168	0.217	0.040	0.059	0.176	0.028	0.060
0.188	0.207	0.447	2.030	0.091	0.030	0.122	0.256
0.822	0.500	0.607	0.206	0.054	0.303	0.636	0.050
0.823	0.149	0.021	0.505	0.031	0.610	0.072	0.100
0.082	0.164	0.844	0.117	0.630	0.152	0.025	1.021
0.041	0.540	0.068	3.569	0.074	0.163	0.073	0.634
0.021	0.055	0.241	0.401	1.122	0.241	0.479	0.164

0.659	0.106	0.226	0.145	0.222	0.357	0.038	0.117
0.035	0.308	0.033	0.167	0.225	0.166	0.062	0.094
0.037	0.112	0.053	0.042	0.178	0.666	0.222	0.123
0.090	0.029	0.477	0.022	0.020	0.251	0.023	0.080
0.046	0.061	0.495	0.262	0.127	0.128	0.161	0.663
0.877	0.042	0.145	0.155	0.505	0.207	0.348	0.148
2.075	0.495	0.801	0.145	0.200	0.338	0.267	0.121
0.026	0.113	0.132	0.876	0.205	1.673	0.046	0.254
0.278	0.080	0.085	0.182	0.371	0.087	0.046	0.141
0.390	0.094	0.139	0.131	0.035	1.083	0.055	0.070
0.100	0.054	0.179	0.200	0.423	0.029	0.779	0.020
0.121	0.023	0.240	0.142	0.056	0.031	0.724	0.785
0.181	0.078	0.595	0.134	4.076	0.063	0.144	0.217
0.461	0.248	0.066	0.033	0.069	0.031	0.070	0.022
0.021	0.420	0.568	0.027	0.191	0.302	0.043	0.033
0.058	0.057	0.024					

C12

0.042	0.465	0.063	0.186	0.048	0.210	0.047	0.664
1.022	0.033	0.330	0.055	0.037	0.044	0.396	0.312
0.025	0.046	0.137	0.131	0.074	0.054	0.031	0.185
0.183	0.026	0.181	0.064	0.116	0.478	0.039	0.072
0.382	0.023	0.123	0.038	0.106	0.026	0.037	0.400
0.137	0.204	0.256	0.115	0.235	0.034	0.044	0.059
0.115	0.044	0.310	0.041	0.038	0.022	0.591	0.283
0.037	0.053	0.258	0.025	0.021	0.021	0.472	0.040
0.167	0.024	0.155	0.072	0.623	0.034	0.061	1.110
0.042	0.034	0.178	0.047	0.107	0.052	0.121	0.135
0.199	0.385	0.180	0.200	0.032	0.045	0.256	0.198
0.040	0.070	0.045	0.028	0.067	0.021	0.115	0.066
0.051	0.241	0.291	0.023	0.020	0.308	0.078	0.041
0.551	0.152	0.049	0.123	0.023	0.690	0.039	0.062
6.840	0.124	0.113	0.046	0.036	0.020	0.024	0.059
0.024	0.173	0.046	0.107	0.042	0.036	0.035	0.025
0.083	0.166	0.072	0.055	0.172	0.222	0.050	0.210
0.033	0.250	0.264	0.041	0.023	0.053	0.034	0.034
0.071	0.401	0.156	1.090	0.021	0.123	0.075	0.032
0.029	0.125	0.117	1.412	0.028	0.028	0.040	0.207
0.032	0.145	0.056	0.102	0.023	0.049	0.045	0.323

0.437	0.117	0.247	0.025	0.451	0.307	0.143	0.521
0.496	0.311	0.034	0.029	0.061	0.255	0.336	0.063
0.298	0.205	0.031	0.028	0.088	0.052	0.196	0.148
0.067	0.211	0.023	0.021	0.119	0.021	0.171	0.049
0.167	0.504	0.129	0.049	0.052	0.229	0.254	0.123
0.023	0.053	0.029	0.031	0.246	0.497	0.176	0.040
0.080	0.038	3.846	0.047	0.059	0.030	0.706	0.020
0.647	0.037	0.409	0.158	0.036	1.299	0.295	0.043
0.033	0.071	0.032	0.046	0.242	0.040	0.031	0.025
0.106	0.179	0.111	0.055	0.055	0.532	0.656	0.185
0.025	0.064	0.561	2.291	0.167	0.451	0.281	0.064
0.337	0.036	0.060	0.273	0.029	0.034	0.048	0.042
0.524	0.037	0.043	0.033	0.029	0.158	0.031	0.030
0.031	0.075	0.029	0.071	0.779	0.027	0.022	0.020
0.046	0.777	0.030	0.076	0.072	0.523	0.184	0.026
0.062	0.038	0.039	0.107	0.035	0.155	0.112	0.081
1.033	0.075	0.025	0.077	0.171	0.031	1.203	0.033
0.051	2.136	0.572	2.451	0.114	0.672	0.066	0.844
0.942	0.450	0.213	0.172	3.146	0.298	0.075	0.784
0.165	0.078	0.229	0.027	0.030	0.281	0.031	1.203
0.755	0.363	0.031	0.120	0.031	0.456	0.242	0.231
0.063	0.105	1.217	0.046	0.027	0.291	0.122	0.745
0.737	0.152	0.026	0.023	0.141	0.967	0.224	0.048
0.261	0.034	0.500	0.027	0.287	0.050	0.196	0.128
0.025	0.108	0.190	0.026	0.036	0.023	0.267	0.074
0.296	0.290	0.037	0.153	0.074	1.668	0.127	0.899
0.566	0.071	0.027	0.066	0.031	0.021	0.162	0.257
0.049	0.103	0.414	4.695	1.557	0.495	0.129	0.154
0.026	0.049	0.225	0.049	0.073	0.370	0.137	0.500
1.066	0.024	0.149	0.076	0.034	0.166	0.151	0.113
0.689	0.037	0.023	0.051	0.156	1.752	0.197	0.207
0.118	0.378	0.032	0.023	3.073	0.020	0.918	0.545
0.025	0.268	0.061	0.283	0.129	0.574	0.024	0.215
0.035	0.303	0.116	0.055	0.021	1.071	0.029	0.391
0.525	0.398	0.216	0.587	0.393	0.188	0.060	0.382
0.028	0.067	0.151	0.035	0.597	0.106	0.029	0.022
0.045	0.303	0.344	2.753	0.029	0.432	0.134	0.031
0.160	0.754	0.055	1.070	0.916	0.223	0.309	0.334
0.250	0.030	1.757	0.164	0.482	0.025	0.066	0.354
0.025	0.048	0.358	0.041	0.202	0.102	0.109	0.420

0.056	0.077	0.020	0.042	0.109	0.083	0.067	0.418
0.055	0.028	0.032	0.193	0.062	0.200	0.160	0.050
0.047	0.065	0.043	1.053	0.022	0.059	0.033	0.027
0.086	0.327	0.253	0.035	0.567	0.112	0.205	0.110
0.044	0.540	0.031	0.666	0.479	0.162	0.102	0.023
0.288	0.191	0.041	0.036	0.732	0.979	0.171	0.396
0.030	0.141	0.023	0.387	0.025	0.796	0.064	0.039
0.022	0.520	0.562	0.192	0.035	0.235	0.108	0.047
0.036	0.150	0.209	0.105	0.132	0.041	0.023	0.051
0.447	0.223	0.025	0.498	0.049	0.338	0.034	0.565
1.099	0.028	0.408	0.043	0.105	0.036	0.024	0.071
0.141	0.062	0.127	0.029	0.064	0.213	0.160	0.021
0.022	0.067	0.053	0.106	0.033	1.090	0.033	0.078

A2

0.883	0.117	0.059	0.066	0.300	0.051	0.074	0.137	0.064	0.378
0.051	0.117	0.057	0.136	0.078	0.197	0.348	0.090	0.071	0.175
0.427	0.097	0.939	0.058	0.376	0.631	0.091	0.058	0.075	0.189
0.235	0.318	0.150	0.056	0.127	0.135	0.347	0.187	0.228	0.085
0.234	0.064	0.093	0.106	0.075	0.276	0.109	0.629	0.085	0.167
0.127	1.176	0.185	0.416	0.093	0.127	0.748	0.160	0.146	0.200
0.247	0.202	0.092	0.167	0.088	0.150	0.067	0.058	0.253	0.130
0.100	0.092	0.064	0.082	0.073	0.112	0.539	0.022	0.109	0.105
0.738	0.064	0.063	1.255	0.381	0.099	0.062	0.099	0.071	0.113
0.489	7.481	0.193	0.122	5.200	0.075	0.125	0.139	0.192	0.066
0.554	0.051	0.101	0.071	0.368	0.254	0.059	0.077	0.164	0.064
0.747	0.309	0.336	0.056	0.089	0.064	0.494	0.193	0.310	0.317
0.306	0.175	0.187	0.059	0.304	0.293	0.195	0.136	0.064	0.109
0.352	0.172	0.267	0.438	0.219	0.063	0.064	0.506	0.064	0.056
0.192	0.095	0.060	0.109	0.236	0.066	0.149	0.206	0.056	0.078
0.217	1.063	0.120	0.112	0.389	0.056	0.068	0.305	0.067	0.067
0.223	0.052	0.102	0.337	0.090	0.074	0.058	0.059	0.300	
0.566	0.323	0.303	0.076	0.070	0.061	0.261	0.275	0.987	
0.726	1.122	0.067	0.159	0.430	0.247	0.113	0.398	0.283	
1.788	0.117	0.113	0.358	0.177	0.069	0.061	0.086	0.088	
0.390	0.952	0.126	0.381	0.126	0.148	0.295	0.114	0.507	
0.387	0.548	0.157	0.201	0.148	0.093	0.057	0.098	0.423	
0.303	0.644	0.268	0.741	0.570	0.362	0.155	0.284	0.142	
0.266	0.214	0.225	0.156	0.165	1.089	0.781	0.060	0.105	

0.075	0.076	0.083	0.233	0.407	0.275	0.093	0.150	0.124	
0.219	0.056	0.570	0.188	0.069	0.234	0.179	0.082	0.080	
0.140	0.100	0.143	0.247	0.149	0.068	0.119	0.142	0.056	
0.315	1.174	0.080	0.096	2.905	0.089	0.516	0.444	0.108	
0.462	0.217	0.499	0.619	2.551	0.134	0.074	0.138	0.093	
0.317	0.329	0.139	0.070	0.869	0.456	0.058	0.087	0.594	
0.808	0.126	0.444	1.424	0.182	0.093	0.414	0.053	0.052	
0.131	0.090	0.507	0.808	0.198	0.266	0.205	0.105	0.051	
0.239	0.491	0.193	0.077	0.137	0.329	1.033	0.395	0.165	
0.393	0.098	0.256	0.191	0.128	2.062	0.122	0.152	0.089	
0.079	0.321	0.245	0.058	0.366	0.386	0.062	1.773	0.113	
0.201	0.298	0.067	0.135	0.551	0.086	0.887	0.092	0.117	
0.067	0.171	0.116	0.140	0.112	0.258	0.113	0.061	0.104	
0.111	0.186	0.157	0.133	0.654	0.072	0.233	0.059	0.095	
0.264	0.116	0.279	0.335	0.521	0.194	0.154	0.071	0.102	
0.087	0.106	0.212	0.074	0.241	0.055	0.263	0.112	0.077	
0.175	0.318	0.086	0.198	0.336	0.177	0.184	0.117	0.103	
0.142	0.136	0.598	1.253	0.465	0.079	0.179	0.050	0.052	
0.246	0.122	0.277	0.059	0.095	0.198	0.068	0.199	0.145	
0.068	0.098	0.156	0.063	0.051	0.114	0.314	0.136	0.051	
0.206	0.056	0.159	0.099	1.054	0.072	0.081	0.478	0.097	
0.104	0.167	0.080	0.106	0.408	0.480	0.052	0.120	0.084	
0.080	0.463	0.058	0.096	0.517	0.207	0.054	0.065	0.059	
0.167	0.089	0.066	0.243	0.056	0.315	0.089	0.020	0.297	
0.094	0.430	0.053	0.603	0.184	0.070	0.280	0.202	0.099	
0.084	0.194	0.281	0.165	0.173	0.070	0.277	0.201	0.430	

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0.036	0.07	0.098	0.086	0.633	0.048	0.154	0.032
0.177	0.085	0.036	0.065	0.029	0.097	0.167	0.096
0.157	0.056	0.501	0.046	0.2	0.496	0.026	0.237
0.034	0.021	0.209	0.155	0.023	0.022	0.258	0.456
0.027	1.203	4.748	0.087	0.144	0.028	0.167	0.033
0.92	0.137	0.241	0.022	0.024	0.023	0.739	0.027
0.077	0.303	0.053	0.08	0.027	0.374	0.231	0.079
0.083	0.279	0.023	0.46	0.11	0.186	0.679	0.054
0.059	0.035	0.202	0.026	0.025	0.066	0.073	0.1
0.143	0.028	0.149	0.042	0.804	0.043	0.055	0.03
0.096	0.081	0.057	0.209	0.427	0.02	0.115	0.175

0.036	0.173	0.121	0.026	0.086	0.022	0.04	0.337
0.034	0.117	0.078	0.056	0.066	0.232	0.065	0.087
0.023	0.07	0.473	0.14	0.05	0.22	0.079	0.07
0.077	0.028	0.047	0.031	0.034	0.45	0.021	0.028
0.027	0.058	0.041	0.32	0.196	0.804	0.025	0.065
0.042	0.022	0.11	0.021	0.502	0.315	0.02	0.067
0.216	0.153	0.03	0.177	0.18	0.727	0.098	0.054
0.167	0.044	0.06	0.036	0.04	0.026	0.076	0.03
0.068	0.024	0.057	0.051	0.022	0.275	0.033	0.091
0.13	0.147	0.022	0.044	0.333	0.153	0.328	0.039
0.131	1.921	0.281	0.069	0.922	0.025	0.163	0.032
0.203	0.029	0.217	3.111	2.862	0.076	0.046	0.041
1.294	0.447	0.336	0.025	0.039	1.147	0.02	0.028
0.341	0.199	0.026	0.538	0.195	5.575	0.311	0.362
0.358	0.057	0.154	0.25	0.043	0.907	0.313	0.126
0.291	0.028	0.159	0.063	0.229	0.379	0.025	0.158
0.074	0.041	0.619	0.444	0.164	0.038	0.788	0.18
2.643	0.829	0.279	0.133	0.055	0.032	0.022	0.023
0.047	0.05	0.323	0.033	0.454	0.798	0.282	0.07
0.294	0.153	4.629	0.082	0.596	0.033	0.029	0.098
0.19	0.264	0.034	0.215	1.247	0.024	0.141	0.064
0.438	0.054	0.02	0.088	2	0.069	0.036	0.034
0.059	0.188	0.212	0.063	1.577	0.021	0.059	0.069
0.04	0.374	0.025	0.304	0.171	0.02	0.247	0.032
0.048	0.036	0.035	0.11	0.07	0.33	0.219	0.031
0.154	0.022	0.061	0.03	0.207	0.338	0.218	0.025
0.076	0.33	0.416	1.127	0.805	0.121	0.662	0.044
0.26	1.11	0.261	1.707	0.029	0.582	0.111	
0.032	0.028	0.059	0.094	0.189	2.408	0.217	
0.028	0.036	0.406	0.425	1.072	0.083	0.227	
0.099	0.024	0.405	0.235	0.041	0.594	0.153	
0.085	0.112	0.5	0.262	0.805	0.562	0.053	
0.23	0.062	0.327	0.512	0.042	0.397	0.16	
0.188	0.036	0.331	0.45	0.213	0.072	0.112	
0.305	0.421	0.175	0.031	0.042	0.05	0.03	
0.218	0.084	0.241	0.263	0.293	0.341	0.057	
0.035	0.837	0.476	2.098	0.128	0.176	0.048	
0.359	0.028	0.17	0.149	0.147	0.028	0.03	
0.186	0.051	0.034	0.407	0.14	0.027	0.31	
0.092	0.53	0.811	0.202	0.094	0.035	0.125	

0.552	2.602	0.872	0.283	0.125	0.247	0.135	
0.17	0.179	0.268	0.457	0.95	1.07	0.046	
0.023	0.096	0.265	0.02	0.021	0.107	0.03	
0.351	0.025	0.076	0.049	0.36	0.025	0.126	
0.024	0.256	1.394	0.04	0.477	0.026	0.077	
0.175	0.083	0.212	0.484	0.055	0.074	0.056	
0.174	0.061	0.376	0.069	0.29	0.141	0.138	
0.52	0.27	0.028	0.14	0.032	0.178	0.041	
0.036	0.594	0.249	0.677	0.152	0.05		
0.037	0.116	0.091	0.262	0.063	0.104		

A6

0.044	0.090	0.033	0.344	2.490	0.049	0.643	0.181
0.038	0.049	0.022	0.024	0.040	4.190	0.062	0.302
0.083	0.026	0.166	0.077	0.136	0.040	0.027	0.289
0.184	0.122	0.030	0.267	0.023	0.224	0.050	0.181
0.314	0.296	0.080	0.046	0.022	0.155	0.025	0.112
0.059	0.033	0.020	0.096	0.023	0.580	0.281	0.161
0.110	0.065	0.021	0.036	0.256	0.084	0.263	0.094
0.049	0.037	0.427	0.187	0.139	0.211	0.139	0.036
4.820	0.030	0.053	0.970	0.229	0.154	0.187	0.079
0.159	0.209	0.040	0.187	0.159	0.099	0.123	0.079
0.044	0.038	1.017	1.544	0.881	0.025	0.389	0.070
0.385	0.068	0.152	0.077	0.229	0.028	0.291	0.079
0.089	0.022	0.074	0.459	0.045	1.481	0.028	0.097
0.169	0.069	1.218	0.025	0.081	2.369	0.191	0.095
0.109	0.379	0.287	0.072	0.224	1.565	0.191	0.078
0.041	0.054	0.266	0.024	1.041	0.305	0.300	0.106
0.212	0.030	0.049	0.727	0.324	0.198	0.034	0.049
0.311	0.267	0.253	0.260	0.023	0.852	0.041	0.023
0.058	0.197	0.119	0.032	0.040	0.142	0.081	
0.084	0.098	0.193	0.225	0.089	0.091	0.052	
0.172	0.192	0.348	0.051	0.111	0.111	0.322	
0.182	0.028	3.137	0.170	1.085	1.083	0.110	
0.041	0.044	0.104	0.026	0.029	0.030	0.215	
0.174	0.036	0.022	1.671	2.316	3.011	0.061	
0.826	0.233	0.095	0.119	0.297	0.247	0.168	
0.053	0.056	0.295	0.212	0.045	0.044	0.174	
0.297	0.432	0.224	0.034	0.099	0.229	0.186	

0.043	0.084	0.926	0.514	0.025	0.045	0.082	
2.023	1.438	0.109	0.167	0.028	0.081	0.038	
1.226	0.491	0.414	0.128	1.083	0.224	0.136	
0.053	0.128	0.211	0.027	2.370	1.041	0.049	
0.589	0.406	0.182	0.521	0.767	0.321	0.042	
0.162	3.028	0.225	0.323	0.305	0.023	0.061	
0.589	0.048	0.185	2.283	0.198	0.040	0.020	
1.078	0.050	0.120	0.880	0.052	0.082	0.114	
0.238	0.391	0.087	0.337	0.014	0.038	0.155	
0.173	0.228	1.109	0.073	0.013	0.136	0.229	
0.059	0.086	0.024	0.165	0.050	0.049	0.118	
0.147	0.257	0.025	0.179	0.033	0.042	0.154	
0.283	0.250	0.093	0.660	0.136	0.061	0.356	
0.470	0.186	0.051	0.026	0.020	0.127	0.055	
0.023	0.077	0.022	0.256	0.036	0.304	0.082	
0.120	0.869	0.069	0.018	0.055	0.329	0.086	
0.022	0.143	0.032	0.062	0.082	0.134	0.020	
0.063	0.043	0.026	0.461	0.086	0.040	0.136	

A8

0.038	0.058	0.032	0.134	0.089	0.085	0.024	0.097
0.297	0.205	0.082	0.36	0.034	0.095	0.023	0.13
0.152	0.106	0.053	0.029	0.814	0.036	0.783	0.035
1.148	0.037	1.762	0.136	0.632	0.573	0.092	0.352
0.122	0.198	0.639	0.701	0.919	0.043	0.45	0.05
0.044	0.388	0.038	0.075	0.034	0.425	0.081	0.314
0.022	0.037	0.058	0.047	0.031	0.134	0.079	0.082
2.678	0.144	0.024	0.038	0.176	0.035	0.029	0.081
0.315	0.021	0.024	0.025	0.184	0.042	0.037	0.048
0.042	0.239	0.048	0.059	0.021	0.038	0.186	0.095
0.19	0.092	0.146	0.151	0.041	0.021	0.305	0.536
0.257	0.119	0.071	0.025	0.106	0.303	0.03	0.126
0.076	0.38	0.036	0.266	0.053	0.042	0.065	0.453
0.216	0.124	0.021	0.734	0.025	0.03	0.18	0.091
0.232	0.022	0.235	0.229	0.057	0.185	0.029	0.022
0.072	0.17	0.044	0.54	0.022	0.077	0.366	0.097
0.184	0.186	0.031	0.857	1.494	0.023	0.038	0.138
0.535	0.176	2.52	0.189	0.03	0.026	0.606	0.125
0.215	0.025	0.313	0.483	0.273	0.2	0.077	0.067

0.286	0.125	2.381	0.255	0.11	0.131	0.577	0.063
0.271	0.092	5.106	0.486	0.052	0.029	0.211	0.05
0.107	2.525	0.021	0.033	0.037	0.038	0.438	0.046
0.532	0.283	0.033	1.247	5.988	0.137	0.129	0.043
0.084	1.012	0.022	0.034	0.213	0.222	0.093	0.022
0.037	0.07	0.026	0.062	0.03	0.03	0.034	0.105
0.092	0.441	0.054	0.129	0.364	0.225	0.119	0.031
0.371	0.032	0.382	0.072	0.189	0.027	0.174	0.049
1.37	0.311	0.02	0.133	0.238	1.77	0.04	0.062
0.078	0.322	0.263	0.185	0.027	0.461	0.075	0.042
0.355	0.131	0.121	0.224	0.027	0.096	0.093	0.099
0.522	0.032	0.062	0.565	0.081	0.049	0.03	0.088
0.079	0.208	0.328	0.71	0.021	0.496	0.065	0.236
0.167	0.443	0.637	0.274	0.055	0.075	0.036	0.021
0.283	0.339	0.091	0.725	0.061	0.05	0.03	0.363
0.472	0.846	0.325	0.323	0.191	0.186	0.031	0.054
0.286	0.034	0.315	0.166	0.203	0.053	0.023	0.03
0.645	0.253	0.707	0.652	0.048	0.067	0.021	0.283
0.072	0.021	0.197	0.281	0.021	0.028	0.19	0.16
0.334	0.097	0.215	0.03	0.114	0.022	0.201	0.027
0.277	0.818	1.064	0.179	0.037	0.072	0.481	0.031
0.497	0.142	0.231	0.034	0.67	1.897	0.031	0.042
0.249	0.282	0.782	0.388	0.803	0.046	0.471	0.114
0.246	0.064	0.786	0.067	0.166	0.045	0.198	0.022
0.123	0.53	0.286	0.118	0.282	0.993	0.057	0.035
0.298	0.486	0.535	0.075	0.047	1.892	0.205	0.036
0.103	0.054	0.073	0.143	0.237	0.993	0.245	
0.034	0.053	0.022	0.022	0.705	0.089	0.3	
0.023	0.266	0.391	0.029	0.109	0.158	0.264	
0.188	0.154	0.226	0.025	0.149	0.081	0.44	
0.063	0.239	0.31	0.112	0.034	1.197		

