# การพัฒนาไฮโดรเจลปิดแผลโดยใช้สารผสมระหว่างพอลิไวนิลแอลกอฮอล์และสารสกัดพอลิแซ็กคาไรด์จากเปลือกผลทุเรียน

นางสาว ภัทรานุช เอกวโรภาส

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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# DEVELOPMENT OF HYDROGEL WOUND DRESSINGS USING MIXTURES OF POLYVINYL ALCOHOL AND POLYSACCHARIDE EXTRACT FROM DURIAN FRUIT-HULLS

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| Thesis Title | DEVELOPMENT OF HYDROGEL WOUND DRESSINGS USING MIXTURES OF POLYVINYL ALCOHOL AND POLYSACCHARIDE EXTRACT FROM DURIAN FRUIT-HULLS |
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ภัทรานุช เอกวโรภาส : การพัฒนาไฮโดรเจลปิดแผลโดยใช้สารผสมระหว่างพอลิไวนิลแอลกอฮอล์และสารสกัดพอลิแซ็กคาไรด์จากเปลือกผลทุเรียน. (DEVELOPMENT OF HYDROGEL WOUND DRESSINGS USING MIXTURES OF POLYVINYL ALCOHOL AND POLYSACCHARIDE EXTRACT FROM DURIAN FRUIT-HULLS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ.ดร.พรรณเพ็ญ วัฒนาอาษากิจ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ.ดร.นฤพร สุตัณฑวิบูลย์, 144 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาแผ่นปิดแผลไฮโดรเจลที่เตรียมด้วยกระบวนการเชื่อมขวางทางกายภาพของพอลิไวนิลแอลกอฮอล์ (พีวีเอ) กับสารสกัดพอลิแซ็กคาไรด์เจลจากเปลือกผลทุเรียน (ดีจี) โดยใช้เทคนิคเยือกแข็ง-อุ่นละลาย ปัจจัยที่มีผลต่อกระบวนการผลิตประกอบด้วยสภาวะเยือกแข็ง-อุ่นละลาย การทำเยือกแข็งที่ -20 องศาเซลเซียส 18 ชั่วโมง และอุ่นละลายที่ 30 องศาเซลเซียส 6 ชั่วโมง (เอฟ18ที6) และการทำเยือกแข็งที่ -20 องศาเซลเซียส 24 ชั่วโมง และอุ่นละลายที่ 30 องศาเซลเซียส 24 ชั่วโมง (เอฟ24ที24) เป็นเวลา 3 4 และ 5 รอบ อุณหภูมิการผสม (อุณหภูมิห้อง 50 70 และ 90 องศาเซลเซียส) ความหนา (1.00 2.00 และ 3.50 มิลลิเมตร) และความเข้มข้นของดีจี (2 3 และ 3.5 เปอร์เซ็นต์โดยน้ำหนัก) ศึกษาคุณสมบัติของแผ่นดีจีพีวีเอไฮโดรเจลด้านปริมาณน้ำในแผ่น การพองตัว คุณสมบัติของพื้นผิว คุณสมบัติทางเชิงกลและโครงสร้าง และฤทธิ์ต้านการเจริญเติบโตของจุลินทรีย์ ดีจีพีวีเอไฮโดรเจลเตรียมโดยกระบวนการเยือกแข็ง-อุ่นละลายมีลักษณะเป็นสีน้ำตาลอ่อนและใส ขณะที่พีวีเอไฮโดรเจลใสไม่มีสี ดีจีพีวีเอไฮโดรเจลที่เตรียมโดยเอฟ24ที24 สามรอบมีคุณสมบัติในการดูดน้ำได้มากกว่าไฮโดรเจลที่เตรียมโดยเอฟ18ที6 สามรอบ การผสมที่อุณหภูมิห้องให้แผ่นไฮโดรเจลที่มีความสามารถดูดน้ำดีกว่าการผสมที่อุณหภูมิสูง ดีจีพีวีเอไฮโดรเจลแผ่นบางดูดน้ำต่อน้ำหนักได้มากกว่าไฮโดรเจลแผ่นหนา แต่แผ่นจะเปื่อยและความแข็งแรงน้อยลงหลังการพองตัว รอบการเยือกแข็ง-อุ่นละลายสูงทำให้ไฮโดรเจลแข็งแรงและยืดได้มาก แต่ความสามารถในการดูดน้ำน้อย ความเข้มข้นของดีจีสูง แผ่นมีความแข็งแรง สามารถยืดขยาย และดูดน้ำได้ดีกว่าแผ่นที่มีดีจีความเข้มข้นน้อย เอฟ24ที24 (สามรอบ) และอุณหภูมิเริ่มต้นการผสมที่อุณหภูมิห้องเป็นสภาวะที่เหมาะสมต่อการเตรียมดีจีพีวีเอไฮโดรเจล จากการเตรียมด้วยสภาวะที่เหมาะสมดังกล่าว ดีจีพีวีเอไฮโดรเจลที่มีดีจี 3.5 เปอร์เซ็นต์โดยน้ำหนัก หนา 3.50 มิลลิเมตร มีปริมาณน้ำในแผ่นสูง 90.10 ± 0.26 เปอร์เซ็นต์ สามารถดูดน้ำสูงที่ 13 ชั่วโมงเป็นปริมาณ 250.96 ± 33.92 เปอร์เซ็นต์ คุณสมบัติทางเชิงกลดี (เปอร์เซ็นต์การยืดตัว 237.77 ± 49.72) ภาพเอสอีเอ็มของแผ่นดีจีพีวีเอไฮโดรเจลแสดงโครงสร้างที่มีการพับซ้อนและช่องขนาดใหญ่ ขณะที่โครงสร้างของพีวีเอไฮโดรเจลมีลักษณะเป็นช่องเส้นใยขนาดเล็ก สภาวะการเก็บส่งผลต่อคุณสมบัติโดยรวมของดีจีพีวีเอไฮโดรเจล การเก็บที่ 4 องศาเซลเซียส คุณสมบัติของไฮโดรเจลเปลี่ยนแปลงน้อยกว่าการเก็บที่ 30 และ 40 องศาเซลเซียสความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ ที่สำคัญดีจีพีวีเอไฮโดรเจลสามารถยับยั้งการเจริญเติบโตของแบคทีเรียในขณะที่พีวีเอไฮโดรเจลไม่แสดงคุณสมบัตินี้ ดีจีพีวีเอไฮโดรเจลแสดงขอบเขตใสของการยับยั้งเชื้อมีค่า 23.3 ± 0.6 ตารางมิลลิเมตร และขอบเขตโปร่งแสงมีค่า 31.3 ± 1.2 ตารางมิลลิเมตร ต่อเชื้อ *Staphylococcus aureus* และ *Escherichia coli* ตามลำดับ การผสมสารสกัดพอลิแซ็กคาไรด์เจลจากเปลือกผลทุเรียนในแผ่นพีวีเอไฮโดรเจลทำให้แผ่นพีวีเอไฮโดรเจลมีคุณสมบัติดีขึ้น เหมาะสำหรับพัฒนาไปใช้ในทางชีวการแพทย์ต่อไป