A10

0.028	0.021	0.028	0.466	0.025	0.044	0.199	0.091
0.284	0.074	0.234	0.037	0.278	0.487	0.108	0.11
0.645	0.024	0.184	0.032	0.21	0.037	0.029	0.086
0.041	0.169	0.638	0.14	0.022	0.046	0.037	0.107
0.064	0.228	3.01	0.492	0.025	0.142	0.026	0.052
1.312	1.187	0.323	0.024	0.887	3.665	0.042	0.053

1.154	0.277	0.034	0.166	0.023	0.196	0.03	0.034
0.046	0.021	0.151	0.056	0.033	0.024	0.489	0.125
0.182	0.024	0.026	0.032	0.193	0.038	0.126	0.021
0.039	0.025	0.042	0.049	0.064	0.039	0.157	0.026
0.036	0.054	0.137	0.057	0.022	0.046	0.228	0.034
0.146	0.119	0.04	0.07	0.041	0.161	0.064	0.023
0.092	0.087	0.042	0.087	0.214	0.074	0.023	0.153
0.65	0.08	0.073	0.154	0.858	0.104	0.045	0.27
0.145	1.295	0.078	0.095	0.124	0.148	0.078	0.775
0.144	0.179	1.389	0.027	2.038	0.039	0.059	0.529
0.135	0.855	1.018	0.426	0.074	0.071	0.045	0.443
0.16	0.089	0.192	0.173	0.097	0.074	0.029	0.362
0.025	0.588	0.038	0.457	0.125	0.06	0.023	0.032
0.027	0.507	0.162	0.185	0.187	0.69	0.05	0.218
4.987	0.293	0.086	0.041	0.173	0.061	0.032	0.032
0.6	4.076	0.037	0.304	0.046	0.057	0.039	0.116
0.038	0.407	4.852	0.162	0.597	0.036	0.095	0.126
0.081	0.847	0.055	0.148	0.3	0.022	0.044	0.146
0.61	0.027	0.18	0.028	0.736	0.649	0.024	0.096
0.119	0.137	0.074	0.135	0.038	0.04	0.039	0.035
0.109	0.074	0.495	0.202	0.179	0.262	0.069	0.022
2.18	0.202	0.049	0.03	0.121	0.132	0.047	0.158
0.145	0.053	0.072	0.346	0.393	0.025	0.021	0.249
0.495	0.212	0.021	0.022	0.031	0.158	0.029	0.036
2.241	0.903	0.518	0.051	0.039	0.41	0.022	0.028
0.28	0.209	0.537	1.38	0.13	0.29	0.048	0.402
0.172	0.022	0.301	0.055	0.116	0.195	0.049	0.049
0.831	0.488	0.363	0.022	0.184	0.129	0.194	0.061
0.388	0.999	0.335	0.703	0.194	0.289	0.077	0.03
0.381	0.405	0.508	0.178	0.076	0.044	0.049	0.291
0.051	0.04	0.14	0.271	0.338	0.127	0.044	0.024
0.026	0.06	1.365	0.45	0.547	0.156	0.022	0.105
0.052	0.474	0.034	0.491	0.157	0.117	0.478	0.174
0.765	0.166	0.397	0.143	0.043	0.029	0.072	0.042
0.163	1.523	1.145	0.456	0.117	0.026	0.022	0.022
0.413	0.354	0.746	0.023	0.193	0.266	0.401	0.139
0.279	0.366	0.045	0.232	0.07	0.03	0.461	0.21
0.055	0.095	0.645	0.476	0.106	2.796	0.328	0.1
0.48	0.025	0.055	0.178	0.194	0.198	0.04	0.034
0.064	0.498	0.567	2.427	0.028	0.122	0.116	0.046

0.143	0.451	0.237	0.022	0.812	0.812	0.198	0.049
0.024	0.082	0.798	0.275	0.345	0.762	0.079	0.048
0.26	0.073	0.221	0.177	0.021	0.102	0.035	0.022
0.064	0.043	0.489	0.849	0.473	0.349	0.113	0.027
0.044	0.063	0.04	0.544	0.037	0.042	0.038	0.025
0.07	0.056	0.146	0.022	0.471	0.395	0.055	
0.028	0.054	0.413	0.06	0.207	0.034		
0.021	0.023	0.021	0.432	0.038	0.122		
0.029	0.052	0.071	0.83	0.035	0.783		
0.141	0.035	0.471	2.611	0.029	0.031		
0.202	0.178	0.038	0.835	0.063	0.245		
0.023	0.164	0.021	0.252	0.207	0.081		
0.193	0.231	0.032	0.046	0.122	0.027		
0.173	0.084	0.137	0.653	0.033	0.03		

A12

0.025	0.061	1.36	0.022	0.03	0.025	0.032	0.07
0.069	0.024	0.063	0.407	2.011	0.059	0.053	0.04
0.05	0.45	0.066	0.17	0.091	0.041	0.102	0.02
0.131	4.144	0.221	0.312	1.622	0.033	0.493	0.056
0.064	0.351	0.077	0.103	0.44	0.102	0.061	0.053
0.026	0.25	0.057	0.037	0.057	0.17	0.142	0.05
0.411	0.065	0.096	0.329	0.487	0.04	0.034	0.212
0.04	0.149	0.122	0.061	0.571	0.053	0.125	0.039
0.028	0.174	3.617	0.271	0.061	0.041	0.199	0.03
0.149	0.436	0.026	0.023	0.062	0.073	0.064	0.039
0.022	0.121	0.21	0.261	0.752	0.208	0.03	0.027
0.554	0.031	0.062	0.154	0.141	0.31	0.04	0.214
0.042	0.669	0.055	0.075	0.84	0.066	0.032	0.081
0.034	0.116	0.071	0.556	0.036	0.128	0.358	0.075
2.589	0.107	0.089	0.056	0.087	0.259	0.021	0.02
0.114	0.096	0.029	0.157	0.139	0.616	0.039	0.064
0.031	0.052	0.249	0.031	0.428	0.056	0.03	0.091
0.035	0.075	0.104	2.75	0.044	0.073	0.044	0.038
0.073	0.198	0.581	0.421	0.216	0.157	0.043	0.024
1.006	0.244	0.023	0.024	0.201	0.154	0.339	0.048
0.895	0.197	0.055	0.169	0.054	0.038	0.403	0.032
0.25	0.045	0.072	0.22	0.032	0.786	0.345	0.062
0.038	0.055	0.354	0.388	0.027	0.624	0.033	0.04

0.206	0.087	0.218	0.121	0.029	0.782	0.157	0.028
6.454	0.076	0.033	0.111	0.028	0.043	0.03	0.706
0.037	0.034	0.212	0.062	0.283	0.04	0.023	0.106
1.289	1.235	0.042	0.389	0.08	0.055	0.073	0.041
0.126	2.37	0.934	0.091	0.031	0.555	0.021	0.056
0.036	0.744	0.049	0.722	0.199	0.108	0.061	0.138
1.389	0.653	0.022	0.042	0.134	0.192	0.147	0.037
0.057	0.196	0.04	0.905	0.599	0.027	0.15	0.039
0.114	0.023	0.053	1.388	0.204	0.932	0.669	0.466
0.404	0.56	0.212	0.449	0.275	0.044	0.052	0.088
0.074	0.229	0.099	1.146	0.664	0.402	0.059	0.037
0.146	0.04	2.445	0.194	2.505	0.041	0.113	0.211
0.281	0.717	0.025	0.482	0.794	0.377	0.135	0.045
1.023	0.421	0.968	0.585	1.765	0.194	0.099	0.024
0.047	0.167	0.455	0.107	1.76	0.032	0.034	0.337
0.039	0.057	0.044	1.058	0.211	0.378	0.494	0.023
0.294	0.525	0.186	0.249	1.201	0.049	0.091	0.075
0.063	1.023	0.025	0.043	0.098	0.034	0.074	0.03
0.406	0.394	0.347	0.819	1.583	3.094	0.057	0.039
0.946	1.413	0.15	0.759	0.067	1.318	0.043	0.021
1.146	0.057	0.033	1.602	0.027	0.065	0.05	0.229
0.085	0.036	0.16	0.342	0.027	0.356	0.342	0.031
0.407	0.022	1.244	0.83	0.088	0.075	0.028	0.02
0.992	0.273	0.035	0.031	0.023	0.064	0.089	0.109
0.454	0.171	0.026	0.421	0.029	0.125	0.114	0.074
0.121	0.124	0.196	0.13	0.082	0.258	0.038	0.021
0.029	0.525	0.51	0.103	0.041	0.322	0.03	0.105
0.723	0.293	0.055	0.076	0.225	0.503	0.128	0.044
0.572	0.528	0.064	0.051	0.443	0.104	0.211	0.028
0.026	0.026	0.956	0.037	0.03	0.534	0.035	
0.103	0.359	0.188	0.041	0.067	0.042	0.028	
0.2	0.078	0.273	0.025	0.063	0.036	0.075	
0.101	0.077	0.102	0.33	0.068	0.029	0.102	
0.035	0.1	0.033	0.021	0.039	0.192	0.027	
0.16	0.022	0.128	0.094	0.381	0.203	0.022	
0.028	0.198	0.053	0.046	0.048	0.04		
0.035	0.022	0.203	0.076	0.095	0.117		
0.072	0.133	0.202	0.054	0.062	0.058		

Density

	Thickness (mm)	Length 1 (mm)	Length 2 (mm)	V (mm ³)	V (cm ³)	Weight (g)	Weight (mg)	Density (mg/cm ³)
Bl	6.71	25.33	24.49	4164.49	4.16	0.1690	169.00	40.58
	6.27	24.33	25.74	3928.70	3.93	0.1686	168.60	42.91
	6.11	24.72	25.39	3834.89	3.83	0.1626	162.60	42.40
	5.66	25.40	25.36	3648.00	3.65	0.1475	147.50	40.43
	6.11	25.38	24.60	3816.85	3.82	0.1534	153.40	40.19
	6.34	25.21	25.26	4037.34	4.04	0.1633	163.30	40.45
	6.06	25.10	25.20	3833.07	3.83	0.1559	155.90	40.67
H2	5.41	23.81	26.00	3347.05	3.35	0.1480	148.00	44.22
	4.97	25.08	25.44	3168.91	3.17	0.1317	131.70	41.56
	6.21	26.10	26.42	4282.18	4.28	0.1974	197.40	46.10
	6.24	25.85	25.73	4148.13	4.15	0.1752	175.20	42.24
	6.04	25.52	25.24	3892.66	3.89	0.1779	177.90	45.70
	5.75	24.14	24.95	3461.18	3.46	0.1604	160.40	46.34
	5.61	24.70	25.00	3462.12	3.46	0.1555	155.50	44.91
H4	5.27	26.99	24.63	3501.09	3.50	0.1551	155.10	44.30
	7.05	26.04	26.40	4848.86	4.85	0.2100	210.00	43.31
	6.64	25.84	25.53	4378.18	4.38	0.1924	192.40	43.95
	5.44	24.62	25.37	3397.88	3.40	0.1549	154.90	45.59
	5.08	24.64	25.80	3227.30	3.23	0.1457	145.70	45.15
	5.21	25.30	25.10	3310.62	3.31	0.1513	151.30	45.70
	5.76	24.55	25.06	3543.68	3.54	0.1552	155.20	43.80
H6	6.39	24.98	24.87	3967.73	3.97	0.1637	163.70	41.26
	6.32	25.90	24.75	4051.28	4.05	0.1675	167.50	41.34
	6.33	24.89	25.30	3988.21	3.99	0.1688	168.80	42.32
	6.80	24.77	24.99	4207.15	4.21	0.2012	201.20	47.82
	6.68	24.54	24.52	4017.49	4.02	0.1891	189.10	47.07
	6.63	24.11	25.40	4062.21	4.06	0.1952	195.20	48.05
	6.58	24.20	24.31	3872.99	3.87	0.1774	177.40	45.80
H8	5.61	25.11	25.28	3561.12	3.56	0.1547	154.70	43.44
	6.46	25.20	25.20	4100.24	4.10	0.1870	187.00	45.61
	6.54	25.11	25.47	4182.67	4.18	0.1898	189.80	45.38
	6.36	24.13	25.59	3925.16	3.93	0.1830	183.00	46.62
	6.04	24.69	25.69	3833.20	3.83	0.1772	177.20	46.23
	6.21	23.92	25.50	3789.88	3.79	0.1760	176.00	46.44
	6.02	24.10	23.89	3467.93	3.47	0.1565	156.50	45.13

H10	6.28	26.42	25.04	4154.58	4.15	0.2016	201.60	48.52
	5.51	25.24	24.85	3453.86	3.45	0.1646	164.60	47.66
	5.54	25.04	24.25	3364.00	3.36	0.1532	153.20	45.54
	5.75	24.51	25.47	3591.63	3.59	0.1606	160.60	44.72
	5.85	24.64	24.23	3494.60	3.49	0.1572	157.20	44.98
	5.61	25.51	25.27	3616.42	3.62	0.1720	172.00	47.56
	5.70	25.60	27.40	3995.87	4.00	0.1852	185.20	46.35
H12	6.28	24.30	25.68	3918.87	3.92	0.1895	189.50	48.36
	6.61	24.82	26.79	4395.17	4.40	0.1911	191.10	43.48
	6.35	25.60	24.77	4028.72	4.03	0.1827	182.70	45.35
	6.44	24.61	25.17	3989.15	3.99	0.1790	179.00	44.87
	6.79	24.87	25.41	4290.92	4.29	0.1990	199.00	46.38
	6.79	25.62	25.89	4503.82	4.50	0.1925	192.50	42.74
	6.51	25.46	25.45	4218.20	4.22	0.1889	188.90	44.78
C2	5.81	25.25	25.24	3700.65	3.70	0.1579	157.90	42.67
	6.38	25.26	24.76	3990.29	3.99	0.1692	169.20	42.40
	6.34	25.33	25.49	4093.50	4.09	0.1756	175.60	42.90
	5.08	26.44	26.37	3541.89	3.54	0.1409	140.90	39.78
	5.29	25.14	26.37	3509.17	3.51	0.1365	136.50	38.90
	5.44	25.83	23.99	3368.89	3.37	0.1280	128.00	37.99
	5.56	24.60	25.20	3444.69	3.44	0.1401	140.10	40.67
C4	5.65	25.33	25.51	3648.70	3.65	0.1585	158.50	43.44
	6.14	25.05	24.41	3754.43	3.75	0.1455	145.50	38.75
	6.23	25.33	25.55	4029.78	4.03	0.1695	169.50	42.06
	6.01	24.00	25.46	3670.31	3.67	0.1557	155.70	42.42
	6.36	24.97	25.16	3997.73	4.00	0.1582	158.20	39.57
	6.27	25.39	24.64	3920.49	3.92	0.1616	161.60	41.22
	6.02	24.60	25.50	3774.26	3.77	0.1558	155.80	41.28
C6	5.37	25.47	24.99	3420.10	3.42	0.1420	142.00	41.52
	6.44	25.09	26.01	4200.51	4.20	0.1737	173.70	41.35
	6.42	25.20	25.50	4127.63	4.13	0.1801	180.10	43.63
	7.42	24.52	27.16	4943.67	4.94	0.1971	197.10	39.87
	7.78	26.15	24.26	4933.51	4.93	0.1925	192.50	39.02
	7.19	26.47	26.01	4952.50	4.95	0.2051	205.10	41.41
	6.58	25.70	24.90	4212.87	4.21	0.1752	175.20	41.59
C8	5.39	25.39	27.07	3702.30	3.70	0.1545	154.50	41.73
	6.21	25.91	24.77	3987.66	3.99	0.1708	170.80	42.83
	6.15	25.21	25.47	3948.91	3.95	0.1764	176.40	44.67
	5.94	24.90	25.39	3755.33	3.76	0.1679	167.90	44.71
	6.42	24.36	24.95	3899.93	3.90	0.1648	164.80	42.26

	5.94	25.79	25.70	3934.84	3.93	0.1683	168.30	42.77
	5.91	24.90	25.50	3750.44	3.75	0.1553	155.30	41.41
C10	6.79	25.24	25.48	4364.61	4.36	0.1942	194.20	44.49
	7.19	24.94	25.74	4617.80	4.62	0.1869	186.90	40.47
	7.06	24.20	26.37	4503.24	4.50	0.1983	198.30	44.03
	7.05	25.22	25.62	4557.42	4.56	0.1951	195.10	42.81
	5.97	24.91	25.10	3730.60	3.73	0.1692	169.20	45.35
	5.73	24.91	25.07	3580.43	3.58	0.1511	151.10	42.20
	6.01	24.56	25.80	3808.22	3.81	0.1587	158.70	41.67
C12	5.78	24.99	24.81	3581.54	3.58	0.1728	172.80	48.25
	5.79	25.22	25.27	3692.15	3.69	0.1770	177.00	47.94
	5.83	23.97	24.97	3487.44	3.49	0.1759	175.90	50.44
	7.46	25.08	24.32	4550.19	4.55	0.1882	188.20	41.36
	7.18	25.66	23.78	4381.20	4.38	0.1827	182.70	41.70
	7.37	25.09	24.27	4489.88	4.49	0.1880	188.00	41.87
	6.90	24.90	25.50	4383.27	4.38	0.1954	195.40	44.58
A2	6.01	25.29	25.08	3814.10	3.81	0.1803	180.30	47.27
	6.04	26.38	24.66	3931.37	3.93	0.1572	157.20	39.99
	6.18	25.34	25.53	4000.19	4.00	0.1595	159.50	39.87
	7.49	24.50	26.74	4909.11	4.91	0.2035	203.50	41.45
	7.92	25.62	27.21	5523.52	5.52	0.2196	219.60	39.76
	6.86	24.60	23.70	4001.46	4.00	0.1669	166.90	41.71
	6.58	25.40	25.30	4226.30	4.23	0.1752	175.20	41.45
	6.47	25.44	25.97	4274.58	4.27	0.1774	177.40	41.50
	6.40	24.86	26.78	4258.59	4.26	0.1825	182.50	42.85
	6.60	24.98	26.78	4412.94	4.41	0.1779	177.90	40.31
	6.94	26.35	26.11	4772.42	4.77	0.2135	213.50	44.74
	7.92	25.62	27.21	5523.52	5.52	0.2196	219.60	39.76
	7.01	24.84	26.69	4649.70	4.65	0.2047	204.70	44.02
	6.59	25.10	24.90	4118.68	4.12	0.1665	166.50	40.43
A6	5.58	25.56	24.94	3554.94	3.55	0.1395	139.50	39.24
	6.22	24.57	26.02	3974.39	3.97	0.1536	153.60	38.65
	6.34	24.88	24.63	3885.12	3.89	0.1586	158.60	40.82
	6.28	24.92	25.19	3944.27	3.94	0.1902	190.20	48.22
	6.33	24.46	24.48	3788.29	3.79	0.1729	172.90	45.64
	6.49	24.36	25.89	4091.01	4.09	0.1767	176.70	43.19
	6.47	25.50	26.00	4287.40	4.29	0.1722	172.20	40.16
A8	6.30	24.39	26.00	3997.20	4.00	0.1486	148.60	37.18
	6.00	24.32	26.02	3798.95	3.80	0.1769	176.90	46.57
	5.75	24.91	26.30	3767.01	3.77	0.1638	163.80	43.48

	5.66	25.50	25.07	3616.22	3.62	0.1586	158.60	43.86
	5.59	25.22	25.75	3632.39	3.63	0.1536	153.60	42.29
	5.46	25.78	26.48	3727.29	3.73	0.1554	155.40	41.69
	5.59	24.67	25.50	3516.59	3.52	0.1502	150.20	42.71
A10	6.53	24.90	25.28	4108.35	4.11	0.1751	175.10	42.62
	5.61	24.67	25.36	3507.71	3.51	0.1466	146.60	41.79
	5.57	24.86	26.28	3641.17	3.64	0.1511	151.10	41.50
	5.69	25.21	26.14	3751.85	3.75	0.1505	150.50	40.11
	5.25	26.40	25.65	3552.83	3.55	0.1563	156.30	43.99
	7.08	24.85	26.25	4618.37	4.62	0.2040	204.00	44.17
	6.73	24.55	24.67	4074.00	4.07	0.1744	174.40	42.81
A12	6.16	25.13	23.31	3608.41	3.61	0.1674	167.40	46.39
	5.89	24.73	25.14	3659.81	3.66	0.1612	161.20	44.05
	5.27	25.49	25.57	3432.70	3.43	0.1558	155.80	45.39
	4.86	24.14	25.17	2954.98	2.95	0.1283	128.30	43.42
	5.19	25.00	26.02	3373.93	3.37	0.1536	153.60	45.53
	7.27	24.70	25.01	4493.08	4.49	0.1912	191.20	42.55
	6.46	25.10	25.40	4120.63	4.12	0.1885	188.50	45.75

	Density (mg/cm ³)	SD
Bl	41.09	1.09
H2	44.44	1.89
H4	44.54	0.94
H6	44.81	3.07
H8	45.55	1.08
H10	46.48	1.47
H12	45.14	1.85
C2	40.76	1.96
C4	41.25	1.63
C6	41.20	1.46
C8	42.91	1.32
C10	43.01	1.72
C12	45.16	3.71
A2	41.64	2.62
A4	41.94	1.95
A6	42.28	3.57
A8	42.54	2.84
A10	42.92	1.43
A12	44.72	1.40

Absorption Test

		Time (minutes)								
		0	1	10	30	60	120	240	480	1440
Bl	Wt	22.4491	23.4151	23.5911	23.4934	23.5243	23.7756	23.8376	23.9667	24.0272
	%	0	4.30	5.09	4.65	4.79	5.91	6.19	6.76	7.03
	Wt	22.8825	23.812	23.898	23.9634	24.127	23.9904	24.006	24.0546	24.6063
	%	0	4.06	4.44	4.72	5.44	4.84	4.91	5.12	7.53
	Wt	13.36	14.0988	14.1511	14.2446	14.2422	14.389	14.349	14.4736	14.5391
	%	0	5.53	5.92	6.62	6.60	7.70	7.40	8.34	8.83
	Wt	13.5793	14.284	14.4154	14.7605	14.9467	14.8871	15.1169	15.1321	15.1441
	%	0	5.19	6.16	8.70	10.07	9.63	11.32	11.44	11.52
	Wt	13.4279	14.167	14.9992	14.3607	14.5866	14.8166	15.1021	15.0823	15.0441
	%	0	5.50	11.70	6.95	8.63	10.34	12.47	12.32	12.04
H2	Wt	13.3528	14.1355	14.2474	14.8308	14.8365	15.0831	15.0802	15.0301	14.7678
	%	0	5.86	6.70	11.07	11.11	12.96	12.94	12.56	10.60
	Wt	13.7615	14.5056	14.6231	14.6856	14.8022	15.0832	15.1783	15.4377	15.7404
	%	0	5.41	6.26	6.72	7.56	9.60	10.30	12.18	14.38
	Wt	13.7443	14.6032	14.9049	14.957	15.2369	15.0223	15.5445	15.6662	16.4402
	%	0	6.25	8.44	8.82	10.86	9.30	13.10	13.98	19.61
	Wt	13.3747	14.392	14.4707	14.475	14.5201	14.7158	15.3271	15.4156	15.5986
	%	0	7.61	8.19	8.23	8.56	10.03	14.60	15.26	16.63
	Wt	13.2132	14.1	14.209	14.2881	14.5058	14.9379	15.4911	15.5433	15.6047
	%	0	6.71	7.54	8.14	9.78	13.05	17.24	17.63	18.10
H4	Wt	23.6483	24.7343	24.7533	24.714	26.2778	25.0208	26.2106	26.146	26.2245
	%	0	4.59	4.67	4.51	11.12	5.80	10.84	10.56	10.89
	Wt	22.5885	23.4117	23.773	23.6672	23.69	24.1531	24.3109	24.4029	23.744
	%	0	3.64	5.24	4.78	4.88	6.93	7.63	8.03	5.12
	Wt	13.8574	14.8093	14.8373	15.2642	15.5742	15.8595	16.1223	16.3858	16.2396
	%	0	6.87	7.07	10.15	12.39	14.45	16.34	18.25	17.19
	Wt	13.7202	14.2597	14.6782	14.7916	15.0006	15.405	15.3104	15.1956	15.1002
	%	0	3.93	6.98	7.81	9.33	12.28	11.59	10.75	10.06
	Wt	13.223	14.0737	14.236	14.374	14.3722	14.4828	15.0396	15.3337	15.407
	%	0	6.43	7.66	8.70	8.69	9.53	13.74	15.96	16.52
Wt	13.8909	14.819	14.8303	15.0036	14.9605	15.5676	15.7073	15.782	15.7654	
%	0	6.68	6.76	8.01	7.70	12.07	13.08	13.61	13.49	
Wt	25.2273	26.4038	26.5451	26.8602	27.4602	27.9276	28.3596	28.7842	29.0256	
%	0	4.66	5.22	6.47	8.85	10.70	12.42	14.10	15.06	
Wt	22.7097	23.4557	23.5876	23.5926	24.78	24.9981	25.3366	25.396	25.7698	
%	0	3.28	3.87	3.89	9.12	10.08	11.57	11.83	13.47	

H6	Wt	22.55	23.3999	23.5683	23.6141	23.7544	24.1634	24.4767	24.8031	25.2338
	%	0	3.77	4.52	4.72	5.34	7.15	8.54	9.99	11.90
	Wt	13.2436	14.0476	14.2147	14.6418	14.7635	15.3951	15.4712	15.549	15.9189
	%	0	6.07	7.33	10.56	11.48	16.25	16.82	17.41	20.20
	Wt	13.6349	14.5671	14.5021	14.6742	15.2865	15.8822	16.0782	16.3326	16.4019
	%	0	6.84	6.36	7.62	12.11	16.48	17.92	19.79	20.29
	Wt	13.3959	14.01	14.2967	14.5282	14.5939	16.1504	16.1795	16.2021	16.1856
	%	0	4.58	6.72	8.45	8.94	20.56	20.78	20.95	20.83
	Wt	13.2626	13.8828	14.0834	14.514	14.3347	14.6034	14.9075	15.7788	15.7487
	%	0	4.68	6.19	9.44	8.08	10.11	12.40	18.97	18.75
	Wt	13.9398	14.613	14.6975	15.0549	15.3272	15.5239	16.419	16.3291	15.8155
	%	0	4.83	5.44	8.00	9.95	11.36	17.79	17.14	13.46
H8	Wt	13.7883	14.7324	14.758	14.7797	15.0366	15.2694	15.3838	15.7044	15.9958
	%	0	6.85	7.03	7.19	9.05	10.74	11.57	13.90	16.01
	Wt	13.3648	14.2776	14.4257	14.4305	14.9782	15.0216	15.2623	15.1302	15.4747
	%	0	6.83	7.94	7.97	12.07	12.40	14.20	13.21	15.79
	Wt	13.625	14.4706	14.4891	14.6222	14.9339	15.47	15.5903	15.418	15.6074
	%	0	6.21	6.34	7.32	9.61	13.54	14.42	13.16	14.55
	Wt	13.9795	14.1423	15.0645	15.0065	16.2581	16.0968	16.5334	16.5126	16.5334
	%	0	1.16	7.76	7.35	16.30	15.15	18.27	18.12	18.27
	Wt	13.7866	14.8263	14.698	14.9859	15.8892	16.8852	17.0018	17.0614	17.0845
	%	0	7.54	6.61	8.70	15.25	22.48	23.32	23.75	23.92
	Wt	13.3706	13.9898	14.0775	15.472	15.0406	15.3205	15.1173	15.1372	14.5296
	%	0	4.63	5.29	15.72	12.49	14.58	13.06	13.21	8.67
H10	Wt	13.2427	14.0025	14.2725	14.3545	14.5195	15.2375	15.3345	15.5685	15.3276
	%	0	5.74	7.78	8.40	9.64	15.06	15.80	17.56	15.74
	Wt	13.9159	14.927	15.3052	15.5238	15.8868	15.8839	15.8754	15.9171	15.8452
	%	0	7.27	9.98	11.55	14.16	14.14	14.08	14.38	13.86
	Wt	13.226	14.0023	14.0527	14.0987	14.5515	15.441	15.6456	15.5751	15.6073
	%	0	5.87	6.25	6.60	10.02	16.75	18.29	17.76	18.00
	Wt	13.7691	14.599	14.7503	14.8124	15.1507	16.2892	16.2994	16.3462	16.7168
	%	0	6.03	7.13	7.58	10.03	18.30	18.38	18.72	21.41
	Wt	25.5024	26.3138	26.3213	26.3586	26.8725	27.58363	28.5802	28.8859	28.9835
	%	0	3.18	3.21	3.36	5.37	8.16	12.07	13.27	13.65
	Wt	22.624	24.1838	24.7264	24.9543	25.2171	25.7786	25.8577	24.8891	24.5666
	%	0	6.89	9.29	10.30	11.46	13.94	14.29	10.01	8.59
H12	Wt	13.6906	14.6172	15.8675	16.027	16.0035	16.0109	16.1645	16.1565	16.2182
	%	0	6.77	15.90	17.07	16.89	16.95	18.07	18.01	18.46
	Wt	13.4393	14.4558	14.5411	14.5524	15.6061	15.9879	16.0924	16.0179	16.1005
	%	0	7.56	8.20	8.28	16.12	18.96	19.74	19.19	19.80
	Wt	25.2642	26.4444	26.4728	27.7335	28.5832	28.7691	29.1547	29.0197	28.9894
%	0	4.67	4.78	9.77	13.14	13.87	15.40	14.86	14.74	

	Wt	25.7689	26.3989	26.9673	27.5229	27.8115	28.724	28.7578	28.8331	28.9758
	%	0	2.44	4.65	6.81	7.93	11.47	11.60	11.89	12.44
	Wt	25.7055	27.258	27.3754	27.4666	27.9041	28.5828	28.756	28.9117	28.9447
	%	0	6.04	6.50	6.85	8.55	11.19	11.87	12.47	12.60
	Wt	26.072	27.1483	27.3143	27.5853	27.997	28.3583	28.4095	28.5728	28.6693
	%	0	4.13	4.76	5.80	7.38	8.77	8.97	9.59	9.96
C2	Wt	13.6257	14.5611	14.6615	14.8022	14.9407	14.9145	15.0932	15.1525	15.4345
	%	0	6.86	7.60	8.63	9.65	9.46	10.77	11.21	13.27
	Wt	13.6166	14.4645	14.7814	14.811	14.9937	14.9159	15.1915	15.2311	15.6186
	%	0	6.23	8.55	8.77	10.11	9.54	11.57	11.86	14.70
	Wt	22.4454	23.4794	23.4146	23.8663	23.9575	24.0354	24.7633	24.9744	24.9152
	%	0	4.61	4.32	6.33	6.74	7.08	10.33	11.27	11.00
	Wt	13.506	14.305	14.3952	14.4591	14.6536	14.5662	14.7996	14.8695	14.9012
	%	0	5.92	6.58	7.06	8.50	7.85	9.58	10.10	10.33
	Wt	22.8483	23.8415	23.9999	24.0406	24.2832	24.5855	24.721	25.8277	25.3677
	%	0	4.35	5.04	5.22	6.28	7.60	8.20	13.04	11.03
	Wt	13.529	14.2658	14.4428	14.6225	14.692	14.7276	14.6211	14.6996	14.8489
	%	0	5.45	6.75	8.08	8.60	8.86	8.07	8.65	9.76
C4	Wt	13.1719	14.0222	14.0129	14.1278	14.2206	14.1251	14.2879	14.3302	14.7826
	%	0	6.46	6.38	7.26	7.96	7.24	8.47	8.79	12.23
	Wt	13.4552	14.2982	14.4647	14.4335	14.6268	14.8871	15.163	15.2446	15.2565
	%	0	6.27	7.50	7.27	8.71	10.64	12.69	13.30	13.39
	Wt	13.8103	14.574	14.7773	14.8473	14.959	15.315	15.551	15.985	15.8026
	%	0	5.53	7.00	7.51	8.32	10.90	12.60	15.75	14.43
	Wt	22.5487	23.5538	23.6947	23.8025	24.3082	24.8644	24.8778	25.3647	25.5115
	%	0	4.46	5.08	5.56	7.80	10.27	10.33	12.49	13.14
	Wt	25.379	26.3518	26.4586	26.6711	27.0005	27.1117	27.0526	27.3414	27.5547
	%	0	3.83	4.25	5.09	6.39	6.83	6.59	7.73	8.57
	Wt	13.9464	14.754	14.9165	14.9634	15.0466	15.2285	15.277	15.7688	15.43
	%	0	5.79	6.96	7.29	7.89	9.19	9.54	13.07	10.64
C6	Wt	13.3276	14.3012	14.2808	14.3295	14.3585	14.5147	14.7051	14.74	15.1147
	%	0	7.31	7.15	7.52	7.74	8.91	10.34	10.60	13.41
	Wt	17.7391	18.6713	18.75	18.8084	18.8454	19.071	19.2426	19.3427	19.4693
	%	0	5.26	5.70	6.03	6.24	7.51	8.48	9.04	9.75
	Wt	22.5788	23.4043	23.6044	23.1515	23.903	24.1092	24.4281	24.6247	24.5122
	%	0	3.66	4.54	2.54	5.86	6.78	8.19	9.06	8.56
	Wt	13.3719	14.3506	14.2867	14.4145	14.6071	14.732	15.0227	15.2129	15.2001
	%	0	7.32	6.84	7.80	9.24	10.17	12.35	13.77	13.67
	Wt	17.7561	18.6498	18.7031	18.8129	19.0574	19.1782	19.4265	20.1365	20.1045
	%	0	5.03	5.33	5.95	7.33	8.01	9.41	13.41	13.23
	Wt	13.4871	14.2002	14.448	14.5988	14.7174	14.7943	15.5544	15.5774	15.6933
	%	0	5.29	7.12	8.24	9.12	9.69	15.33	15.50	16.36

C8	Wt	13.5902	14.501	14.6818	14.7802	14.8857	15.2109	15.701	15.9584	16.1005
	%	0	6.70	8.03	8.76	9.53	11.93	15.53	17.43	18.47
	Wt	23.8795	24.8979	25.0633	25.2317	25.3183	25.6005	26.2024	26.5484	26.9989
	%	0	4.26	4.96	5.66	6.03	7.21	9.73	11.18	13.06
	Wt	13.6129	14.407	14.556	14.6479	14.7523	14.9654	15.104	15.5016	15.9884
	%	0	5.83	6.93	7.60	8.37	9.94	10.95	13.87	17.45
	Wt	13.876	14.8646	14.9233	15.1947	15.2589	15.318	15.3848	15.5832	15.7987
	%	0	7.12	7.55	9.50	9.97	10.39	10.87	12.30	13.86
	Wt	23.8749	24.959	24.902	24.9288	25.3212	25.3975	25.6226	25.5879	25.8879
	%	0	4.54	4.30	4.41	6.06	6.38	7.32	7.17	8.43
	Wt	13.4905	14.2795	14.6072	14.5795	14.9059	15.0275	15.2452	15.892	14.8604
	%	0	5.85	8.28	8.07	10.49	11.39	13.01	17.80	10.15
C10	Wt	13.518	14.2678	14.7205	14.5322	14.6164	15.186	15.2247	15.5133	15.5592
	%	0	5.55	8.90	7.50	8.13	12.34	12.63	14.76	15.10
	Wt	22.6541	23.5465	23.6708	24.155	24.1192	24.5551	24.7846	25.0948	25.2802
	%	0	3.94	4.49	6.63	6.47	8.39	9.40	10.77	11.59
	Wt	13.7042	14.6147	14.8127	14.916	15.5717	15.672	16.2645	16.6176	16.7866
	%	0	6.64	8.09	8.84	13.63	14.36	18.68	21.26	22.49
	Wt	13.3712	14.2887	14.3784	14.7406	15.0113	14.999	15.5001	15.6171	15.8599
	%	0	6.86	7.53	10.24	12.27	12.17	15.92	16.80	18.61
	Wt	22.8095	24.1006	24.6233	24.7167	24.8361	24.9972	25.1083	25.2425	25.3931
	%	0	5.66	7.95	8.36	8.88	9.59	10.08	10.67	11.33
	Wt	13.8686	14.6423	14.8343	14.9508	15.0506	15.3894	15.565	15.9929	15.6873
	%	0	5.58	6.96	7.80	8.52	10.97	12.23	15.32	13.11
C12	Wt	13.6085	14.4082	14.5647	14.7542	14.8771	14.9962	15.0078	15.3728	15.5886
	%	0	5.88	7.03	8.42	9.32	10.20	10.28	12.96	14.55
	Wt	13.9131	14.7581	14.9765	15.051	15.4774	15.6885	15.8784	16.5872	16.9925
	%	0	6.07	7.64	8.18	11.24	12.76	14.13	19.22	22.13
	Wt	23.641	25.1835	25.36	25.5946	25.8805	26.7436	26.9775	26.9887	26.8469
	%	0	6.52	7.27	8.26	9.47	13.12	14.11	14.16	13.56
	Wt	22.899	23.9763	24.2519	24.371	24.965	24.7805	25.0012	26.11	26.15
	%	0	4.70	5.91	6.43	9.02	8.22	9.18	14.02	14.20
	Wt	23.7913	24.3537	24.7466	24.8143	25.4046	25.5825	25.7239	26.4368	25.2643
	%	0	2.36	4.02	4.30	6.78	7.53	8.12	11.12	6.19
	Wt	14.0667	14.8292	15.083	15.5421	15.8142	15.9306	16.14	16.5364	16.8003
	%	0	5.42	7.22	10.49	12.42	13.25	14.74	17.56	19.43
A2	Wt	13.9806	14.8666	14.9236	14.9476	15.078	15.1134	15.0166	15.2072	15.8692
	%	0	6.34	6.75	6.92	7.85	8.10	7.41	8.77	13.51
	Wt	25.7516	26.726	26.9317	27.3247	27.5039	27.5959	27.9781	28.0726	28.1205
	%	0	3.78	4.58	6.11	6.80	7.16	8.65	9.01	9.20
	Wt	13.7715	14.4816	14.6085	14.6652	14.8283	15.0375	15.3301	15.6738	15.5566
%	0	5.16	6.08	6.49	7.67	9.19	11.32	13.81	12.96	

	Wt	13.8329	14.5772	14.788	14.885	15.2264	15.3261	15.4217	15.5815	15.5800
	%	0	5.38	6.90	7.61	10.07	10.79	11.49	12.64	12.63
	Wt	13.7254	14.534	14.915	15.0615	15.4314	15.7273	15.963	16.0049	15.9978
	%	0	5.89	8.67	9.73	12.43	14.59	16.30	16.61	16.56
	Wt	13.9193	14.5095	14.7853	15.009	15.3086	15.3232	15.6105	15.6193	16.0081
	%	0	4.24	6.22	7.83	9.98	10.09	12.15	12.21	15.01
A4	Wt	13.5172	14.3221	14.4251	14.5055	14.5952	14.6247	14.8015	15.1642	15.3823
	%	0	5.95	6.72	7.31	7.98	8.19	9.50	12.18	13.80
	Wt	25.4051	26.3595	26.4269	26.8192	27.0904	27.3712	27.571	27.8376	28.9591
	%	0	3.76	4.02	5.57	6.63	9.57	13.99	14.03	13.99
	Wt	13.978	14.9845	15.0126	15.1035	15.3035	15.3226	15.6055	15.8442	15.8024
	%	0	7.20	7.40	8.05	9.48	9.62	11.64	13.35	13.05
	Wt	14.0644	14.4963	14.6828	14.8017	15.3002	15.192	15.3061	15.3312	15.3521
	%	0	3.07	4.40	5.24	8.79	8.02	8.83	9.01	9.16
	Wt	13.6442	14.6248	14.6186	14.8409	15.2869	15.1564	15.3734	16.0816	15.9988
	%	0	7.19	7.14	8.77	12.04	11.08	12.67	17.86	17.26
	Wt	14.1374	14.893	15.132	15.2085	15.5772	16.2115	16.5091	16.5105	16.519
	%	0	5.34	7.04	7.58	10.18	14.67	16.78	16.79	16.85
A6	Wt	13.3375	14.0423	14.306	14.3626	14.7667	14.7939	14.8966	15.4012	15.476
	%	0	5.28	7.26	7.69	10.72	10.92	11.69	15.47	16.03
	Wt	23.6215	24.5647	24.9801	25.2794	25.5285	26.3286	26.7443	27.2994	27.587
	%	0	3.99	5.75	7.02	8.07	11.46	13.22	15.57	16.79
	Wt	13.3457	14.3188	14.2783	14.4081	14.5153	14.6512	15.12	15.3335	15.4244
	%	0	7.29	6.99	7.96	8.76	9.78	13.29	14.89	15.58
	Wt	13.6554	14.3618	14.4602	14.6173	15.1099	15.1754	15.3076	15.5204	15.6345
	%	0	5.17	5.89	7.04	10.65	11.13	12.10	13.66	14.49
	Wt	25.7634	27.1831	27.9012	27.9246	28.1004	29.3535	29.365	30.157	30.2947
	%	0	5.51	8.30	8.39	9.07	13.93	13.98	17.05	17.59
	Wt	13.6698	14.5379	14.485	14.6613	15.0666	15.1705	15.3232	15.8018	15.8168
	%	0	6.35	5.96	7.25	10.22	10.98	12.10	15.60	15.71
A8	Wt	13.3262	14.0851	14.2013	14.306	14.7176	14.8992	14.9582	15.3191	15.5943
	%	0	5.69	6.57	7.35	10.44	11.80	12.25	14.95	17.02
	Wt	25.2623	26.2268	26.3885	26.5585	27.1562	27.3155	27.5059	27.7778	27.8072
	%	0	3.82	4.46	5.13	7.50	8.13	8.88	9.96	10.07
	Wt	13.8745	14.7065	14.7369	14.8907	14.975	15.2083	15.2302	15.443	15.5295
	%	0	6.00	6.22	7.32	7.93	9.61	9.77	11.30	11.93
	Wt	22.4391	23.6009	23.5809	23.7997	24.2275	24.4505	24.378	24.5107	24.3378
	%	0	5.18	5.09	6.06	7.97	8.96	8.64	9.23	8.46
	Wt	13.3305	14.207	14.2044	14.4002	14.8727	15.395	15.7814	15.9107	15.9213
	%	0	6.58	6.56	8.02	11.57	15.49	18.39	19.36	19.44
	Wt	13.678	14.6869	14.6896	14.7125	15.17	15.3332	15.6634	15.6503	15.7797
	%	0	7.38	7.40	7.56	10.91	12.10	14.52	14.42	15.37

A10	Wt	14.1907	14.9755	15.2431	15.2272	15.7371	15.9796	16.0014	16.3762	16.304
	%	0	5.53	7.42	7.30	10.90	12.61	12.76	15.40	14.89
	Wt	13.8466	14.8801	14.9422	15.1175	15.3515	15.6408	15.5489	15.5135	15.4681
	%	0	7.46	7.91	9.18	10.87	12.96	12.29	12.04	11.71
	Wt	13.4252	14.5036	14.4412	14.6316	14.9317	15.0064	15.1145	15.6832	15.685
	%	0	8.03	7.57	8.99	11.22	11.78	12.58	16.82	16.83
	Wt	13.5961	14.6118	14.8984	14.9669	15.1906	15.507	15.5545	15.4676	15.3938
	%	0	7.47	9.58	10.08	11.73	14.05	14.40	13.76	13.22
	Wt	23.0725	23.8607	24.0792	24.1802	24.2215	24.2706	24.8263	24.8408	24.723
	%	0	3.42	4.36	4.80	4.98	5.19	7.60	7.66	7.15
	Wt	13.8362	14.6309	14.9973	14.9473	15.0555	15.606	15.9051	16.2208	16.4255
	%	0	5.74	8.39	8.03	8.81	12.79	14.95	17.23	18.71
A12	Wt	13.6874	14.6607	14.8759	14.9984	15.1193	15.5201	15.6933	15.8716	15.7653
	%	0	7.11	8.68	9.58	10.46	13.39	14.66	15.96	15.18
	Wt	22.4889	23.5349	23.7976	23.9963	24.2076	24.2092	24.3351	24.4274	24.8275
	%	0	4.65	5.82	6.70	7.64	7.65	8.21	8.62	10.40
	Wt	23.8631	25.5652	25.7112	26.076	26.5045	27.3508	27.4467	27.6719	27.1733
	%	0	7.13	7.74	9.27	11.07	14.62	15.02	15.96	13.87
	Wt	25.3486	26.6686	26.8097	26.8511	27.0085	27.3852	27.3545	27.1852	27.4542
	%	0	5.21	5.76	5.93	6.55	8.03	7.91	7.25	8.31
	Wt	24.0825	24.979	24.9548	25.4725	25.5851	25.9166	25.9176	25.9584	26.2501
	%	0	3.72	3.62	5.77	6.24	7.62	7.62	7.79	9.00
	Wt	14.282	15.3675	15.4724	15.466	15.9481	16.3584	16.788	16.8909	17.0469
	%	0	7.60	8.33	8.29	11.67	14.54	17.55	18.27	19.36

	Time (minutes)								
	0	1	10	30	60	120	240	480	1440
Bl	0	5.08	6.67	7.12	7.77	8.56	9.20	9.42	9.59
SD	0	0.73	2.59	2.46	2.56	3.01	3.46	3.13	2.10
H2	0	5.70	6.73	6.86	8.79	9.12	12.28	12.94	14.12
SD	0	1.45	1.57	1.86	2.35	2.54	3.42	3.43	5.36
H4	0	5.31	6.26	7.51	9.35	11.52	13.12	14.08	14.30
SD	0	1.55	1.43	2.14	1.59	1.80	1.79	2.73	2.58
H6	0	5.13	6.09	8.13	9.32	13.65	15.71	17.37	17.57
SD	0	1.12	0.99	1.98	2.46	4.95	4.44	3.89	3.88
H8	0	5.54	6.83	9.04	12.46	14.81	15.81	15.89	16.20
SD	0	2.36	0.98	3.32	2.91	4.07	4.30	4.30	4.97
H10	0	5.83	7.27	7.96	10.12	14.39	15.48	15.28	15.21
SD	0	1.43	2.42	2.89	2.86	3.48	2.51	3.34	4.35
H12	0	5.27	7.47	9.10	11.67	13.54	14.27	14.34	14.67
SD	0	1.88	4.36	4.14	4.27	3.84	4.16	3.72	3.80

C2	0	5.57	6.48	7.35	8.31	8.40	9.75	11.02	11.68
SD	0	0.97	1.57	1.41	1.53	1.03	1.41	1.51	1.90
C4	0	5.39	6.20	6.66	7.84	9.18	10.04	11.85	12.07
SD	0	1.04	1.26	1.05	0.79	1.77	2.38	3.02	2.13
C6	0	5.64	6.12	6.35	7.59	8.51	10.68	11.90	12.50
SD	0	1.43	1.08	2.09	1.41	1.31	2.73	2.71	2.85
C8	0	5.72	6.67	7.34	8.41	9.54	11.24	13.29	13.57
SD	0	1.14	1.66	1.93	1.96	2.26	2.81	4.02	3.94
C10	0	5.71	7.32	8.23	9.65	11.30	13.16	14.93	15.37
SD	0	1.04	1.53	1.24	2.72	2.13	3.55	3.98	4.41
C12	0	5.16	6.51	7.68	9.71	10.85	11.76	14.84	15.01
SD	0	1.50	1.36	2.10	1.95	2.57	2.90	3.00	5.50
A2	0	5.13	6.53	7.45	9.14	9.99	11.22	12.18	13.31
SD	0	0.97	1.33	1.30	2.08	2.61	3.10	2.97	2.49
A4	0	5.42	6.12	7.09	9.18	10.19	12.24	13.87	14.02
SD	0	1.72	1.50	1.40	1.86	2.46	2.94	3.20	2.94
A6	0	5.60	6.69	7.56	9.58	11.37	12.73	15.37	16.03
SD	0	1.12	1.00	0.55	1.10	1.38	0.89	1.10	1.07
A8	0	5.77	6.05	6.91	9.39	11.02	12.07	13.20	13.71
SD	0	1.22	1.08	1.09	1.78	2.69	3.82	3.80	4.25
A10	0	6.28	7.54	8.06	9.75	11.56	12.43	13.82	13.75
SD	0	1.73	1.74	1.87	2.54	3.21	2.60	3.58	4.08
A12	0	5.90	6.66	7.59	8.94	10.97	11.83	12.31	12.69
SD	0	1.59	1.94	1.68	2.41	3.54	4.40	4.94	4.25

Desorption Test

		Time (minutes)							
		0	30	60	120	240	480	1440	2880
Bl	Wt	2.0561	1.9938	1.9304	2	1.4734	1.1054	0.4988	0.3514
	%	0	-3.03	-6.11	-11.02	-28.34	-46.24	-75.74	-82.91
	Wt	1.6325	1.6094	1.5868	1.481	1.1277	0.9894	0.4765	0.3055
	%	0	-1.42	-2.80	-9.28	-30.92	-39.39	-70.81	-81.29
	Wt	1.6389	1.58932	1.525	1.3789	1.0904	1.0099	0.4225	0.3172
	%	0	-3.03	-6.95	-15.86	-33.47	-38.38	-74.22	-80.65
	Wt	1.6468	1.58	1.5485	1.3773	1.1544	1.0003	0.5161	0.3189
	%	0	-4.06	-5.97	-16.37	-29.90	-39.26	-68.66	-80.64
	Wt	1.5932	1.5055	1.4387	1.3763	1.2818	1.0445	0.3444	0.2263
	%	0	-5.50	-9.70	-13.61	-19.55	-34.44	-78.38	-85.80
	Wt	1.9685	1.8601	1.7647	1.5157	1.0478	1.1023	0.5145	0.3248
	%	0	-5.51	-10.35	-23.00	-46.77	-44.00	-73.86	-83.50
H2	Wt	1.854	1.7985	1.7359	1.6408	1.4063	1.0432	0.4546	0.4054
	%	0	-2.99	-6.37	-11.50	-24.15	-43.73	-75.48	-78.13
	Wt	1.3205	1.2722	1.2243	1.127	0.7889	0.7531	0.4044	0.2534
	%	0	-3.66	-7.29	-14.65	-40.26	-42.97	-69.38	-80.81
	Wt	2.5689	2.4918	2.4353	2.2836	2.0008	1.7354	0.8819	0.468
	%	0	-3.00	-5.20	-11.11	-22.11	-32.45	-65.67	-81.78
	Wt	2.6671	2.608	2.4587	2.3643	2.1226	1.843	1.0454	0.6058
	%	0	-2.22	-7.81	-11.35	-20.42	-30.90	-60.80	-77.29
	Wt	1.1639	1.1275	1.0913	1.0544	0.9162	0.7457	0.4179	0.3088
	%	0	-3.13	-6.24	-9.41	-21.28	-35.93	-64.09	-73.47
	Wt	2.1952	2.094	1.997	1.866	1.7745	1.2309	0.6011	0.3699
	%	0	-4.61	-9.03	-15.00	-19.16	-43.93	-72.62	-83.15
H4	Wt	1.9024	1.8392	1.7771	1.6924	1.4692	1.132	0.6266	0.4026
	%	0	-0.80	-4.15	-8.72	-20.76	-38.94	-66.20	-78.28
	Wt	2.624	2.5617	2.5004	2.3818	1.9533	1.6456	0.6435	0.5425
	%	0	-2.37	-4.71	-9.23	-25.56	-37.29	-75.48	-79.33
	Wt	1.8006	1.7316	1.6833	1.5622	1.354	1.1591	0.7472	0.4138
	%	0	-3.83	-6.51	-13.24	-24.80	-35.63	-58.50	-77.02
	Wt	2.1105	2.059	1.9322	1.8591	1.6802	1.4648	0.812	0.4566
	%	0	-2.44	-8.45	-11.91	-20.39	-30.59	-61.53	-78.37
	Wt	2.2114	2.1214	2.0231	1.8978	1.6004	1.1374	0.7369	0.4372
	%	0	-4.07	-8.51	-14.18	-27.63	-48.57	-66.68	-80.23
	Wt	2.7065	2.5869	2.4789	2.352	2.1345	1.6316	0.596	0.479
	%	0	-4.42	-8.41	-13.10	-21.13	-39.72	-77.98	-82.30
H6	Wt	2.1035	2.0441	1.9891	1.9028	1.6725	1.3353	0.6759	0.3969
	%	0	-2.82	-5.44	-9.54	-20.49	-36.52	-67.87	-81.13
	Wt	2.1015	2.0486	1.9979	1.9115	1.5853	1.3221	0.2911	0.29

	%	0	-2.52	-4.93	-9.04	-24.56	-37.09	-86.15	-86.20
	Wt	2.6722	2.5974	2.541	2.3988	2.1415	1.8823	1.1126	0.3604
	%	0	-2.80	-4.91	-10.23	-19.86	-29.56	-58.36	-86.51
	Wt	1.84	1.8254	1.7887	1.6686	1.5983	1.429	1.2193	0.6135
	%	0	-0.79	-2.79	-9.32	-13.14	-22.34	-33.73	-66.66
	Wt	1.8362	1.7607	1.674	1.5666	1.3173	0.9507	0.4094	0.371
	%	0	-4.11	-8.83	-14.68	-28.26	-48.22	-77.70	-79.80
	Wt	2.5504	2.4402	2.3439	2.2266	2.0114	1.6603	0.3704	0.3457
	%	0	-4.32	-8.10	-12.70	-21.13	-34.90	-85.48	-86.45
H8	Wt	2.0306	1.9746	1.9127	1.8248	1.5752	1.1888	0.5152	0.3252
	%	0	-2.76	-5.81	-10.13	-22.43	-41.46	-74.63	-83.99
	Wt	2.3372	2.2798	2.2231	2.1225	1.7548	1.4692	0.6156	0.4138
	%	0	-2.46	-4.88	-9.19	-24.92	-37.14	-73.66	-82.30
	Wt	2.713	2.6386	2.5809	2.439	2.1796	1.9247	1.1275	0.3473
	%	0	-2.74	-4.87	-10.10	-19.66	-29.06	-58.44	-87.20
	Wt	2.1564	2.1015	1.9742	1.8011	1.7178	1.4832	0.803	0.346
	%	0	-2.55	-8.45	-16.48	-20.34	-31.22	-62.76	-83.95
	Wt	1.746	1.6712	1.581	1.4682	1.2035	0.8239	0.3631	0.3605
	%	0	-4.28	-9.45	-15.91	-31.07	-52.81	-79.20	-79.35
	Wt	2.4584	2.3423	2.2412	2.1145	1.9886	1.4321	0.4048	0.3748
	%	0	-4.72	-8.84	-13.99	-19.11	-41.75	-83.53	-84.75
H10	Wt	2.0032	1.9016	1.8124	1.673	1.5135	1.1251	0.4876	0.3542
	%	0	-5.07	-9.52	-16.48	-24.45	-43.83	-75.66	-82.32
	Wt	1.6423	1.554	1.4759	1.3561	1.1679	0.9206	0.5173	0.4238
	%	0	-5.38	-10.13	-17.43	-28.89	-43.94	-68.50	-74.19
	Wt	1.632	1.5562	1.4778	1.362	1.1908	0.9233	0.3345	0.3256
	%	0	-4.64	-9.45	-16.54	-27.03	-43.43	-79.50	-80.05
	Wt	2.0196	1.9412	1.8543	1.716	1.4793	1.1879	0.7378	0.3491
	%	0	-3.88	-8.18	-15.03	-26.75	-41.18	-63.47	-82.71
	Wt	1.8394	1.7723	1.7359	1.6559	1.5069	1.3014	0.7158	0.4772
	%	0	-3.65	-5.63	-9.98	-18.08	-29.25	-61.09	-74.06
	Wt	1.264	1.2013	1.1447	1.0158	0.903	0.7123	0.4602	0.3501
	%	0	-4.96	-9.44	-19.64	-28.56	-43.65	-63.59	-72.30
H12	Wt	1.8525	1.7514	1.6644	1.5312	1.3042	1.1298	0.4733	0.3984
	%	0	-5.46	-10.15	-17.34	-29.60	-39.01	-74.45	-78.49
	Wt	1.9643	1.8653	1.779	1.649	1.4305	1.2402	0.4091	0.3618
	%	0	-5.04	-9.43	-16.05	-27.18	-36.86	-79.17	-81.58
	Wt	1.3968	1.3275	1.2575	1.1505	0.9819	0.8724	0.4344	0.3154
	%	0	-4.96	-9.97	-17.63	-29.70	-37.54	-68.90	-77.42
	Wt	2.2088	2.1424	2.1048	1.9514	1.6682	1.3375	0.808	0.3596
	%	0	-3.01	-4.71	-11.65	-24.47	-39.45	-63.42	-83.72
	Wt	2.574	2.501	2.4224	2.2827	2.0163	1.5925	0.6595	0.5795

	%	0	-2.84	-5.89	-11.32	-21.67	-38.13	-74.38	-77.49
	Wt	2.6855	2.5813	2.5479	2.3856	2.1036	1.6302	0.5736	0.465
	%	0	-3.88	-5.12	-11.17	-21.67	-39.30	-78.64	-82.68
C2	Wt	1.802	1.7482	1.6755	1.5855	1.3471	0.9856	0.4293	0.3183
	%	0	-2.99	-7.02	-12.01	-25.24	-45.31	-76.18	-82.34
	Wt	1.6474	1.5885	1.539	1.4465	1.2331	1.1402	0.3834	0.3029
	%	0	-3.58	-6.58	-12.19	-25.15	-30.79	-76.73	-81.61
	Wt	1.6615	1.5866	1.5296	1.3894	1.2206	0.8623	0.3275	0.3164
	%	0	-4.51	-7.94	-16.38	-26.54	-48.10	-80.29	-80.96
	Wt	1.4495	1.3965	1.2725	1.2034	1.0266	0.823	0.4809	0.2853
	%	0	-3.66	-12.21	-16.98	-29.18	-43.22	-66.82	-80.32
	Wt	1.5021	1.4282	1.329	1.2091	1.0388	0.893	0.5505	0.301
	%	0	-4.92	-11.52	-19.51	-30.84	-40.55	-63.35	-79.96
	Wt	2.1169	1.9959	1.8999	1.7729	1.5445	1.0995	0.4469	0.378
	%	0	-5.72	-10.25	-16.25	-27.04	-48.06	-78.89	-82.14
C4	Wt	1.482	1.4321	1.3476	1.2427	0.962	0.8174	0.4074	0.318
	%	0	-3.37	-9.07	-16.15	-35.09	-44.84	-72.51	-78.54
	Wt	1.7503	1.6875	1.637	1.5425	1.3248	0.9746	0.5117	0.4105
	%	0	-3.59	-6.47	-11.87	-24.31	-44.32	-70.77	-76.55
	Wt	2.0693	1.9934	1.837	1.6929	1.5133	1.2346	0.5458	0.3092
	%	0	-3.67	-11.23	-18.19	-26.87	-40.34	-73.62	-85.06
	Wt	2.3862	2.3016	2.1866	2.1043	1.8962	1.6292	0.9538	0.3895
	%	0	-3.55	-8.36	-11.81	-20.53	-31.72	-60.03	-83.68
	Wt	1.8162	1.7471	1.6536	1.5395	1.2932	0.9398	0.429	0.366
	%	0	-3.80	-8.95	-15.24	-28.80	-48.25	-76.38	-79.85
	Wt	2.3594	2.2504	2.1519	2.0122	1.8875	1.1706	0.3821	0.3829
	%	0	-4.62	-8.79	-14.72	-20.00	-50.39	-83.81	-83.77
C6	Wt	1.7598	1.7092	1.6312	1.54	1.3016	0.9454	0.482	0.4104
	%	0	-2.88	-7.31	-12.49	-26.04	-46.28	-72.61	-76.68
	Wt	1.769	1.7373	1.6912	1.6079	1.3343	1.1402	0.6772	0.2923
	%	0	-1.79	-4.40	-9.11	-24.57	-35.55	-61.72	-83.48
	Wt	1.8019	1.7653	1.6758	1.5278	1.243	1.02	0.4521	0.3665
	%	0	-2.03	-7.00	-15.21	-31.02	-43.39	-74.91	-79.66
	Wt	2.3803	2.2148	2.0748	2.0906	1.8593	1.6044	0.9635	0.4658
	%	0	-6.95	-12.83	-12.17	-21.89	-32.60	-59.52	-80.43
	Wt	1.884	1.8114	1.7092	1.5872	1.3178	0.9105	0.431	0.3788
	%	0	-3.85	-9.28	-15.75	-30.05	-51.67	-77.12	-79.89
	Wt	2.3932	2.2948	2.1946	2.0694	1.8875	1.3552	0.5217	0.4328
	%	0	-4.11	-8.30	-13.53	-21.13	-43.37	-78.20	-81.92
C8	Wt	1.9135	1.859	1.7844	1.6985	1.472	1.0918	0.4186	0.3195
	%	0	-2.85	-6.75	-11.24	-23.07	-42.94	-78.12	-83.30
	Wt	2.4603	2.3771	2.3054	2.2156	1.8316	1.532	0.5156	0.3246

	%	0	-3.38	-6.30	-9.95	-25.55	-37.73	-79.04	-86.81
	Wt	1.8986	1.7525	1.7826	1.645	1.3972	1.1797	0.5556	0.3319
	%	0	-7.70	-6.11	-13.36	-26.41	-37.86	-70.74	-82.52
	Wt	2.5322	2.4552	2.34	2.2628	2.0486	1.8138	1.08	0.3556
	%	0	-3.04	-7.59	-10.64	-19.10	-28.37	-57.35	-85.96
	Wt	1.6834	1.6048	1.5196	1.4233	1.1747	0.8052	0.5358	0.3492
	%	0	-4.67	-9.73	-15.45	-30.22	-52.17	-68.17	-79.26
	Wt	2.1369	2.0374	1.9315	1.812	1.6446	1.165	0.5045	0.3956
	%	0	-4.66	-9.61	-15.20	-23.04	-45.48	-76.39	-81.49
C10	Wt	3.0965	2.9813	2.8627	2.7135	2.1792	1.7287	0.8589	0.5545
	%	0	-3.72	-7.55	-12.37	-29.62	-44.17	-72.26	-82.09
	Wt	3.0292	2.918	2.8157	2.6635	2.4223	1.9403	0.6425	0.3446
	%	0	-3.67	-7.05	-12.07	-20.03	-35.95	-78.79	-88.62
	Wt	3.7741	3.5876	3.4936	3.2406	2.9772	2.1035	1.0325	0.352
	%	0	-4.94	-7.43	-14.14	-21.11	-44.26	-72.64	-90.67
	Wt	2.8066	2.7273	2.6398	2.5053	2.3499	1.9598	0.5728	0.474
	%	0	-2.83	-5.94	-10.74	-16.27	-30.17	-79.59	-83.11
	Wt	1.1065	1.0551	1.0016	0.9509	0.8816	0.6147	0.3075	0.3076
	%	0	-4.65	-9.48	-14.06	-20.33	-44.45	-72.21	-72.20
	Wt	2.7577	2.5863	2.351	2.0089	1.9511	1.509	0.9776	0.5203
	%	0	-6.22	-14.75	-27.15	-29.25	-45.28	-64.55	-81.13
C12	Wt	1.775	1.6867	1.6073	1.4864	1.244	0.9576	0.3606	0.3443
	%	0	-4.97	-9.45	-16.26	-29.92	-46.05	-79.68	-80.60
	Wt	1.363	1.3274	1.303	1.1869	0.9939	0.7575	0.3256	0.3069
	%	0	-2.61	-4.40	-12.92	-27.08	-44.42	-76.11	-77.48
	Wt	1.21	1.1363	1.0662	0.9596	0.8928	0.6243	0.4836	0.3147
	%	0	-6.09	-11.88	-20.69	-26.21	-48.40	-60.03	-73.99
	Wt	2.352	2.2652	2.1765	2.036	1.7742	1.4044	0.4583	0.3905
	%	0	-3.69	-7.46	-13.44	-24.57	-40.29	-80.51	-83.40
	Wt	1.1578	1.0884	1.0348	0.9226	0.902	0.6142	0.4787	0.3282
	%	0	-5.99	-10.62	-20.31	-22.09	-46.95	-58.65	-71.65
	Wt	2.9561	2.861	2.8284	2.6772	2.4145	1.8897	0.5238	0.4541
	%	0	-3.22	-4.32	-9.43	-18.32	-36.07	-82.28	-84.64
A2	Wt	2.1117	2.0529	1.9656	1.8679	1.614	1.2082	0.5574	0.3446
	%	0	-2.78	-6.92	-11.55	-23.57	-42.79	-73.60	-83.68
	Wt	1.5659	1.4897	1.4446	1.366	1.0935	0.8819	0.2785	0.2785
	%	0	-4.87	-7.75	-12.77	-30.17	-43.68	-82.21	-82.21
	Wt	2.6758	2.6105	2.5531	2.4043	2.1382	1.8776	1.0685	0.465
	%	0	-2.44	-4.59	-10.15	-20.09	-29.83	-60.07	-82.62
	Wt	1.7718	1.7045	1.5842	1.5079	1.3407	1.157	0.6425	0.3776
	%	0	-3.80	-10.59	-14.89	-24.33	-34.70	-63.74	-78.69
	Wt	1.7011	1.6443	1.5602	1.4573	1.2428	0.9116	0.4177	0.3318

	%	0	-3.34	-8.28	-14.33	-26.94	-46.41	-75.45	-80.49
	Wt	1.9135	1.8264	1.7351	1.6313	1.445	1.047	0.3225	0.3224
	%	0	-4.55	-9.32	-14.75	-24.48	-45.28	-83.15	-83.15
A4	Wt	1.9868	1.9322	1.8445	1.7398	1.4549	1.0098	0.3835	0.3279
	%	0	-2.75	-7.16	-12.43	-26.77	-49.17	-80.70	-83.50
	Wt	2.2805	2.1802	2.127	2.0326	1.7098	1.4495	0.4836	0.3223
	%	0	-4.40	-6.73	-10.87	-25.03	-36.44	-78.79	-85.87
	Wt	1.5639	1.5346	1.4493	1.313	1.0766	0.8612	0.4106	0.3905
	%	0	-1.87	-7.33	-16.04	-31.16	-44.93	-73.75	-75.03
	Wt	1.6503	1.5849	1.4856	1.397	1.2335	1.0436	0.5331	0.357
	%	0	-3.96	-9.98	-15.35	-25.26	-36.76	-67.70	-78.37
	Wt	2.3994	2.3332	2.2301	2.1073	1.8344	1.4086	0.6164	0.391
	%	0	-2.76	-7.06	-12.17	-23.55	-41.29	-74.31	-83.70
	Wt	2.3795	2.2743	2.1679	2.0428	1.6467	1.3262	0.3644	0.365
	%	0	-4.42	-8.89	-14.15	-30.80	-44.27	-84.69	-84.66
A6	Wt	2.0763	2.0289	1.9481	1.8451	1.5885	1.1933	0.5116	0.2881
	%	0	-2.28	-6.17	-11.14	-23.49	-42.53	-75.36	-86.12
	Wt	1.937	1.841	1.7916	1.7018	1.3948	1.1586	0.2892	0.2885
	%	0	-4.96	-7.51	-12.14	-27.99	-40.19	-85.07	-85.11
	Wt	1.5655	1.5022	1.4603	1.3345	1.1186	0.9255	0.4233	0.3623
	%	0	-4.04	-6.72	-14.76	-28.55	-40.88	-72.96	-76.86
	Wt	2.6267	2.5602	2.4287	2.3562	2.1535	1.9225	1.221	0.3382
	%	0	-2.53	-7.54	-10.30	-18.01	-26.81	-53.52	-87.12
	Wt	2.0852	2.0249	1.9285	1.8136	1.5573	1.1503	0.49	0.3558
	%	0	-2.89	-7.51	-13.03	-25.32	-44.84	-76.50	-82.94
	Wt	2.4165	2.3151	2.2124	2.0849	1.8845	1.3574	0.3366	0.3369
	%	0	-4.20	-8.45	-13.72	-22.02	-43.83	-86.07	-86.06
A8	Wt	2.0763	2.027	1.9485	1.8532	1.6034	1.2476	0.6053	0.324
	%	0	-2.37	-6.16	-10.75	-22.78	-39.91	-70.85	-84.40
	Wt	2.2621	2.1612	2.111	2.0136	1.6624	1.3872	0.2954	0.2945
	%	0	-4.46	-6.68	-10.99	-26.51	-38.68	-86.94	-86.98
	Wt	1.7298	1.659	1.621	1.4902	1.258	1.0445	0.4228	0.3247
	%	0	-4.09	-6.29	-13.85	-27.27	-39.62	-75.56	-81.23
	Wt	1.233	1.1739	1.0577	0.9912	0.8361	0.6821	0.3551	0.315
	%	0	-4.79	-14.22	-19.61	-32.19	-44.68	-71.20	-74.45
	Wt	1.2249	1.1691	1.1025	0.9989	0.8645	0.6922	0.3269	0.3274
	%	0	-4.56	-9.99	-18.45	-29.42	-43.49	-73.31	-73.27
	Wt	2.4383	2.3371	2.2342	2.084	1.8875	1.2417	0.3327	0.3328
	%	0	-4.15	-8.37	-14.53	-22.59	-49.08	-86.36	-86.35
A10	Wt	2.5549	2.4597	2.3774	2.244	2.0093	1.7289	0.8444	0.3115
	%	0	-3.73	-6.95	-12.17	-21.36	-32.33	-66.95	-87.81
	Wt	1.4879	1.4016	1.3573	1.2153	1.0085	0.7604	0.315	0.314

	%	0	-5.80	-8.78	-18.32	-32.22	-48.89	-78.83	-78.90
	Wt	1.1507	1.1078	1.012	0.9573	0.7828	0.5799	0.3455	0.3271
	%	0	-3.73	-12.05	-16.81	-31.97	-49.60	-69.97	-71.57
	Wt	2.1143	2.036	1.9613	1.8372	1.616	1.3133	0.4774	0.3435
	%	0	-3.70	-7.24	-13.11	-23.57	-37.88	-77.42	-83.75
	Wt	1.9236	1.8129	1.7862	1.6605	1.4156	1.0305	0.507	0.3974
	%	0	-5.75	-7.14	-13.68	-26.41	-46.43	-73.64	-79.34
	Wt	2.9206	2.8247	2.7926	2.6375	2.3725	1.9539	0.6094	0.4449
	%	0	-3.28	-4.38	-9.69	-18.77	-33.10	-79.13	-84.77
A12	Wt	1.9732	1.8888	1.813	1.6974	1.5022	1.2058	0.4438	0.321
	%	0	-4.28	-8.12	-13.98	-23.87	-38.89	-77.51	-83.73
	Wt	2.0562	1.9649	1.881	1.7493	1.5102	1.1881	0.4052	0.3112
	%	0	-4.44	-8.52	-14.93	-26.55	-42.22	-80.29	-84.87
	Wt	3.1679	3.0786	3.0042	2.8654	2.5776	2.2204	0.5923	0.4462
	%	0	-2.82	-5.17	-9.55	-18.63	-29.91	-81.30	-85.91
	Wt	2.9814	2.8523	2.7067	2.5516	2.1533	1.692	0.7965	0.4352
	%	0	-4.33	-9.21	-14.42	-27.78	-43.25	-73.28	-85.40
	Wt	2.3558	2.2487	2.1209	2.0026	1.4974	1.0334	0.4003	0.3006
	%	0	-4.55	-9.97	-14.99	-36.44	-56.13	-83.01	-87.24
	Wt	1.6203	1.5335	1.5048	1.3711	1.1407	0.8231	0.3576	0.3581
	%	0	-5.36	-7.13	-15.38	-29.60	-49.20	-77.93	-77.90

	time (minutes)						
	30	60	120	240	480	1440	2880
Bl	-3.76	-6.98	-14.86	-31.49	-40.29	-73.61	-82.46
SD	1.60	2.76	4.84	8.86	4.22	3.47	2.02
H2	-3.27	-6.99	-12.17	-24.56	-38.32	-68.01	-79.11
SD	0.80	1.35	2.19	7.87	5.96	5.51	3.53
H4	-2.99	-6.79	-11.73	-23.38	-38.46	-67.73	-79.25
SD	1.37	1.99	2.26	3.02	5.92	7.64	1.84
H6	-3.25	-7.05	-12.63	-22.92	-38.90	-72.04	-83.59
SD	0.99	2.09	3.22	4.53	8.57	9.63	2.62
H8	-3.25	-7.05	-12.63	-22.92	-38.90	-72.04	-83.59
SD	0.99	2.09	3.22	4.53	8.57	9.63	2.62
H10	-4.60	-8.73	-15.85	-25.63	-40.88	-68.63	-77.61
SD	0.69	1.65	3.25	4.02	5.79	7.44	4.62
H12	-4.20	-7.55	-14.19	-25.71	-38.38	-73.16	-80.23
SD	1.12	2.57	3.13	3.67	1.04	6.04	2.77
C2	-4.23	-9.25	-15.55	-27.33	-42.67	-73.71	-81.22
SD	1.01	2.40	2.92	2.26	6.50	6.93	0.97
C4	-3.77	-8.81	-14.66	-25.93	-43.31	-72.85	-81.24

SD	0.44	1.52	2.49	5.65	6.65	7.77	3.41
C6	-3.60	-8.19	-13.04	-25.78	-42.14	-70.68	-80.34
SD	1.89	2.81	2.40	4.10	7.01	8.06	2.30
C8	-4.38	-7.68	-12.64	-24.57	-40.76	-71.64	-83.22
SD	1.80	1.62	2.38	3.76	8.10	8.20	2.81
C10	-4.34	-8.70	-15.09	-22.77	-40.71	-73.34	-82.97
SD	1.19	3.18	6.05	5.43	6.22	5.46	6.50
C12	-4.43	-8.02	-15.51	-24.70	-43.70	-72.88	-78.63
SD	1.47	3.19	4.44	4.07	4.66	10.68	5.18
A2	-3.63	-7.91	-13.07	-24.93	-40.45	-73.04	-81.81
SD	0.96	2.07	1.93	3.39	6.64	9.46	1.88
A4	-3.36	-7.86	-13.50	-27.09	-42.14	-76.65	-81.85
SD	1.05	1.28	2.01	3.18	4.98	5.99	4.21
A6	-3.48	-7.32	-12.51	-24.23	-39.84	-74.91	-84.03
SD	1.07	0.78	1.66	3.95	6.62	11.76	3.79
A8	-4.07	-8.62	-14.70	-26.79	-42.58	-77.37	-81.11
SD	0.87	3.12	3.70	3.75	3.96	7.39	5.98
A10	-4.33	-7.76	-13.96	-25.72	-41.37	-74.33	-81.02
SD	1.13	2.54	3.14	5.55	7.90	5.04	5.73
A12	-4.29	-8.02	-13.87	-27.15	-43.27	-78.89	-84.18
SD	0.82	1.70	2.17	5.94	8.94	3.44	3.29



% Weight loss

	Initial weight	Dehydrated weight	% Weight loss		
Bl	0.1690	0.1683	-0.41	Average	-0.15
	0.1686	0.1676	-0.59	SD	0.34
	0.1626	0.1625	-0.06		
	0.1485	0.1484	-0.07		
	0.1534	0.1540	0.39		
	0.1633	0.1630	-0.18		
H2	0.1480	0.1468	-0.81	Average	-0.93
	0.1317	0.1312	-0.38	SD	0.46
	0.1974	0.1944	-1.52		
	0.2052	0.2032	-0.97		

	0.1809	0.1800	-0.50		
	0.1644	0.1621	-1.40		
H4	0.1651	0.1626	-1.51	Average	-1.68
	0.2100	0.2070	-1.43	SD	0.41
	0.1824	0.1792	-1.75		
	0.1549	0.1513	-2.32		
	0.2159	0.2134	-1.16		
	0.1613	0.1582	-1.92		
	H6	0.1737	0.1735	-0.12	Average
0.1675		0.1625	-2.99	SD	1.94
0.1688		0.1624	-3.79		
0.1812		0.1812	0.00		
0.1799		0.1725	-4.11		
0.1752		0.1681	-4.05		
H8	0.1547	0.1490	-3.68	Average	-4.24
	0.1870	0.1835	-1.87	SD	1.43
	0.1898	0.1802	-5.06		
	0.1830	0.1751	-4.32		
	0.1772	0.1695	-4.35		
	0.1760	0.1652	-6.14		
H10	0.2016	0.1906	-5.46	Average	-5.17
	0.1646	0.1564	-4.98	SD	0.88
	0.1532	0.1456	-4.96		
	0.1606	0.1508	-6.10		
	0.1572	0.1515	-3.63		
	0.1720	0.1619	-5.87		
H12	0.1895	0.1784	-5.86	Average	-5.49
	0.1911	0.1870	-2.15	SD	1.78
	0.1827	0.1702	-6.84		
	0.1790	0.1671	-6.65		
	0.1990	0.1892	-4.92		
	0.1925	0.1800	-6.49		
C2	0.1579	0.1575	-0.25	Average	-0.15
	0.1692	0.1693	0.06	SD	0.22
	0.1756	0.1756	0.00		
	0.1407	0.1403	-0.28		
	0.1365	0.1366	0.07		
	0.1280	0.1274	-0.47		
C4	0.1685	0.1683	-0.12	Average	-0.18
	0.1795	0.1791	-0.22	SD	0.08

	0.1790	0.1787	-0.17		
	0.1659	0.1657	-0.12		
	0.1582	0.1577	-0.32		
	0.1616	0.1614	-0.12		
C6	0.1420	0.1420	0.00	Average	-0.17
	0.1737	0.1729	-0.46	SD	0.16
	0.1701	0.1699	-0.12		
	0.1791	0.1789	-0.11		
	0.1925	0.1922	-0.16		
	0.2051	0.2047	-0.20		
C8	0.1545	0.1554	0.58	Average	-0.27
	0.1708	0.1705	-0.18	SD	0.84
	0.1764	0.1762	-0.11		
	0.1679	0.1685	0.36		
	0.1648	0.1640	-0.49		
	0.1683	0.1653	-1.78		
C10	0.1942	0.1936	-0.31	Average	-0.33
	0.1869	0.1854	-0.80	SD	0.38
	0.1983	0.1990	0.35		
	0.1951	0.1944	-0.36		
	0.1692	0.1686	-0.35		
	0.1519	0.1511	-0.53		
C12	0.1728	0.1710	-1.04	Average	-0.37
	0.1770	0.1761	-0.51	SD	0.66
	0.1739	0.1726	-0.75		
	0.1882	0.1872	-0.53		
	0.1827	0.1823	-0.22		
	0.1880	0.1896	0.85		
A2	0.1803	0.1793	-0.55	Average	-1.18
	0.1572	0.1544	-1.78	SD	0.48
	0.1595	0.1568	-1.69		
	0.2035	0.2016	-0.93		
	0.2196	0.2169	-1.23		
	0.1669	0.1654	-0.90		
A4	0.1670	0.1635	-2.10	Average	-2.86
	0.1825	0.1752	-4.00	SD	0.68
	0.1779	0.1724	-3.09		
	0.1835	0.1786	-2.67		
	0.1965	0.1920	-2.29		
	0.2047	0.1985	-3.03		

A6	0.2095	0.2035	-2.86	Average	-3.70
	0.1836	0.1764	-3.92		
	0.1886	0.1818	-3.61		
	0.1902	0.1824	-4.10		
	0.1829	0.1756	-3.99		
	0.1767	0.1701	-3.74		
A8	0.1486	0.1412	-4.98	Average	-5.27
	0.1769	0.1655	-6.44		
	0.1638	0.1545	-5.68		
	0.1586	0.1508	-4.92		
	0.1536	0.1452	-5.47		
	0.1554	0.1490	-4.12		
A10	0.1751	0.1628	-7.02	Average	-5.25
	0.1466	0.1369	-6.62		
	0.1511	0.1446	-4.30		
	0.1505	0.1501	-0.27		
	0.1563	0.1456	-6.85		
	0.2040	0.1908	-6.47		
A12	0.1674	0.1592	-4.90	Average	-6.40
	0.1612	0.1492	-7.44		
	0.1758	0.1658	-5.69		
	0.1783	0.1668	-6.45		
	0.1536	0.1442	-6.12		
	0.1912	0.1763	-7.79		

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY
Water Vapor Transmission Test

		Time (minutes)						
		0	30	60	120	240	480	1440
Bl	weight (g)	95.1363	95.1322	95.1151	95.0866	95.0328	94.8875	93.9884
	dif wt.		0.0041	0.0171	0.0285	0.0538	0.1453	0.8991
	Rate (g H ₂ O/m ² /min)		0.19	0.81	0.67	0.63	0.86	1.32
	weight (g)	93.9205	93.9126	93.8960	93.8659	93.7713	93.5412	92.9560
	dif wt.		0.0079	0.0166	0.0301	0.0946	0.2301	0.5852
	Rate (g H ₂ O/m ² /min)		0.37	0.78	0.71	1.11	1.36	0.86
	weight (g)	93.9067	93.9011	93.8865	93.8471	93.8077	93.6242	93.1958

	dif wt.		0.0056	0.0146	0.0394	0.0394	0.1835	0.4284
	Rate (g H ₂ O/m ² /min)		0.26	0.69	0.93	0.46	1.08	0.63
	weight (g)	94.6638	94.6524	94.6399	94.6011	94.5295	94.3898	93.9018
	dif wt.		0.0114	0.0125	0.0388	0.0716	0.1397	0.4880
	Rate (g H ₂ O/m ² /min)		0.54	0.59	0.91	0.84	0.82	0.72
	weight (g)	93.8986	93.8801	93.8723	93.8351	93.7545	93.6190	92.8537
	dif wt.		0.0185	0.0078	0.0372	0.0806	0.1355	0.7653
	Rate (g H ₂ O/m ² /min)		0.87	0.37	0.88	0.95	0.80	1.13
H2	weight (g)	94.8379	94.8295	94.8171	94.7902	94.7298	94.6356	93.9641
	dif wt.		0.0084	0.0124	0.0269	0.0604	0.0942	0.6715
	Rate (g H ₂ O/m ² /min)		0.40	0.58	0.63	0.71	0.56	0.99
	weight (g)	94.1816	94.1809	94.1760	94.1515	94.0789	93.9093	93.4428
	dif wt.		0.0007	0.0049	0.0245	0.0726	0.1696	0.4665
	Rate (g H ₂ O/m ² /min)		0.03	0.23	0.58	0.86	1.00	0.69
	weight (g)	93.9557	93.9480	93.9343	93.8982	93.8623	93.6462	93.2700
	dif wt.		0.0077	0.0137	0.0361	0.0359	0.2161	0.3762
	Rate (g H ₂ O/m ² /min)		0.36	0.65	0.85	0.42	1.27	0.55
	weight (g)	94.5472	94.5401	94.5206	94.4954	94.4455	94.2515	93.7395
	dif wt.		0.0071	0.0195	0.0252	0.0499	0.1940	0.5120
	Rate (g H ₂ O/m ² /min)		0.33	0.92	0.59	0.59	1.14	0.75
	weight (g)	95.3296	95.3245	95.3090	95.2842	95.2299	95.1265	94.6860
	dif wt.		0.0051	0.0155	0.0248	0.0543	0.1034	0.4405
	Rate (g H ₂ O/m ² /min)		0.24	0.73	0.58	0.64	0.61	0.65
H4	weight (g)	94.2373	94.2299	94.2188	94.1964	94.1462	93.9864	93.4497
	dif wt.		0.0074	0.0111	0.0224	0.0502	0.1598	0.5367
	Rate (g H ₂ O/m ² /min)		0.35	0.52	0.53	0.59	0.94	0.79
	weight (g)	97.7638	97.7611	97.7460	97.7062	97.6224	97.4287	97.2059
	dif wt.		0.0027	0.0151	0.0398	0.0838	0.1937	0.2228
	Rate (g H ₂ O/m ² /min)		0.13	0.71	0.94	0.99	1.14	0.33
	weight (g)	93.2920	93.2836	93.2700	93.2396	93.2086	93.0918	92.5003

	dif wt.		0.0084	0.0136	0.0304	0.0310	0.1168	0.5915
	Rate (g H ₂ O/m ² /min)		0.40	0.64	0.72	0.37	0.69	0.87
	weight (g)	94.3915	94.3879	94.3743	94.3673	94.3004	94.1786	93.7744
	dif wt.		0.0036	0.0136	0.0070	0.0669	0.1218	0.4042
	Rate (g H ₂ O/m ² /min)		0.17	0.64	0.16	0.79	0.72	0.60
	weight (g)	97.0304	97.0246	97.0134	96.9794	96.9425	96.7896	96.3105
	dif wt.		0.0058	0.0112	0.0340	0.0369	0.1529	0.4791
	Rate (g H ₂ O/m ² /min)		0.27	0.53	0.80	0.43	0.90	0.71
H6	weight (g)	94.1160	94.1083	94.0981	94.0577	94.0129	93.8577	93.4580
	dif wt.		0.0077	0.0102	0.0404	0.0448	0.1552	0.3997
	Rate (g H ₂ O/m ² /min)		0.36	0.48	0.95	0.53	0.91	0.59
	weight (g)	95.1690	95.1659	95.1597	95.1361	95.0636	94.9759	94.6139
	dif wt.		0.0031	0.0062	0.0236	0.0725	0.0877	0.3620
	Rate (g H ₂ O/m ² /min)		0.15	0.29	0.56	0.85	0.52	0.53
	weight (g)	93.3970	93.3896	93.3770	93.3497	93.3035	93.1201	92.6885
	dif wt.		0.0074	0.0126	0.0273	0.0462	0.1834	0.4316
	Rate (g H ₂ O/m ² /min)		0.35	0.59	0.64	0.54	1.08	0.64
	weight (g)	94.3479	94.3403	94.3222	94.3112	94.2686	94.1083	93.5240
	dif wt.		0.0076	0.0181	0.0110	0.0426	0.1603	0.5843
	Rate (g H ₂ O/m ² /min)		0.36	0.85	0.26	0.50	0.94	0.86
	weight (g)	94.5329	94.5270	94.5131	94.4864	94.4309	94.3057	93.8458
	dif wt.		0.0059	0.0139	0.0267	0.0555	0.1252	0.4599
	Rate (g H ₂ O/m ² /min)		0.28	0.66	0.63	0.65	0.74	0.68
	H8	weight (g)	93.5544	93.5481	93.5362	93.5157	93.4717	93.3320
dif wt.			0.0063	0.0119	0.0205	0.0440	0.1397	0.5974
Rate (g H ₂ O/m ² /min)			0.30	0.56	0.48	0.52	0.82	0.88
weight (g)		94.7435	94.7356	94.7301	94.7066	94.6402	94.4789	93.9979
dif wt.			0.0079	0.0055	0.0235	0.0664	0.1613	0.4810
Rate (g H ₂ O/m ² /min)			0.37	0.26	0.55	0.78	0.95	0.71
weight (g)		93.3900	93.3821	93.3652	93.3126	93.2456	93.0828	92.5334

	dif wt.		0.0079	0.0169	0.0526	0.0670	0.1628	0.5494
	Rate (g H ₂ O/m ² /min)		0.37	0.80	1.24	0.79	0.96	0.81
	weight (g)	93.5169	93.5105	93.4942	93.4801	93.4367	93.2929	92.7889
	dif wt.		0.0064	0.0163	0.0141	0.0434	0.1438	0.5040
	Rate (g H ₂ O/m ² /min)		0.30	0.77	0.33	0.51	0.85	0.74
	weight (g)	94.4042	94.3980	94.3817	94.3624	94.3120	94.2175	93.8596
	dif wt.		0.0062	0.0163	0.0193	0.0504	0.0945	0.3579
	Rate (g H ₂ O/m ² /min)		0.29	0.77	0.45	0.59	0.56	0.53
H10	weight (g)	94.4023	94.3950	94.3800	94.3574	94.3135	94.1774	93.6343
	dif wt.		0.0073	0.0150	0.0226	0.0439	0.1361	0.5431
	Rate (g H ₂ O/m ² /min)		0.34	0.71	0.53	0.52	0.80	0.80
	weight (g)	95.1959	95.1889	95.1698	95.1390	95.0788	94.9005	94.4403
	dif wt.		0.0070	0.0191	0.0308	0.0602	0.1783	0.4602
	Rate (g H ₂ O/m ² /min)		0.33	0.90	0.73	0.71	1.05	0.68
	weight (g)	97.6533	97.6496	97.6410	97.6153	97.5679	97.4366	96.9516
	dif wt.		0.0037	0.0086	0.0257	0.0474	0.1313	0.4850
	Rate (g H ₂ O/m ² /min)		0.17	0.41	0.61	0.56	0.77	0.71
	weight (g)	93.4870	93.4802	93.4683	93.4377	93.3837	93.2334	92.6229
	dif wt.		0.0068	0.0119	0.0306	0.0540	0.1503	0.6105
	Rate (g H ₂ O/m ² /min)		0.32	0.56	0.72	0.64	0.89	0.90
	weight (g)	94.8875	94.8785	94.8721	94.8369	94.7823	94.6645	94.3054
	dif wt.		0.0090	0.0064	0.0352	0.0546	0.1178	0.3591
	Rate (g H ₂ O/m ² /min)		0.42	0.30	0.83	0.64	0.69	0.53
H12	weight (g)	93.8225	93.8128	93.7965	93.7697	93.7151	93.5416	92.9106
	dif wt.		0.0097	0.0163	0.0268	0.0546	0.1735	0.6310
	Rate (g H ₂ O/m ² /min)		0.46	0.77	0.63	0.64	1.02	0.93
	weight (g)	93.9846	93.9778	93.9628	93.9285	93.8811	93.7323	93.2992
	dif wt.		0.0068	0.0150	0.0343	0.0474	0.1488	0.4331
	Rate (g H ₂ O/m ² /min)		0.32	0.71	0.81	0.56	0.88	0.64
	weight (g)	95.1908	95.1860	95.1771	95.1538	95.1148	94.9938	94.6536

	dif wt.		0.0048	0.0089	0.0233	0.0390	0.1210	0.3402
	Rate (g H ₂ O/m ² /min)		0.23	0.42	0.55	0.46	0.71	0.50
	weight (g)	94.2050	94.1979	94.1889	94.1631	94.1188	93.9992	93.5070
	dif wt.		0.0071	0.0090	0.0258	0.0443	0.1196	0.4922
	Rate (g H ₂ O/m ² /min)		0.33	0.42	0.61	0.52	0.70	0.73
	weight (g)	93.4450	93.4400	93.4286	93.4158	93.3378	93.1969	92.7467
	dif wt.		0.0050	0.0114	0.0128	0.0780	0.1409	0.4502
	Rate (g H ₂ O/m ² /min)		0.24	0.54	0.30	0.92	0.83	0.66
C2	weight (g)	94.2548	94.2491	94.2371	94.2071	94.1101	93.8764	93.1021
	dif wt.		0.0057	0.0120	0.0300	0.0970	0.2337	0.7743
	Rate (g H ₂ O/m ² /min)		0.27	0.57	0.71	1.14	1.38	1.14
	weight (g)	95.0874	95.0724	95.0615	95.0396	94.9652	94.7931	94.4424
	dif wt.		0.0150	0.0109	0.0219	0.0744	0.1721	0.3507
	Rate (g H ₂ O/m ² /min)		0.71	0.51	0.52	0.88	1.01	0.52
	weight (g)	93.2491	93.2417	93.2282	93.1988	93.1697	92.9925	92.4508
	dif wt.		0.0074	0.0135	0.0294	0.0291	0.1772	0.5417
	Rate (g H ₂ O/m ² /min)		0.35	0.64	0.69	0.34	1.04	0.80
	weight (g)	93.8285	93.8214	93.8047	93.7554	93.7078	93.5812	93.0161
	dif wt.		0.0071	0.0167	0.0493	0.0476	0.1266	0.5651
	Rate (g H ₂ O/m ² /min))		0.33	0.79	1.16	0.56	0.75	0.83
	weight (g)	94.7186	94.7041	94.6902	94.6655	94.6096	94.5000	93.9562
	dif wt.		0.0145	0.0139	0.0247	0.0559	0.1096	0.5438
	Rate (g H ₂ O/m ² /min)		0.68	0.66	0.58	0.66	0.65	0.80
	C4	weight (g)	94.0217	94.0166	94.0045	93.9802	93.9295	93.7708
dif wt.			0.0051	0.0121	0.0243	0.0507	0.1587	0.9364
Rate (g H ₂ O/m ² /min)			0.24	0.57	0.57	0.60	0.94	1.38
weight (g)		94.3164	94.3012	94.2952	94.2496	94.1552	93.9031	93.4724
dif wt.			0.0152	0.0060	0.0456	0.0944	0.2521	0.4307
Rate (g H ₂ O/m ² /min)			0.72	0.28	1.07	1.11	1.49	0.63
weight (g)		93.3582	93.3573	93.3366	93.3082	93.2571	93.0879	92.6371

	dif wt.		0.0009	0.0207	0.0284	0.0511	0.1692	0.4508
	Rate (g H ₂ O/m ² /min)		0.04	0.98	0.67	0.60	1.00	0.66
	weight (g)	93.1854	93.1802	93.1641	93.1185	93.0887	92.9650	92.4502
	dif wt.		0.0052	0.0161	0.0456	0.0298	0.1237	0.5148
	Rate (g H ₂ O/m ² /min)		0.25	0.76	1.07	0.35	0.73	0.76
	weight (g)	93.8118	93.7978	93.7823	93.7565	93.6864	93.5705	93.2400
	dif wt.		0.0140	0.0155	0.0258	0.0701	0.1159	0.3305
	Rate (g H ₂ O/m ² /min)		0.66	0.73	0.61	0.83	0.68	0.49
C6	weight (g)	95.3408	95.3356	95.3271	95.2929	95.2309	95.0452	94.4649
	dif wt.		0.0052	0.0085	0.0342	0.0620	0.1857	0.5803
	Rate (g H ₂ O/m ² /min)		0.25	0.40	0.81	0.73	1.09	0.85
	weight (g)	94.6988	94.6918	94.6755	94.6549	94.5771	94.4279	93.6648
	dif wt.		0.0070	0.0163	0.0206	0.0778	0.1492	0.7631
	Rate (g H ₂ O/m ² /min)		0.33	0.77	0.49	0.92	0.88	1.12
	weight (g)	95.7335	95.7266	95.7126	95.6805	95.6475	95.4560	95.0018
	dif wt.		0.0069	0.0140	0.0321	0.0330	0.1915	0.4542
	Rate (g H ₂ O/m ² /min)		0.33	0.66	0.76	0.39	1.13	0.67
	weight (g)	95.8128	95.8080	95.7922	95.7644	95.7145	95.5703	94.9859
	dif wt.		0.0048	0.0158	0.0278	0.0499	0.1442	0.5844
	Rate (g H ₂ O/m ² /min)		0.23	0.74	0.66	0.59	0.85	0.86
	weight (g)	94.8400	94.8338	94.8175	94.7854	94.7134	94.5855	94.3745
	dif wt.		0.0062	0.0163	0.0321	0.0720	0.1279	0.2110
	Rate (g H ₂ O/m ² /min)		0.29	0.77	0.76	0.85	0.75	0.31
	C8	weight (g)	93.1565	93.1505	93.1403	93.1180	93.0655	92.8395
dif wt.			0.0060	0.0102	0.0223	0.0525	0.2260	0.5874
Rate (g H ₂ O/m ² /min)			0.28	0.48	0.53	0.62	1.33	0.87
weight (g)		94.2026	94.1970	94.1904	94.1595	94.1040	93.9569	93.5131
dif wt.			0.0056	0.0066	0.0309	0.0555	0.1471	0.4438
Rate (g H ₂ O/m ² /min)			0.26	0.31	0.73	0.65	0.87	0.65
weight (g)		93.7275	93.7202	93.7033	93.6690	93.6186	93.4669	92.9448

	dif wt.		0.0073	0.0169	0.0343	0.0504	0.1517	0.5221
	Rate (g H ₂ O/m ² /min)		0.34	0.80	0.81	0.59	0.89	0.77
	weight (g)	94.4352	94.4302	94.4123	94.3917	94.3216	94.1521	93.3132
	dif wt.		0.0050	0.0179	0.0206	0.0701	0.1695	0.8389
	Rate (g H ₂ O/m ² /min)		0.24	0.84	0.49	0.83	1.00	1.24
	weight (g)	94.6579	94.6517	94.6353	94.6062	94.5453	94.4334	94.1467
	dif wt.		0.0062	0.0164	0.0291	0.0609	0.1119	0.2867
	Rate (g H ₂ O/m ² /min)		0.29	0.77	0.69	0.72	0.66	0.42
C10	weight (g)	93.5830	93.5755	93.5633	93.5423	93.4929	93.3805	92.9185
	dif wt.		0.0075	0.0122	0.0210	0.0494	0.1124	0.4620
	Rate (g H ₂ O/m ² /min)		0.35	0.58	0.49	0.58	0.66	0.68
	weight (g)	94.4496	94.4436	94.4278	94.4085	94.3271	94.1112	93.5830
	dif wt.		0.0060	0.0158	0.0193	0.0814	0.2159	0.5282
	Rate (g H ₂ O/m ² /min)		0.28	0.74	0.45	0.96	1.27	0.78
	weight (g)	94.5493	94.5451	94.5360	94.5036	94.4159	94.2600	93.5596
	dif wt.		0.0042	0.0091	0.0324	0.0877	0.1559	0.7004
	Rate (g H ₂ O/m ² /min)		0.20	0.43	0.76	1.03	0.92	1.03
	weight (g)	94.2538	94.2466	94.2287	94.2032	94.1622	94.0075	93.5227
	dif wt.		0.0072	0.0179	0.0255	0.0410	0.1547	0.4848
	Rate (g H ₂ O/m ² /min)		0.34	0.84	0.60	0.48	0.91	0.71
	weight (g)	93.6877	93.6752	93.6657	93.6347	93.5945	93.4785	93.0345
	dif wt.		0.0125	0.0095	0.0310	0.0402	0.1160	0.4440
	Rate (g H ₂ O/m ² /min)		0.59	0.45	0.73	0.47	0.68	0.65
	C12	weight (g)	93.6131	93.6052	93.6011	93.5680	93.5275	93.3980
dif wt.			0.0079	0.0041	0.0331	0.0405	0.1295	0.5162
Rate (g H ₂ O/m ² /min)			0.37	0.19	0.78	0.48	0.76	0.76
weight (g)		94.0484	94.0472	94.0285	94.0115	93.9730	93.8496	93.3376
dif wt.			0.0012	0.0187	0.0170	0.0385	0.1234	0.5120
Rate (g H ₂ O/m ² /min)			0.06	0.88	0.40	0.45	0.73	0.75
weight (g)		95.4323	95.4271	95.4180	95.3930	95.3129	95.2295	94.4286

	dif wt.		0.0052	0.0091	0.0250	0.0801	0.0834	0.8009
	Rate (g H ₂ O/m ² /min)		0.25	0.43	0.59	0.94	0.49	1.18
	weight (g)	94.7770	94.7691	94.7603	94.7365	94.6939	94.4595	93.9371
	dif wt.		0.0079	0.0088	0.0238	0.0426	0.2344	0.5224
	Rate (g H ₂ O/m ² /min)		0.37	0.41	0.56	0.50	1.38	0.77
	weight (g)	95.4285	95.4230	95.4166	95.3858	95.3464	95.1563	94.9527
	dif wt.		0.0055	0.0064	0.0308	0.0394	0.1901	0.2036
	Rate (g H ₂ O/m ² /min)		0.26	0.30	0.73	0.46	1.12	0.30
A2	weight (g)	94.3470	94.3411	94.3325	94.3127	94.2698	94.1347	93.2624
	dif wt.		0.0059	0.0086	0.0198	0.0429	0.1351	0.8723
	Rate (g H ₂ O/m ² /min)		0.28	0.41	0.47	0.51	0.80	1.28
	weight (g)	95.0029	94.9890	94.9744	94.9337	94.8140	94.6328	94.2516
	dif wt.		0.0139	0.0146	0.0407	0.1197	0.1812	0.3812
	Rate (g H ₂ O/m ² /min)		0.66	0.69	0.96	1.41	1.07	0.56
	weight (g)	93.2317	93.2254	93.2117	93.1802	93.1468	92.9381	92.4059
	dif wt.		0.0063	0.0137	0.0315	0.0334	0.2087	0.5322
	Rate (g H ₂ O/m ² /min)		0.30	0.65	0.74	0.39	1.23	0.78
	weight (g)	93.7332	93.7286	93.7109	93.6580	93.6047	93.4735	93.0059
	dif wt.		0.0046	0.0177	0.0529	0.0533	0.1312	0.4676
	Rate (g H ₂ O/m ² /min)		0.22	0.83	1.25	0.63	0.77	0.69
	weight (g)	94.7908	94.7822	94.7679	94.7417	94.6881	94.5890	94.2680
	dif wt.		0.0086	0.0143	0.0262	0.0536	0.0991	0.3210
	Rate (g H ₂ O/m ² /min)		0.41	0.67	0.62	0.63	0.58	0.47
A4	weight (g)	94.1891	94.1842	94.1730	94.1525	94.1070	93.9725	93.1346
	dif wt.		0.0049	0.0112	0.0205	0.0455	0.1345	0.8379
	Rate (g H ₂ O/m ² /min)		0.23	0.53	0.48	0.54	0.79	1.23
	weight (g)	93.5901	93.5827	93.5763	93.5528	93.4794	93.3109	92.8523
	dif wt.		0.0074	0.0064	0.0235	0.0734	0.1685	0.4586
	Rate (g H ₂ O/m ² /min)		0.35	0.30	0.55	0.86	0.99	0.68
	weight (g)	94.6252	94.6186	94.6042	94.5770	94.5476	94.3397	94.1849

	dif wt.		0.0066	0.0144	0.0272	0.0294	0.2079	0.1548
	Rate (g H ₂ O/m ² /min)		0.31	0.68	0.64	0.35	1.23	0.23
	weight (g)	93.8914	93.8874	93.8706	93.8245	93.7504	93.6022	93.1928
	dif wt.		0.0040	0.0168	0.0461	0.0741	0.1482	0.4094
	Rate (g H ₂ O/m ² /min)		0.19	0.79	1.09	0.87	0.87	0.60
	weight (g)	93.5075	93.5007	93.4846	93.4604	93.4075	93.3052	92.8405
	dif wt.		0.0068	0.0161	0.0242	0.0529	0.1023	0.4647
	Rate (g H ₂ O/m ² /min)		0.32	0.76	0.57	0.62	0.60	0.68
A6	weight (g)	94.3064	94.3022	94.2899	94.2676	94.2160	94.0618	93.4800
	dif wt.		0.0042	0.0123	0.0223	0.0516	0.1542	0.5818
	Rate (g H ₂ O/m ² /min)		0.20	0.58	0.53	0.61	0.91	0.86
	weight (g)	92.9735	92.9652	92.9595	92.9352	92.8606	92.6826	92.1540
	dif wt.		0.0083	0.0057	0.0243	0.0746	0.1780	0.5286
	Rate (g H ₂ O/m ² /min)		0.39	0.27	0.57	0.88	1.05	0.78
	weight (g)	94.2473	94.2413	94.2282	94.2010	94.1730	93.9935	93.5426
	dif wt.		0.0060	0.0131	0.0272	0.0280	0.1795	0.4509
	Rate (g H ₂ O/m ² /min)		0.28	0.62	0.64	0.33	1.06	0.66
	weight (g)	94.6639	94.6592	94.6425	94.6075	94.5575	94.3834	93.9879
	dif wt.		0.0047	0.0167	0.0350	0.0500	0.1741	0.3955
	Rate (g H ₂ O/m ² /min)		0.22	0.79	0.82	0.59	1.03	0.58
	weight (g)	94.3240	94.3174	94.3023	94.2766	94.2225	94.1260	93.7976
	dif wt.		0.0066	0.0151	0.0257	0.0541	0.0965	0.3284
	Rate (g H ₂ O/m ² /min)		0.31	0.71	0.61	0.64	0.57	0.48
	A8	weight (g)	97.4690	97.4645	97.4517	97.4252	97.3677	97.1994
dif wt.			0.0045	0.0128	0.0265	0.0575	0.1683	0.7485
Rate (g H ₂ O/m ² /min)			0.21	0.60	0.62	0.68	0.99	1.10
weight (g)		94.3230	94.3148	94.3085	94.2832	94.1971	94.0525	93.4554
dif wt.			0.0082	0.0063	0.0253	0.0861	0.1446	0.5971
Rate (g H ₂ O/m ² /min)			0.39	0.30	0.60	1.01	0.85	0.88
weight (g)		96.8146	96.8096	96.7956	96.7678	96.7398	96.5598	96.2704

	dif wt.		0.0050	0.0140	0.0278	0.0280	0.1800	0.2894
	Rate (g H ₂ O/m ² /min)		0.24	0.66	0.66	0.33	1.06	0.43
	weight (g)	97.0812	97.0774	97.0597	97.0234	96.9884	96.8595	96.4980
	dif wt.		0.0038	0.0177	0.0363	0.0350	0.1289	0.3615
	Rate (g H ₂ O/m ² /min)		0.18	0.83	0.86	0.41	0.76	0.53
	weight (g)	93.8129	93.8047	93.7889	93.7600	93.7069	93.5772	93.0547
	dif wt.		0.0082	0.0158	0.0289	0.0531	0.1297	0.5225
	Rate (g H ₂ O/m ² /min)		0.39	0.74	0.68	0.63	0.76	0.77
A10	weight (g)	94.0765	94.0687	94.0543	94.0310	93.9865	93.8351	93.6258
	dif wt.		0.0078	0.0144	0.0233	0.0445	0.1514	0.2093
	Rate (g H ₂ O/m ² /min)		0.37	0.68	0.55	0.52	0.89	0.31
	weight (g)	94.9222	94.9150	94.8971	94.8754	94.8035	94.6769	94.1435
	dif wt.		0.0072	0.0179	0.0217	0.0719	0.1266	0.5334
	Rate (g H ₂ O/m ² /min)		0.34	0.84	0.51	0.85	0.75	0.79
	weight (g)	94.6773	94.6717	94.6628	94.6273	94.5585	94.4185	93.8252
	dif wt.		0.0056	0.0089	0.0355	0.0688	0.1400	0.5933
	Rate (g H ₂ O/m ² /min)		0.26	0.42	0.84	0.81	0.82	0.87
	weight (g)	95.6397	95.6316	95.6224	95.5977	95.5532	95.4143	94.9884
	dif wt.		0.0081	0.0092	0.0247	0.0445	0.1389	0.4259
	Rate (g H ₂ O/m ² /min)		0.38	0.43	0.58	0.52	0.82	0.63
	weight (g)	96.7850	96.7756	96.7643	96.7246	96.6821	96.5878	96.2039
	dif wt.		0.0094	0.0113	0.0397	0.0425	0.0943	0.3839
	Rate (g H ₂ O/m ² /min)		0.44	0.53	0.94	0.50	0.56	0.57
A12	weight (g)	93.8162	93.8083	93.7947	93.7618	93.7255	93.5830	93.1129
	dif wt.		0.0079	0.0136	0.0329	0.0363	0.1425	0.4701
	Rate (g H ₂ O/m ² /min)		0.37	0.64	0.78	0.43	0.84	0.69
	weight (g)	96.6555	96.6495	96.6322	96.6024	96.5425	96.3977	95.9701
	dif wt.		0.0060	0.0173	0.0298	0.0599	0.1448	0.4276
	Rate (g H ₂ O/m ² /min)		0.28	0.82	0.70	0.71	0.85	0.63
	weight (g)	95.2808	95.2764	95.2677	95.2405	95.1902	95.1177	94.5272

dif wt.		0.0044	0.0087	0.0272	0.0503	0.0725	0.5905
Rate (g H ₂ O/m ² /min)		0.21	0.41	0.64	0.59	0.43	0.87
weight (g)	93.3775	93.3692	93.3603	93.3318	93.2772	93.1091	92.7407
dif wt.		0.0083	0.0089	0.0285	0.0546	0.1681	0.3684
Rate (g H ₂ O/m ² /min)		0.39	0.42	0.67	0.64	0.99	0.54
weight (g)	94.5578	94.5523	94.5338	94.5118	94.4536	94.2984	93.8058
dif wt.		0.0055	0.0185	0.0220	0.0582	0.1552	0.4926
Rate (g H ₂ O/m ² /min)		0.26	0.87	0.52	0.69	0.91	0.73

time (minutes)	30	60	120	240	480	1440
Bl	0.45	0.65	0.82	0.80	0.98	0.93
	0.27	0.18	0.12	0.26	0.24	0.29
2%HPMC	0.27	0.62	0.65	0.64	0.92	0.73
	0.15	0.25	0.12	0.16	0.32	0.16
4%HPMC	0.26	0.61	0.63	0.63	0.88	0.66
	0.11	0.08	0.30	0.26	0.18	0.21
6%HPMC	0.30	0.58	0.61	0.62	0.84	0.66
	0.09	0.21	0.25	0.15	0.22	0.12
8%HPMC	0.33	0.63	0.61	0.64	0.83	0.73
	0.04	0.23	0.36	0.14	0.16	0.13
10%HPMC	0.32	0.58	0.68	0.61	0.84	0.72
	0.09	0.24	0.12	0.08	0.14	0.14
12%HPMC	0.31	0.57	0.58	0.62	0.83	0.69
	0.09	0.16	0.18	0.18	0.13	0.16
2%CLMW	0.47	0.63	0.73	0.72	0.97	0.82
	0.21	0.10	0.25	0.31	0.29	0.22
4%CLMW	0.38	0.66	0.80	0.70	0.97	0.78
	0.29	0.26	0.25	0.29	0.32	0.35
6%CLMW	0.28	0.67	0.69	0.69	0.94	0.76
	0.05	0.16	0.13	0.21	0.16	0.30
8%CLMW	0.28	0.64	0.65	0.68	0.95	0.79
	0.04	0.23	0.14	0.09	0.25	0.30
10%CLMW	0.35	0.61	0.61	0.71	0.89	0.77
	0.15	0.18	0.14	0.27	0.25	0.15
12%CLMW	0.26	0.44	0.61	0.57	0.90	0.75
	0.13	0.26	0.15	0.21	0.35	0.31

2%Alg	0.37	0.65	0.81	0.71	0.89	0.76
	0.17	0.15	0.31	0.40	0.26	0.32
4%Alg	0.28	0.61	0.67	0.65	0.90	0.69
	0.07	0.20	0.24	0.22	0.23	0.36
6%Alg	0.28	0.59	0.63	0.61	0.92	0.67
	0.08	0.20	0.11	0.19	0.21	0.15
8%Alg	0.28	0.63	0.68	0.61	0.89	0.74
	0.10	0.20	0.10	0.27	0.14	0.27
10%Alg	0.36	0.58	0.68	0.64	0.77	0.63
	0.07	0.18	0.19	0.17	0.13	0.22
12%Alg	0.30	0.63	0.66	0.61	0.81	0.69
	0.08	0.22	0.09	0.11	0.22	0.12

Tensile strength Test

	Tensile strength (x 10 ⁻² MPa)	SD	Elongation (%)	SD
B1	5.24	0.38	171.52	26.76
H2	4.95	0.59	171.89	17.58
H4	5.27	0.59	171.87	10.85
H6	5.08	0.62	159.55	10.50
H8	4.94	0.66	149.09	10.04
H10	4.48	0.68	134.49	13.82
H12	4.53	0.68	129.14	12.91
C2	5.14	0.70	179.09	17.11
C4	5.12	0.61	168.55	12.60
C6	4.83	0.47	156.75	17.22
C8	5.34	0.45	166.03	10.01
C10	4.26	0.38	145.79	13.10
C12	4.18	0.38	143.17	23.23
A2	5.64	0.41	185.56	15.04
A4	5.09	0.36	178.99	13.43
A6	5.21	0.34	179.08	24.49
A8	5.15	0.34	185.60	11.98
A10	4.95	0.42	163.76	11.04
A12	4.93	0.41	171.93	12.62

Compression Test

	Compressive strength ($\times 10^{-3}$ MPa)					
	25% strain	SD	50% strain	SD	75% strain	SD
Bl	3.61	0.65	4.60	1.05	10.73	3.15
H2	3.70	0.44	5.02	0.60	11.53	1.58
H4	4.00	0.42	5.41	0.65	12.44	1.90
H6	4.15	0.56	5.69	0.79	13.13	2.39
H8	4.20	0.69	5.76	1.07	13.31	3.19
H10	4.14	0.35	5.91	0.50	14.85	1.80
H12	4.16	0.48	5.88	0.65	14.13	1.91
C2	3.48	0.57	4.63	0.82	10.69	1.72
C4	3.69	0.79	5.08	1.05	11.47	2.36
C6	3.67	0.51	5.18	1.14	11.61	2.20
C8	3.78	0.65	5.03	1.08	11.56	2.47
C10	3.93	0.70	5.19	1.34	13.98	3.06
C12	3.75	0.20	5.38	0.42	14.20	2.47
A2	3.95	0.32	4.98	0.55	11.68	2.06
A4	3.85	0.28	4.99	0.66	11.70	2.11
A6	3.97	0.74	5.29	0.93	12.50	2.14
A8	3.70	0.50	4.89	0.79	11.71	1.90
A10	3.75	0.26	4.98	0.36	11.71	1.01
A12	3.57	0.46	4.56	0.63	9.85	2.46

Silver Releasing test

BL-0.4Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0.000	0.000	0.000	14.4	18.04	0.000	0.000	0.000
2	0.4	0.105	0.131	0.525	14.4	18.04	1.894	0.810	0.131
4	0.4	0.078	0.098	0.390	14.4	18.04	1.932	0.826	0.134
8	0.4	0.048	0.060	0.240	14.4	18.04	1.781	0.762	0.123
12	0.4	0.044	0.055	0.220	14.4	18.04	1.949	0.833	0.135
24	0.4	0.043	0.054	0.216	14.4	18.04	2.154	0.921	0.149

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.4	0.050	0.063	0.250	15.6	19.44	0.972	0.367	0.063
4	0.4	0.065	0.081	0.325	15.6	19.44	1.513	0.571	0.097
8	0.4	0.048	0.060	0.240	15.6	19.44	1.508	0.569	0.097
12	0.4	0.054	0.068	0.270	15.6	19.44	1.865	0.704	0.120
24	0.4	0.157	0.196	0.786	15.6	19.44	4.139	1.562	0.266

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0.000	0	0.0000
2	0.4	0.234	0.293	1.170	15.2	19.00	4.446	1.901	0.2925
4	0.4	0.087	0.109	0.435	15.2	19.00	2.823	1.207	0.1857
8	0.4	0.040	0.050	0.200	15.2	19.00	2.365	1.011	0.1556
12	0.4	0.112	0.140	0.560	15.2	19.00	3.933	1.682	0.2588

24	0.4	0.131	0.164	0.655	15.2	19.00	4.854	2.076	0.3193
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Unit 4	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	0.839	839.393

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0.000	0.000	0	15.1	18.83	0	0	0
2	0.4	0.123	0.154	0.615	15.1	18.83	2.317	1.104	0.154
4	0.4	0.128	0.160	0.640	15.1	18.83	3.026	1.442	0.201
8	0.4	0.076	0.095	0.380	15.1	18.83	2.686	1.280	0.178
12	0.4	0.095	0.119	0.475	15.1	18.83	3.424	1.632	0.227
24	0.4	0.171	0.214	0.855	15.1	18.83	5.331	2.540	0.354

BL-0.6Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.6	0.717	0.896	3.585	14.4	18.04	12.936	5.532	0.896
4	0.6	0.584	0.730	2.920	14.4	18.04	14.121	6.038	0.978
8	0.6	0.386	0.483	1.930	14.4	18.04	13.469	5.760	0.933
12	0.6	0.225	0.281	1.125	14.4	18.04	12.494	5.343	0.866
24	0.6	0.181	0.226	0.905	14.4	18.04	12.826	5.484	0.889

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	1.307	1307.287

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.6	0.159	0.199	0.795	14.0	17.44	2.773	1.273	0.199
4	0.6	0.198	0.248	0.990	14.0	17.44	4.248	1.950	0.304
8	0.6	0.060	0.075	0.300	14.0	17.44	2.831	1.299	0.203
12	0.6	0.066	0.083	0.330	14.0	17.44	3.236	1.485	0.232
24	0.6	0.102	0.128	0.510	14.0	17.44	4.194	1.925	0.301

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.6	0.123	0.154	0.615	14.4	18.04	2.219	0.949	0.154
4	0.6	0.604	0.755	3.020	14.4	18.04	11.512	4.923	0.798
8	0.6	0.265	0.331	1.325	14.4	18.04	8.416	3.599	0.583
12	0.6	0.213	0.266	1.065	14.4	18.04	8.803	3.764	0.610
24	0.6	0.189	0.236	0.945	14.4	18.04	9.435	4.034	0.654

Unit 4

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.65	1.589	1589.422

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.6	0.038	0.0475	0.190	15.6	19.44	0.739	0.279	0.048
4	0.6	0.171	0.2138	0.855	15.6	19.44	3.514	1.326	0.226
8	0.6	0.109	0.1363	0.545	15.6	19.44	3.164	1.194	0.203
12	0.6	0.127	0.1588	0.635	15.6	19.44	4.059	1.532	0.261
24	0.6	0.224	0.2800	1.120	15.6	19.44	6.579	2.484	0.423

BL-0.8Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0.000	0	0	15.2	19.00	0	0	0
2	0.8	0.871	1.089	4.355	15.2	19.00	16.549	7.077	1.089
4	0.8	0.477	0.596	2.385	15.2	19.00	13.418	5.738	0.883
8	0.8	0.160	0.200	0.800	15.2	19.00	9.780	4.182	0.643
12	0.8	0.147	0.184	0.735	15.2	19.00	10.333	4.419	0.680
24	0.8	0.817	1.021	4.085	15.2	19.00	23.798	10.176	1.566

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	1.679	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	1.036	1.295	5.180	14.4	18.04	18.687	8.905	1.295
4	0.8	0.472	0.590	2.360	14.4	18.04	13.694	6.526	0.949
8	0.8	0.561	0.701	2.805	14.4	18.04	17.659	8.415	1.224
12	0.8	0.455	0.569	2.275	14.4	18.04	18.552	8.841	1.286
24	0.8	0.386	0.483	1.930	14.4	18.04	19.582	9.332	1.357

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	1.743	1743.049

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.8	0.994	1.243	4.970	14.0	17.44	17.333	7.955	1.243
4	0.8	0.296	0.370	1.480	14.0	17.44	10.132	4.650	0.726
8	0.8	0.183	0.229	0.915	14.0	17.44	9.641	4.425	0.691
12	0.8	0.254	0.318	1.270	14.0	17.44	11.794	5.413	0.845
24	0.8	0.994	1.243	4.970	14.0	17.44	25.968	11.918	1.861

Unit 4	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	1.851	1851.400

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	0.8	0.846	1.058	4.230	13.4	16.77	14.188	6.131	1.058
4	0.8	0.391	0.489	1.955	13.4	16.77	10.787	4.661	0.804
8	0.8	0.241	0.301	1.205	13.4	16.77	10.227	4.419	0.762
12	0.8	0.345	0.431	1.725	13.4	16.77	13.176	5.693	0.982
24	0.8	0.741	0.926	3.705	13.4	16.77	21.542	9.308	1.606

Bl-1.0Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	2.179	2178.811

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	1	3.078	3.848	15.390	14.0	17.44	53.673	24.634	3.848
4	1	1.807	2.259	9.035	14.0	17.44	46.900	21.525	3.362
8	1	1.074	1.343	5.370	14.0	17.44	43.153	19.806	3.093
12	1	1.001	1.251	5.005	14.0	17.44	47.250	21.686	3.387
24	1	1.131	1.414	5.655	14.0	17.44	54.522	25.024	3.908

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	2.754	3.443	13.770	13.4	16.77	46.187	19.958	3.443
4	1	2.900	3.625	14.500	13.4	16.77	62.405	26.966	4.651
8	1	0.986	1.233	4.930	13.4	16.77	44.806	19.361	3.340
12	1	1.024	1.280	5.120	13.4	16.77	50.373	21.767	3.755
24	1	1.008	1.260	5.040	13.4	16.77	55.225	23.863	4.116

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	2.461	3.076	12.305	13.4	16.77	41.273	17.834	3.076
4	1	2.178	2.723	10.890	13.4	16.77	48.832	21.101	3.640
8	1	1.613	2.016	8.065	13.4	16.77	50.246	21.712	3.745
12	1	1.554	1.943	7.770	13.4	16.77	57.322	24.769	4.272

24	1	1.655	2.069	8.275	13.4	16.77	66.786	28.858	4.978
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Unit 4	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	1.278	1.598	6.390	13.4	16.77	21.433	9.261	1.598
4	1	1.105	1.381	5.525	13.4	16.77	24.922	10.769	1.858
8	1	1.403	1.754	7.015	13.4	16.77	35.444	15.316	2.642
12	1	1.412	1.765	7.060	13.4	16.77	42.610	18.412	3.176
24	1	1.451	1.814	7.255	13.4	16.77	50.324	21.745	3.751

Total amount/area orifice (mcg/cm²)

time (hr)	Bl-0.4Ag	SD	Bl-0.6Ag	SD	Bl-0.8Ag	SD	Bl-1.0Ag	SD
0	0	0	0	0	0	0	0	0
2	1.05	0.65	0.83	0.51	7.52	1.19	17.92	6.43
4	1.01	0.39	2.73	1.92	5.39	0.91	20.09	6.76
8	0.91	0.31	2.03	1.36	5.36	2.04	19.05	2.69
12	1.21	0.52	2.26	1.30	6.09	1.91	21.66	2.60
24	1.77	0.70	2.81	1.09	10.18	1.22	24.87	2.98

Conc (ppm)

time (hr)	Bl-0.4Ag	SD	Bl-0.6Ag	SD	Bl-0.8Ag	SD	Bl-1.0Ag	SD
0	0	0	0	0	0	0	0	0
2	0.16	0.10	0.32	0.08	1.17	0.12	2.99	0.98
4	0.15	0.05	0.58	0.31	0.84	0.10	3.38	1.15
8	0.14	0.04	0.48	0.22	0.83	0.27	3.20	0.46
12	0.19	0.07	0.49	0.21	0.95	0.26	3.65	0.48
24	0.27	0.09	0.57	0.18	1.60	0.21	4.19	0.55

Silver Releasing Test

H4-0.4Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.4	0.692	0.865	3.460	14.4	18.04	12.485	5.339	0.865
4	0.4	0.577	0.721	2.885	14.4	18.04	13.870	5.931	0.961
8	0.4	0.802	1.003	4.010	14.4	18.04	20.814	8.900	1.442
12	0.4	0.856	1.070	4.280	14.4	18.04	25.799	11.032	1.787
24	0.4	0.852	1.065	4.260	14.4	18.04	30.007	12.831	2.079

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.4	0.852	1.065	4.260	15.6	19.44	16.561	6.252	1.065
4	0.4	0.896	1.120	4.480	15.6	19.44	21.676	8.183	1.394
8	0.4	0.792	0.990	3.960	15.6	19.44	24.135	9.111	1.552
12	0.4	0.798	0.998	3.990	15.6	19.44	28.211	10.650	1.814
24	0.4	0.832	1.040	4.160	15.6	19.44	32.862	12.405	2.113

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.18	0.872	871.525

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.4	0.911	1.139	4.555	14.0	17.44	15.886	7.291	1.139
4	0.4	0.918	1.148	4.590	14.0	17.44	20.563	9.438	1.474
8	0.4	0.867	1.084	4.335	14.0	17.44	24.263	11.136	1.739
12	0.4	0.697	0.871	3.485	14.0	17.44	25.634	11.765	1.838
24	0.4	0.791	0.989	3.955	14.0	17.44	30.758	14.117	2.205

H4-0.6Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	1.389	1388.550

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	0.6	0.778	0.973	3.89	13.4	16.77	13.048	5.638	0.973
4	0.6	0.802	1.003	4.01	13.4	16.77	17.340	7.493	1.292
8	0.6	0.855	1.069	4.28	13.4	16.77	22.239	9.610	1.658
12	0.6	1.016	1.270	5.08	13.4	16.77	29.214	12.624	2.177
24	0.6	0.829	1.036	4.15	13.4	16.77	31.158	13.464	2.322

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	0.6	0.783	0.979	3.915	15.2	19.00	14.877	6.362	0.979
4	0.6	0.856	1.070	4.280	15.2	19.00	20.179	8.629	1.328
8	0.6	0.890	1.113	4.450	15.2	19.00	25.105	10.735	1.652
12	0.6	0.874	1.093	4.370	15.2	19.00	29.251	12.508	1.924
24	0.6	0.837	1.046	4.185	15.2	19.00	32.918	14.076	2.166

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	1.259	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	0.6	0.656	0.820	3.280	15.1	18.83	12.355	5.887	0.820
4	0.6	0.728	0.910	3.640	15.1	18.83	16.991	8.097	1.128
8	0.6	0.840	1.050	4.200	15.1	18.83	22.740	10.836	1.509

12	0.6	1.488	1.860	7.440	15.1	18.83	39.144	18.653	2.598
24	0.6	1.106	1.383	5.530	15.1	18.83	39.390	18.771	2.614

H4-0.8Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	0.847	1.059	4.235	14.4	18.04	15.281	6.534	1.059
4	0.8	0.682	0.853	3.410	14.4	18.04	16.539	7.072	1.146
8	0.8	1.512	1.890	7.560	14.4	18.04	34.924	14.934	2.420
12	0.8	1.483	1.854	7.415	14.4	18.04	41.961	17.943	2.907
24	0.8	1.369	1.711	6.845	14.4	18.04	47.319	20.234	3.278

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	2.119	2119.229

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.8	1.002	1.253	5.010	15.6	19.44	19.476	7.352	1.253
4	0.8	1.020	1.275	5.100	15.6	19.44	24.836	9.376	1.597
8	0.8	1.893	2.366	9.465	15.6	19.44	46.905	17.707	3.016
12	0.8	1.704	2.130	8.520	15.6	19.44	52.697	19.893	3.389
24	0.8	1.673	2.091	8.365	15.6	19.44	60.614	22.882	3.898

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	1.743	1743.049

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
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0	0	0	0	0	14.0	17.44	0.0000	0.000	0
2	0.8	1.118	1.398	5.590	14.0	17.44	19.495	8.948	1.398
4	0.8	1.314	1.643	6.570	14.0	17.44	28.503	13.082	2.043
8	0.8	1.662	2.078	8.310	14.0	17.44	41.141	18.882	2.949
12	0.8	1.394	1.743	6.970	14.0	17.44	44.778	20.552	3.210
24	0.8	1.314	1.643	6.570	14.0	17.44	50.353	23.110	3.610

H4-1.0Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	2.148	2.685	10.740	13.4	16.77	36.024	15.566	2.685
4	1	1.783	2.229	8.915	13.4	16.77	40.642	17.562	3.029
8	1	1.283	1.604	6.415	13.4	16.77	41.172	17.791	3.069
12	1	1.942	2.428	9.710	13.4	16.77	58.639	25.338	4.371
24	1	1.753	2.191	8.765	13.4	16.77	65.179	28.164	4.858

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	2.339	2338.575

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.0	0	0	0
2	1	1.432	1.790	7.160	15.2	19.0	27.208	11.634	1.790
4	1	1.245	1.556	6.225	15.2	19.0	30.815	13.177	2.027
8	1	2.643	3.304	13.215	15.2	19.0	63.602	27.197	4.184
12	1	3.166	3.958	15.830	15.2	19.0	86.754	37.097	5.708
24	1	2.074	2.593	10.370	15.2	19.0	81.836	34.994	5.384

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	2.098	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	1	1.126	1.408	5.630	15.1	18.83	21.206	10.106	1.408
4	1	1.331	1.664	6.655	15.1	18.83	30.697	14.628	2.037
8	1	1.719	2.149	8.595	15.1	18.83	44.660	21.282	2.964
12	1	2.314	2.893	11.570	15.1	18.83	64.460	30.718	4.278
24	1	1.687	2.109	8.435	15.1	18.83	64.222	30.604	4.263

H6-0.4Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.4	0.508	0.635	2.540	14.4	18.04	9.165	3.919	0.635
4	0.4	0.541	0.676	2.705	14.4	18.04	12.301	5.260	0.852
8	0.4	0.621	0.776	3.105	14.4	18.04	16.449	7.034	1.140
12	0.4	0.530	0.663	2.650	14.4	18.04	17.912	7.659	1.241
24	0.4	0.706	0.883	3.530	14.4	18.04	23.737	10.150	1.645

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.438	0	0	0
2	0.4	0.668	0.835	3.340	15.6	19.438	12.984	4.901	0.835
4	0.4	0.932	1.165	4.660	15.6	19.438	21.456	8.099	1.380
8	0.4	0.835	1.044	4.175	15.6	19.438	24.230	9.147	1.558
12	0.4	0.740	0.925	3.700	15.6	19.438	26.559	10.026	1.708
24	0.4	0.956	1.195	4.780	15.6	19.438	34.457	13.007	2.216

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.18	0.8715	871.5246

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.438	0	0	0
2	0.4	0.776	0.970	3.880	14.0	17.438	13.532	6.210	0.970
4	0.4	0.929	1.161	4.645	14.0	17.438	20.079	9.216	1.439
8	0.4	0.886	1.108	4.430	14.0	17.438	23.975	11.004	1.719
12	0.4	0.886	1.108	4.430	14.0	17.438	28.405	13.037	2.036
24	0.4	0.533	0.666	2.665	14.0	17.438	26.679	12.245	1.912

H6-0.6Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.31	1.389	1388.550

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	0.6	0.837	1.046	4.185	13.4	16.77	14.037	6.066	1.046
4	0.6	2.072	2.590	10.360	13.4	16.77	38.934	16.824	2.902
8	0.6	1.435	1.794	7.175	13.4	16.77	38.611	16.684	2.878
12	0.6	0.654	0.818	3.270	13.4	16.77	32.688	14.125	2.436
24	0.6	1.772	2.215	8.860	13.4	16.77	54.708	23.640	4.078

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0

2	0.6	0.945	1.181	4.725	15.2	19.00	17.955	7.678	1.181
4	0.6	2.134	2.668	10.670	15.2	19.00	45.271	19.358	2.978
8	0.6	1.951	2.439	9.755	15.2	19.00	52.464	22.434	3.452
12	0.6	1.168	1.460	5.840	15.2	19.00	47.342	20.244	3.115
24	0.6	1.116	1.395	5.580	15.2	19.00	52.194	22.319	3.434

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	1.259	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	0.6	0.508	0.635	2.540	15.1	18.83	9.567	4.559	0.635
4	0.6	1.876	2.345	9.380	15.1	18.83	37.871	18.047	2.514
8	0.6	1.900	2.375	9.500	15.1	18.83	47.703	22.732	3.166
12	0.6	1.804	2.255	9.020	15.1	18.83	55.395	26.398	3.677
24	0.6	1.727	2.159	8.635	15.1	18.83	62.965	30.005	4.179

H6-0.8Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	0.944	1.180	4.720	14.4	18.04	17.031	7.283	1.180
4	0.8	1.204	1.505	6.020	14.4	18.04	26.442	11.307	1.832
8	0.8	2.275	2.844	11.375	14.4	18.04	51.785	22.144	3.588
12	0.8	2.881	3.601	14.405	14.4	18.04	74.093	31.683	5.133
24	0.8	1.945	2.431	9.725	14.4	18.04	71.611	30.622	4.962

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	2.119	2119.229

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.8	1.101	1.376	5.505	15.6	19.44	21.401	8.079	1.376
4	0.8	0.921	1.151	4.605	15.6	19.44	23.407	8.836	1.505
8	0.8	2.803	3.504	14.015	15.6	19.44	64.593	24.384	4.154
12	0.8	2.578	3.223	12.890	15.6	19.44	74.235	28.023	4.774
24	0.8	2.247	2.809	11.235	15.6	19.44	80.691	30.461	5.189

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	1.743	1743.049

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.8	1.068	1.335	5.340	14.0	17.44	18.623	8.547	1.335
4	0.8	2.660	3.325	13.300	14.0	17.44	51.724	23.739	3.708
8	0.8	2.245	2.806	11.225	14.0	17.44	57.787	26.522	4.142
12	0.8	2.147	2.684	10.735	14.0	17.44	67.303	30.890	4.825
24	0.8	1.923	2.404	9.615	14.0	17.44	74.132	34.024	5.314

H6-1.0Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	0.995	1.244	4.975	13.4	16.77	16.687	7.211	1.244
4	1	1.162	1.453	5.810	13.4	16.77	24.463	10.570	1.823
8	1	2.543	3.179	12.715	13.4	16.77	53.433	23.089	3.983
12	1	2.887	3.609	14.435	13.4	16.77	71.917	31.076	5.360
24	1	2.364	2.955	11.820	13.4	16.77	77.581	33.523	5.782

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	2.339	2338.575

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	1	0.748	0.935	3.740	15.2	19.00	14.212	6.077	0.935
4	1	2.553	3.191	12.765	15.2	19.00	52.247	22.341	3.437
8	1	2.413	3.016	12.065	15.2	19.00	62.352	26.662	4.102
12	1	2.679	3.349	13.395	15.2	19.00	79.471	33.983	5.228
24	1	2.213	2.766	11.065	15.2	19.00	84.012	35.924	5.527

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	2.098	2098.482

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	1	1.023	1.279	5.115	15.1	18.83	19.267	9.181	1.279
4	1	0.921	1.151	4.605	15.1	18.83	22.461	10.703	1.491
8	1	1.645	2.056	8.225	15.1	18.83	40.701	19.395	2.701
12	1	2.335	2.919	11.675	15.1	18.83	61.921	29.507	4.110
24	1	2.556	3.195	12.780	15.1	18.83	77.758	37.054	5.161

CHULALONGKORN UNIVERSITY

Amount/area orifice

time (hr)	H4-0.4Ag	SD	H4-0.6Ag	SD	H4-0.8Ag	SD	H4-1.0Ag	SD	H6-0.4Ag	SD
0	0	0	0	0	0	0	0	0	0	0
2	6.294	0.977	5.962	0.368	7.611	1.227	12.435	2.817	5.010	1.150
4	7.850	1.777	8.073	0.568	9.843	3.032	15.122	2.234	7.525	2.040
8	9.716	1.235	10.394	0.681	17.174	2.027	22.090	4.755	9.061	1.986
12	11.149	0.567	14.595	3.515	19.462	1.357	31.051	5.886	10.241	2.695
24	13.118	0.891	15.437	2.903	22.075	1.599	31.254	3.461	11.801	1.479

time (hr)	H6-0.6Ag	SD	H6-0.8Ag	SD	H6-1.0Ag	SD
0	0	0	0	0	0	0
2	6.101	1.560	7.970	0.639	7.490	1.571
4	18.076	1.268	14.627	7.987	14.538	6.758
8	20.617	3.409	24.350	2.189	23.049	3.634
12	20.255	6.137	30.199	1.925	31.522	2.271
24	25.321	4.110	31.702	2.013	35.501	1.803

Concentration (ppm)

time (hr)	H4-0.4Ag	SD	H4-0.6Ag	SD	H4-0.8Ag	SD	H4-1.0Ag	SD	H6-0.4Ag	SD
0	0	0	0	0	0	0	0	0	0	0
2	1.023	0.142	0.924	0.090	1.236	0.170	1.961	0.656	0.813	0.169
4	1.276	0.276	1.249	0.107	1.595	0.449	2.365	0.576	1.224	0.323
8	1.578	0.150	1.606	0.084	2.795	0.327	3.406	0.676	1.472	0.299
12	1.813	0.025	2.233	0.340	3.169	0.243	4.785	0.800	1.662	0.400
24	2.132	0.065	2.367	0.228	3.595	0.310	4.835	0.561	1.924	0.286

time (hr)	H6-0.6Ag	SD	H6-0.8Ag	SD	H6-1.0Ag	SD
0	0	0	0	0	0	0
2	0.954	0.358	1.297	0.103	1.153	0.189
4	2.798	0.487	2.348	1.189	2.250	1.041
8	3.165	0.537	3.961	0.324	3.595	0.777
12	3.076	0.569	4.911	0.195	4.899	0.687
24	3.897	0.119	5.155	0.179	5.490	0.312

Silver Releasing Test

C4-0.4Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.4	0.022	0.028	0.110	14.4	18.04	0.397	0.170	0.028
4	0.4	0.521	0.651	2.605	14.4	18.04	9.510	4.066	0.659
8	0.4	0.734	0.918	3.670	14.4	18.04	15.958	6.824	1.106
12	0.4	0.934	1.168	4.670	14.4	18.04	23.236	9.936	1.610
24	0.4	0.851	1.064	4.255	14.4	18.04	26.408	11.293	1.830

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.4	0.027	0.034	0.135	15.6	19.44	0.525	0.198	0.034
4	0.4	0.430	0.538	2.150	15.6	19.44	8.493	3.206	0.546
8	0.4	0.535	0.669	2.675	15.6	19.44	12.684	4.788	0.816
12	0.4	1.033	1.291	5.165	15.6	19.44	25.039	9.452	1.610
24	0.4	0.755	0.944	3.775	15.6	19.44	24.800	9.362	1.595

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	0.872	871.525

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.4	0.079	0.099	0.395	14.0	17.44	1.378	0.632	0.099
4	0.4	0.578	0.723	2.890	14.0	17.44	10.474	4.807	0.751
8	0.4	0.667	0.834	3.335	14.0	17.44	14.916	6.846	1.069
12	0.4	0.652	0.815	3.260	14.0	17.44	17.989	8.256	1.290
24	0.4	0.764	0.955	3.820	14.0	17.44	23.202	10.649	1.663

C4-0.6Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	1.389	1388.550

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	0.6	0.128	0.160	0.640	13.4	16.77	2.147	0.928	0.160
4	0.6	0.423	0.529	2.115	13.4	16.77	7.734	3.342	0.576
8	0.6	1.108	1.385	5.540	13.4	16.77	21.337	9.220	1.590
12	0.6	0.992	1.240	4.960	13.4	16.77	24.932	10.773	1.858
24	0.6	0.845	1.056	4.225	13.4	16.77	27.426	11.851	2.044

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	0.6	0.089	0.111	0.445	15.2	19.00	1.691	0.723	0.111
4	0.6	0.147	0.184	0.735	15.2	19.00	3.238	1.385	0.213
8	0.6	0.948	1.185	4.740	15.2	19.00	19.192	8.207	1.263
12	0.6	1.044	1.305	5.220	15.2	19.00	25.756	11.014	1.694
24	0.6	0.958	1.198	4.790	15.2	19.00	29.342	12.547	1.930

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	1.259	1259.089

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	0.6	0.092	0.115	0.460	15.1	18.83	1.733	0.826	0.115
4	0.6	0.778	0.973	3.890	15.1	18.83	15.112	7.202	1.003
8	0.6	0.458	0.573	2.290	15.1	18.83	12.976	6.183	0.861
12	0.6	1.045	1.306	5.225	15.1	18.83	26.321	12.543	1.747

24	0.6	0.985	1.231	4.925	15.1	18.83	30.416	14.494	2.019
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C4-0.8Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	0.162	0.2025	0.81	14.4	18.04	2.923	1.250	0.203
4	0.8	1.055	1.31875	5.275	14.4	18.04	19.844	8.485	1.375
8	0.8	2.538	3.1725	12.69	14.4	18.04	51.875	22.182	3.594
12	0.8	2.127	2.65875	10.635	14.4	18.04	57.150	24.438	3.960
24	0.8	1.623	2.02875	8.115	14.4	18.04	58.692	25.097	4.066

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	2.119	2119.229

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.8	0.092	0.115	0.460	15.6	19.44	1.788	0.675	0.115
4	0.8	2.533	3.166	12.665	15.6	19.44	49.695	18.760	3.196
8	0.8	1.637	2.046	8.185	15.6	19.44	44.944	16.966	2.890
12	0.8	2.182	2.728	10.910	15.6	19.44	63.723	24.055	4.098
24	0.8	1.940	2.425	9.700	15.6	19.44	69.929	26.398	4.497

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
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2.18	1.743	1743.049
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time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.8	0.103	0.129	0.515	14.0	17.44	1.796	0.824	0.129
4	0.8	0.736	0.920	3.680	14.0	17.44	13.349	6.127	0.957
8	0.8	2.026	2.533	10.130	14.0	17.44	39.523	18.140	2.833
12	0.8	2.003	2.504	10.015	14.0	17.44	49.252	22.605	3.531
24	0.8	1.496	1.870	7.480	14.0	17.44	50.427	23.144	3.615

C4-1.0Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	0.042	0.053	0.210	13.4	16.77	0.704	0.304	0.053
4	1	0.163	0.204	0.815	13.4	16.77	2.944	1.272	0.219
8	1	2.947	3.684	14.735	13.4	16.77	50.449	21.799	3.760
12	1	1.747	2.184	8.735	13.4	16.77	45.059	19.470	3.358
24	1	1.896	2.370	9.480	13.4	16.77	56.293	24.324	4.196

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	2.339	2338.575

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	1	0.022	0.028	0.110	15.2	19.00	0.418	0.179	0.028
4	1	0.403	0.504	2.015	15.2	19.00	7.767	3.321	0.511
8	1	2.280	2.850	11.400	15.2	19.00	45.445	19.433	2.990
12	1	2.944	3.680	14.720	15.2	19.00	69.461	29.702	4.570

24	1	1.511	1.889	7.555	15.2	19.00	56.954	24.354	3.747
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Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	2.098	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	1	0.436	0.545	2.180	15.1	18.83	8.211	3.913	0.545
4	1	0.931	1.164	4.655	15.1	18.83	19.714	9.394	1.308
8	1	2.902	3.628	14.510	15.1	18.83	61.489	29.302	4.081
12	1	2.331	2.914	11.655	15.1	18.83	65.246	31.092	4.330
24	1	1.708	2.135	8.540	15.1	18.83	65.167	31.055	4.325

C6-0.4Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.4	0.036	0.045	0.180	14.4	18.04	0.650	0.278	0.045
4	0.4	0.245	0.306	1.225	14.4	18.04	4.600	1.967	0.319
8	0.4	0.647	0.809	3.235	14.4	18.04	13.078	5.592	0.906
12	0.4	0.854	1.068	4.270	14.4	18.04	20.048	8.573	1.389
24	0.4	0.778	0.973	3.890	14.4	18.04	22.946	9.812	1.590

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.4	0.050	0.063	0.250	15.6	19.44	0.972	0.367	0.063

								area orifice (mcg/cm ²)	
0	0	0	0	0	15.2	19.00	0	0	0
2	0.6	0.116	0.145	0.580	15.2	19.00	2.204	0.942	0.145
4	0.6	0.695	0.869	3.475	15.2	19.00	13.785	5.895	0.907
8	0.6	1.990	2.488	9.950	15.2	19.00	41.865	17.902	2.754
12	0.6	1.075	1.344	5.375	15.2	19.00	34.430	14.723	2.265
24	0.6	0.984	1.230	4.920	15.2	19.00	38.076	16.282	2.505

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.098	1.259	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	0.6	0.101	0.126	0.505	15.1	18.83	1.902	0.813	0.126
4	0.6	0.702	0.878	3.510	15.1	18.83	13.726	5.869	0.911
8	0.6	1.036	1.295	5.180	15.1	18.83	23.526	10.060	1.561
12	0.6	1.946	2.433	9.730	15.1	18.83	45.845	19.604	3.043
24	0.6	1.876	2.345	9.380	15.1	18.83	54.256	23.201	3.601

C6-0.8Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	0.038	0.048	0.190	14.4	18.04	0.686	0.293	0.048
4	0.8	0.822	1.028	4.110	14.4	18.04	15.020	6.423	1.041
8	0.8	1.562	1.953	7.810	14.4	18.04	32.481	13.889	2.250
12	0.8	2.147	2.684	10.735	14.4	18.04	50.845	21.742	3.523
24	0.8	2.169	2.711	10.845	14.4	18.04	61.977	26.502	4.294

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.65	2.119	2119.229

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.8	0.096	0.120	0.480	15.6	19.44	1.866	0.704	0.120
4	0.8	0.741	0.926	3.705	15.6	19.44	14.883	5.618	0.957
8	0.8	1.993	2.491	9.965	15.6	19.44	42.924	16.204	2.760
12	0.8	2.305	2.881	11.525	15.6	19.44	58.953	22.255	3.791
24	0.8	1.920	2.400	9.600	15.6	19.44	62.995	23.780	4.051

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.18	1.743	1743.049

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.8	0.224	0.280	1.120	14.0	17.44	3.906	1.793	0.280
4	0.8	0.589	0.736	2.945	14.0	17.44	11.391	5.228	0.817
8	0.8	2.869	3.586	14.345	14.0	17.44	54.093	24.827	3.878
12	0.8	1.320	1.650	6.600	14.0	17.44	41.428	19.014	2.970
24	0.8	1.956	2.445	9.780	14.0	17.44	59.118	27.133	4.238

C6-1.0Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.78	0	0	0
2	1	0.053	0.066	0.265	13.4	16.78	0.889	0.384	0.066
4	1	0.662	0.828	3.310	13.4	16.78	11.370	4.913	0.847
8	1	2.770	3.463	13.850	13.4	16.78	50.042	21.623	3.729
12	1	2.651	3.314	13.255	13.4	16.78	61.896	26.745	4.612
24	1	1.666	2.083	8.330	13.4	16.78	58.627	25.333	4.369

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	2.339	2338.575

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	1	0.111	0.13875	0.555	15.2	19.00	2.109	0.902	0.139
4	1	0.507	0.63375	2.535	15.2	19.00	10.188	4.356	0.670
8	1	1.959	2.44875	9.795	15.2	19.00	40.311	17.237	2.652
12	1	2.663	3.32875	13.315	15.2	19.00	63.482	27.146	4.176
24	1	2.594	3.2425	12.97	15.2	19.00	75.486	32.279	4.966

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	2.098	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	1	0.156	0.195	0.780	15.1	18.83	2.938	1.400	0.195
4	1	0.506	0.633	2.530	15.1	18.83	10.309	4.913	0.684
8	1	1.936	2.420	9.680	15.1	18.83	39.770	18.952	2.640
12	1	2.720	3.400	13.600	15.1	18.83	64.214	30.600	4.262
24	1	2.045	2.556	10.225	15.1	18.83	65.102	31.024	4.321

Amount/Area orifice

time (hr)	C4-0.4Ag	SD	C4-0.6Ag	SD	C4-0.8Ag	SD	C4 -1.0Ag	SD	C6-0.4Ag	SD
0	0	0	0	0	0	0	0	0	0	0
2	0.33	0.26	0.83	0.10	0.92	0.30	1.47	2.12	0.29	0.07
4	4.03	0.80	3.98	2.96	11.12	6.72	4.66	4.22	2.19	1.11
8	6.15	1.18	7.87	1.55	19.10	2.74	23.51	5.15	6.08	0.45
12	9.21	0.86	11.44	0.96	23.70	0.97	26.75	6.35	7.93	0.99
24	10.43	0.98	12.96	1.37	24.88	1.64	26.58	3.88	9.57	1.58

time (hr)	C6-0.6Ag	SD	C6-0.8Ag	SD	C6-1.0Ag	SD
0	0	0	0	0	0	0
2	0.83	0.11	0.93	0.77	0.90	0.51
4	5.24	1.10	5.76	0.61	4.73	0.32
8	12.17	5.02	18.31	5.76	19.27	2.21
12	16.16	3.00	21.00	1.74	28.16	2.12
24	18.04	4.55	25.81	1.78	29.55	3.70

Concentration (ppm)

time (hr)	C4-0.4Ag	SD	C4-0.6Ag	SD	C4-0.8Ag	SD	C4-1.0Ag	SD	C6-0.4Ag	SD
0	0	0	0	0	0	0	0	0	0	0
2	0.05	0.04	0.13	0.03	0.15	0.05	0.21	0.29	0.05	0.01
4	0.65	0.10	0.60	0.40	1.84	1.19	0.68	0.56	0.35	0.16
8	1.00	0.16	1.24	0.37	3.11	0.42	3.61	0.56	0.99	0.10
12	1.50	0.19	1.77	0.08	3.86	0.30	4.09	0.64	1.29	0.12
24	1.70	0.12	2.00	0.06	4.06	0.44	4.09	0.30	1.55	0.19

time (hr)	C6-0.6Ag	SD	C6-0.8Ag	SD	C6-1.0Ag	SD
0	0	0	0	0	0	0
2	0.13	0.01	0.15	0.12	0.13	0.06
4	0.83	0.13	0.94	0.11	0.73	0.10
8	1.93	0.72	2.96	0.83	3.01	0.63
12	2.58	0.41	3.43	0.42	4.35	0.23
24	2.88	0.63	4.19	0.13	4.55	0.36

Silver Releasing Test

A4-0.4Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.4	0.390	0.488	1.950	14.4	18.04	7.036	3.009	0.488
4	0.4	0.637	0.796	3.185	14.4	18.04	13.443	5.748	0.931
8	0.4	0.769	0.961	3.845	14.4	18.04	19.009	8.128	1.317
12	0.4	1.400	1.750	7.000	14.4	18.04	34.238	14.641	2.372
24	0.4	0.708	0.885	3.540	14.4	18.04	28.754	12.295	1.992

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.4	0.646	0.808	3.230	15.6	19.44	12.557	4.740	0.808
4	0.4	0.578	0.723	2.890	15.6	19.44	14.465	5.460	0.930
8	0.4	1.133	1.416	5.665	15.6	19.44	28.143	10.624	1.810
12	0.4	0.996	1.245	4.980	15.6	19.44	31.145	11.757	2.003
24	0.4	0.862	1.078	4.310	15.6	19.44	33.520	12.654	2.156

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	0.872	871.525

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.4	0.448	0.560	2.240	14.0	17.44	7.812	3.585	0.560
4	0.4	1.089	1.361	5.445	14.0	17.44	21.229	9.744	1.522
8	0.4	1.142	1.428	5.710	14.0	17.44	27.599	12.667	1.978
12	0.4	0.648	0.810	3.240	14.0	17.44	24.695	11.334	1.770
24	0.4	0.698	0.873	3.490	14.0	17.44	28.806	13.221	2.065

A4-0.6Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	1.389	1388.550

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	0.6	0.152	0.190	0.760	13.4	16.77	2.549	1.102	0.190
4	0.6	0.828	1.035	4.140	13.4	16.77	14.646	6.329	1.092
8	0.6	1.148	1.435	5.740	13.4	16.77	24.153	10.437	1.800
12	0.6	1.770	2.213	8.850	13.4	16.77	40.324	17.424	3.006
24	0.6	1.535	1.919	7.675	13.4	16.77	45.233	19.546	3.371

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	0.6	0.341	0.426	1.705	15.2	19.00	6.479	2.770	0.426
4	0.6	0.901	1.126	4.505	15.2	19.00	18.824	8.049	1.238
8	0.6	1.035	1.294	5.175	15.2	19.00	25.875	11.064	1.702
12	0.6	1.038	1.298	5.190	15.2	19.00	31.107	13.302	2.047
24	0.6	1.365	1.706	6.825	15.2	19.00	42.510	18.178	2.797

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	1.259	1259.089

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	0.6	0.207	0.259	1.035	15.1	18.83	3.899	1.858	0.259
4	0.6	0.444	0.555	2.220	15.1	18.83	9.397	4.478	0.624

8	0.6	1.310	1.638	6.550	15.1	18.83	27.927	13.308	1.854
12	0.6	1.587	1.984	7.935	15.1	18.83	39.694	18.915	2.635
24	0.6	1.516	1.895	7.580	15.1	18.83	46.291	22.059	3.072

A4-0.8Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	1.172	1.465	5.860	14.4	18.04	21.145	9.042	1.465
4	0.8	1.535	1.919	7.675	14.4	18.04	33.554	14.348	2.325
8	0.8	0.970	1.213	4.850	14.4	18.04	31.035	13.271	2.150
12	0.8	1.017	1.271	5.085	14.4	18.04	36.733	15.708	2.545
24	0.8	1.953	2.441	9.765	14.4	18.04	58.705	25.103	4.067

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	2.119	2119.229

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.8	1.162	1.453	5.810	15.6	19.44	22.586	8.526	1.453
4	0.8	1.354	1.693	6.770	15.6	19.44	32.128	12.128	2.066
8	0.8	2.043	2.554	10.215	15.6	19.44	52.291	19.740	3.363
12	0.8	1.290	1.613	6.450	15.6	19.44	47.869	18.070	3.078
24	0.8	1.955	2.444	9.775	15.6	19.44	67.245	25.385	4.324

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	1.743	1743.049

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.8	1.046	1.308	5.230	14.0	17.44	18.240	8.371	1.308
4	0.8	0.957	1.196	4.785	14.0	17.44	21.918	10.059	1.571
8	0.8	1.568	1.960	7.840	14.0	17.44	37.357	17.146	2.678
12	0.8	1.498	1.873	7.490	14.0	17.44	43.976	20.184	3.152
24	0.8	1.221	1.526	6.105	14.0	17.44	46.636	21.404	3.343

A4-1.0Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	1.186	1.483	5.930	13.4	16.77	19.890	8.595	1.483
4	1	1.436	1.795	7.180	13.4	16.77	30.013	12.969	2.237
8	1	3.262	4.078	16.310	13.4	16.77	67.816	29.304	5.055
12	1	1.235	1.544	6.175	13.4	16.77	50.132	21.662	3.737
24	1	1.297	1.621	6.485	13.4	16.77	57.347	24.780	4.274

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	2.339	2338.575

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	1	0.523	0.654	2.615	15.2	19.00	9.937	4.249	0.654
4	1	1.941	2.426	9.705	15.2	19.00	39.494	16.888	2.598
8	1	2.651	3.314	13.255	15.2	19.00	62.689	26.806	4.124
12	1	1.086	1.358	5.430	15.2	19.00	46.209	19.759	3.040
24	1	1.448	1.810	7.240	15.2	19.00	58.517	25.023	3.850

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.10	2.098	2098.482

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	1	0.900	1.125	4.500	15.1	18.83	16.950	8.077	1.125
4	1	2.123	2.654	10.615	15.1	18.83	44.483	21.198	2.952
8	1	1.623	2.029	8.115	15.1	18.83	45.682	21.769	3.032
12	1	2.613	3.266	13.065	15.1	18.83	72.442	34.521	4.808
24	1	1.732	2.165	8.660	15.1	18.83	68.914	32.840	4.574

A6-0.4Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.34	0.935	935.430

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.4	0.168	0.210	0.840	14.4	18.04	3.031	1.296	0.210
4	0.4	0.414	0.518	2.070	14.4	18.04	8.309	3.553	0.576
8	0.4	0.980	1.225	4.900	14.4	18.04	20.591	8.805	1.427
12	0.4	0.936	1.170	4.680	14.4	18.04	24.697	10.561	1.711
24	0.4	1.208	1.510	6.040	14.4	18.04	34.284	14.660	2.375

Unit 2

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.65	1.060	1059.615

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.4	0.549	0.686	2.745	15.6	19.44	10.671	4.028	0.686
4	0.4	1.150	1.438	5.750	15.6	19.44	25.098	9.474	1.614
8	0.4	1.031	1.289	5.155	15.6	19.44	28.535	10.772	1.835

12	0.4	0.864	1.080	4.320	15.6	19.44	30.444	11.492	1.958
24	0.4	0.693	0.866	3.465	15.6	19.44	31.440	11.869	2.022

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.18	0.872	871.525

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.4	0.216	0.270	1.080	14.0	17.44	3.767	1.729	0.270
4	0.4	0.951	1.189	4.755	14.0	17.44	17.663	8.107	1.266
8	0.4	1.089	1.361	5.445	14.0	17.44	24.824	11.394	1.780
12	0.4	0.582	0.728	2.910	14.0	17.44	21.429	9.835	1.536
24	0.4	1.238	1.548	6.190	14.0	17.44	35.778	16.421	2.565

A6-0.6Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.31	1.389	1388.550

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	0.6	0.597	0.746	2.985	13.4	16.77	10.012	4.326	0.746
4	0.6	1.051	1.314	5.255	13.4	16.77	20.611	8.906	1.536
8	0.6	1.312	1.640	6.560	13.4	16.77	30.243	13.068	2.254
12	0.6	1.538	1.923	7.690	13.4	16.77	40.594	17.541	3.026
24	0.6	1.403	1.754	7.015	13.4	16.77	46.019	19.885	3.430

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.403	1403.145

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
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0	0	0	0	0	15.2	19.00	0	0	0
2	0.6	0.909	1.136	4.545	15.2	19.00	17.271	7.385	1.136
4	0.6	0.982	1.228	4.910	15.2	19.00	23.203	9.922	1.527
8	0.6	1.102	1.378	5.510	15.2	19.00	30.393	12.996	2.000
12	0.6	1.262	1.578	6.310	15.2	19.00	38.943	16.652	2.562
24	0.6	1.130	1.413	5.650	15.2	19.00	42.745	18.278	2.812

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	1.259	1678.785

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	0.6	0.580	0.725	2.900	15.1	18.83	10.923	4.671	0.725
4	0.6	0.825	1.031	4.125	15.1	18.83	18.438	7.884	1.224
8	0.6	1.012	1.265	5.060	15.1	18.83	26.084	11.154	1.731
12	0.6	1.238	1.548	6.190	15.1	18.83	35.401	15.138	2.350
24	0.6	1.089	1.361	5.445	15.1	18.83	38.785	16.585	2.574

A6-0.8Ag

Unit 1	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	1.871	1870.860

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.4	18.04	0	0	0
2	0.8	1.315	1.644	6.575	14.4	18.04	23.725	10.145	1.644
4	0.8	1.510	1.888	7.550	14.4	18.04	33.818	14.461	2.343
8	0.8	2.185	2.731	10.925	14.4	18.04	53.546	22.897	3.710
12	0.8	3.274	4.093	16.370	14.4	18.04	84.118	35.970	5.828
24	0.8	1.932	2.415	9.660	14.4	18.04	76.277	32.617	5.285

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.65	2.119	2119.229

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.6	19.44	0	0	0
2	0.8	1.354	1.693	6.770	15.6	19.44	26.318	9.935	1.693
4	0.8	1.205	1.506	6.025	15.6	19.44	30.192	11.397	1.942
8	0.8	2.994	3.743	14.970	15.6	19.44	70.991	26.799	4.565
12	0.8	1.993	2.491	9.965	15.6	19.44	66.504	25.105	4.277
24	0.8	1.879	2.349	9.395	15.6	19.44	74.253	28.030	4.775

Unit 3

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.18	1.743	1743.049

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	14.0	17.44	0	0	0
2	0.8	1.168	1.460	5.840	14.0	17.44	20.367	9.348	1.460
4	0.8	1.785	2.231	8.925	14.0	17.44	36.966	16.966	2.650
8	0.8	1.969	2.461	9.845	14.0	17.44	49.099	22.535	3.520
12	0.8	2.341	2.926	11.705	14.0	17.44	65.431	30.031	4.690
24	0.8	2.430	3.038	12.150	14.0	17.44	78.688	36.115	5.641

A6-1.0Ag

Unit 1

Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
2.31	2.314	2314.250

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	13.4	16.77	0	0	0
2	1	1.381	1.726	6.905	13.4	16.77	23.161	10.008	1.726
4	1	2.252	2.815	11.260	13.4	16.77	44.673	19.303	3.330
8	1	2.960	3.700	14.800	13.4	16.77	67.807	29.300	5.054
12	1	1.790	2.238	8.950	13.4	16.77	62.985	27.216	4.695
24	1	2.012	2.515	10.060	13.4	16.77	75.658	32.692	5.639

Unit 2	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.34	2.339	2338.575

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.2	19.00	0	0	0
2	1	1.971	2.464	9.855	15.2	19.00	37.449	16.014	2.464
4	1	2.623	3.279	13.115	15.2	19.00	59.692	25.525	3.927
8	1	3.841	4.801	19.205	15.2	19.00	95.949	41.029	6.312
12	1	2.941	3.676	14.705	15.2	19.00	98.054	41.929	6.451
24	1	1.912	2.390	9.560	15.2	19.00	93.208	39.857	6.132

Unit 3	Area (cm ²)	Amount of Ag (mg/cm ²)	Amount of Ag (mcg/cm ²)
	2.10	2.09848163	1678.7853

time (hr)	Ag conc (mg/cm ²)	conc. (mcg/ml)	factor (1.25)	Amount Ag in 4 ml (mcg)	Volume cell	dilution factor	Total Amount (mcg)	Total amount / area orifice (mcg/cm ²)	Conc (ppm, mcg/ml)
0	0	0	0	0	15.1	18.83	0	0	0
2	1	0.769	0.961	3.845	15.1	18.83	14.483	6.902	0.961
4	1	1.638	2.048	8.190	15.1	18.83	34.694	16.533	2.303
8	1	2.540	3.175	12.700	15.1	18.83	59.872	28.531	3.974
12	1	3.690	4.613	18.450	15.1	18.83	94.230	44.904	6.254
24	1	3.020	3.775	15.100	15.1	18.83	100.062	47.683	6.641

Amount/area orifice

time (hr)	A4-0.4Ag	SD	A4-0.6Ag	SD	A4-0.8Ag	SD	A4-1.0Ag	SD	A6-0.4Ag	SD
0	0	0	0	0	0	0	0	0	0	0
2	3.78	0.88	1.91	0.84	8.65	0.35	6.97	2.37	2.35	1.47
4	6.98	2.39	6.29	1.79	12.18	2.14	17.02	4.12	7.04	3.10
8	10.47	2.27	11.60	1.51	16.72	3.26	25.96	3.84	10.32	1.35
12	12.58	1.80	16.55	2.91	17.99	2.24	25.31	8.03	10.63	0.83
24	12.72	0.47	19.93	1.97	23.96	2.22	27.55	4.59	14.32	2.30

time (hr)	A6-0.6Ag	SD	A6-0.8Ag	SD	A6-1.0Ag	SD
0	0	0	0	0	0	0
2	5.46	1.68	9.81	0.41	10.97	4.63
4	8.90	1.02	14.27	2.79	20.45	4.61
8	12.41	1.09	24.08	2.36	32.95	7.00
12	16.44	1.22	30.37	5.44	38.02	9.47
24	18.25	1.65	32.25	4.05	40.08	7.50

Concentration (ppm)

time (hr)	A4-0.4Ag	SD	A4-0.6Ag	SD	A4-0.8Ag	SD	A4-1.0Ag	SD	A6-0.4Ag	SD
0	0	0	0	0	0	0	0	0	0	0
2	0.62	0.17	0.29	0.12	1.41	0.09	1.09	0.42	0.39	0.26
4	1.13	0.34	0.98	0.32	1.99	0.38	2.60	0.36	1.15	0.53
8	1.70	0.34	1.79	0.08	2.73	0.61	4.07	1.01	1.68	0.22
12	2.05	0.30	2.56	0.48	2.93	0.33	3.86	0.89	1.74	0.21
24	2.07	0.08	3.08	0.29	3.91	0.51	4.23	0.36	2.32	0.28

time (hr)	A6-0.6Ag	SD	A6-0.8Ag	SD	A6-1.0Ag	SD
0	0	0	0	0	0	0
2	0.87	0.23	1.60	0.12	1.72	0.75
4	1.43	0.18	2.31	0.36	3.19	0.82
8	1.99	0.26	3.93	0.56	5.11	1.17
12	2.65	0.35	4.93	0.80	5.80	0.96
24	2.94	0.44	5.23	0.44	6.14	0.50

VITA

Miss Nantaporn Namviriyachote was born on 27th October 1985, in Bangkok, Thailand. She received the degree of Bachelor of Science in Pharmacy (Second Class Honours) in 2009 and of Master of Science in Pharmacy (Clinical Pharmacy Program) in 2012 from Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand. After graduation, she worked as a clinical research associate at Bangkok Botanica Co., Ltd. for one year before attending the Doctoral of Philosophy Program in Pharmaceutical Technology since the first semester of the academic year 2013.