ภาควิชา…….วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม.. ลายมือชื่อนิสิต.....

สาขาวิชา……เภสัชอุตสาหกรรม……..…… ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก……..…...

ปีการศึกษา….2552……..…….. ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม…….……..…..

## 4976587633 : MAJOR INDUSTRIAL PHARMACY
KEYWORDS: POLYSACCHARIDE FROM DURIAN FRUIT-HULLS/ POLYVINYL ALCOHOL/ FREEZE-THAW PROCESS/ PROCESS PARAMETERS/ ANTIMICROBIAL/ HYDROGEL DRESSINGS

PATTARANUT EAKWAROPAS: DEVELOPMENT OF HYDROGEL WOUND DRESSINGS USING MIXTURES OF POLYVINYL ALCOHOL AND POLYSACCHARIDE EXTRACT FROM DURIAN FRUIT-HULLS. THESIS ADVISOR: PHANPHEN WATTANAARSAKIT, Ph.D., THESIS CO-ADVISOR: NARUEPORN SUTANTHAVIBUL, Ph.D., 144 pp.

The purpose of this study was to develop hydrogel dressing prepared by physically cross-linked polyvinyl alcohol (PVA) with polysaccharide gel extracted from durian fruit-hulls (DG) using freeze-thaw technique. Processing parameters included freeze-thaw duration (freezing at -20°C 18 hrs and thawing at 30°C 6 hrs (F18T6) and freezing at -20°C 24 hrs and thawing at 30°C 24 hrs (F24T24)); freeze-thaw cycles (3, 4 and 5 cycles); mixing temperatures (room temperature: RT, 50°C, 70°C and 90°C); thickness (1.00, 2.00 and 3.50 mm); and DG concentrations (2, 3 and 3.5%w/w). The DG/PVA hydrogel membranes were characterized by its water content, swelling property, surface properties, mechanical/structural behaviors and antimicrobial activity. DG/PVA hydrogels prepared by freeze-thaw process were light-tan in color and transparent while PVA hydrogels were transparent and colorless. DG/PVA hydrogels prepared by F24T24 (3 cycles) absorbed higher amount of water than F18T6 (3 cycles). Mixing hydrogel at RT resulted in hydrogel membranes with better water absorption capacity than mixing at higher temperatures. Thinner DG/PVA hydrogels absorbed higher amount of water per weight than thicker hydrogels but were more fragile and showed decreased strength after swelling. High freeze-thaw cycles resulted in the hydrogels with higher strength and ductility but less water absorption ability. With higher DG concentrations, membranes became stronger, were able to elongate and absorbed higher amount of water than at lower concentrations. F24T24 (3 cycles) and initial mixing at RT was the optimal condition for the preparation of DG/PVA hydrogels. By using this optimal condition, DG/PVA hydrogel with 3.5%w/w of DG and thickness of 3.50 mm showed high water content (90.10 ± 0.26%), high water absorption capacity at 13 hrs (250.96 ± 33.92%) and good mechanical properties with percent elongation of 237.77 ± 49.72%. Scanning electron photomicrographs of DG/PVA hydrogel showed folded structure with large pores, while PVA hydrogel structure composed of small fibers which created its elongated cavities. Storage condition affected overall properties of DG/PVA hydrogel. When stored at 4°C, hydrogel properties changed less than when stored at 30°C and 40°C, 75%RH. Most importantly, DG/PVA hydrogel was able to inhibit bacterial growth while PVA hydrogel did not. DG/PVA hydrogels exhibited clear zone (23.3 ± 0.6 $mm^2$) and translucent zone (31.3 ± 1.2 $mm^2$) against *Staphylococcus aureus* and *Escherichia coli*, respectively. The addition of polysaccharide gel from durian fruit-hulls extract into polyvinyl alcohol hydrogel resulted in an improved membrane properties suitable for further development in biomedical applications.

Department: …Pharmaceutics and Industrial Pharmacy.. Student's signature: .....

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

Academic year: 2009……...….... Co-advisor's signature: ……..

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| % | = | Percentage |
| ATCC | = | American Type Culture Collection, Maryland, USA |
| °C | = | degree celcius |
| CDC | = | critical point dryer |
| CFU | = | colony forming unit |
| DG | = | polysaccharide gel extracted from Durian fruit-hulls |
| cm | = | centimeter |
| DSC | = | differential scanning calorimetry |
| et al. | = | *et alii*, 'and others' |
| FTIR | = | Fourier Transformed Infrared Spectrometry |
| g | = | gram |
| hr | = | hour |
| hrs | = | hours |
| kGy | = | kilogray |
| mg | = | milligram |
| MHA | = | Mueller Hinton Agar |
| min | = | minute |
| ml | = | milliliter |
| mm | = | millimeter |

| | | |
|---|---|---|
| mo | = | month or months |
| MW. | = | molecular weight |
| N | = | newton |
| nm | = | nanometer |
| no. | = | number |
| PVA | = | polyvinyl alcohol |
| PVP | = | polyvinyl pyrrolidone |
| RH | = | relative humidity |
| RT | = | room temperature |
| sec | = | second |
| SEM | = | Scanning Electron microscopy |
| w/w | = | weight by weight |

# CHAPTER I

# INTRODUCTION

Wound management is improvability of healing rate, normal healing process and no scar. Dry dressings were used for wound dressings in the past times but from Winter's study in 1962, it was found that wet dressings could promote wound healing process due to keeping moist environment in the wound bed (Winter, 1962, cited in Kim et al., 2008; Kokabi, Sirousazar and Hasssan, 2007). Moisture affects on faster healing process because it supports epithelialization, new epithelial cells can move into wound (Wiseman, Rovee and Alvarez, 1992).

Hydrogels are three-dimensional polymeric networks which can hold high amount of water in structure (Varshney, 2007). Hydrogels show many interesting properties when used as wound dressings mainly as pain decreasing, easy application, transparency to observe healing process, wound fluid absorbent and environmental bacteria barrier (Kokabi, et al., 2007). PVA hydrogels prepared by freeze-thaw process are transparent, have good mechanical properties, high ability of swelling and widely used as synthetic hydrogel systems (Kim et al., 2008). Polysaccharide hydrogels have been attentive in the present due to their natural/ biomedical properties but most polysaccharides able to dissolve in water. Thus polysaccharide hydrogels should prepared by mixing with synthetic materials (Kunal, Banthia and Majumdar, 2006).

Polysaccharide gel extracted from dried fruit-hulls of durian (*Durio zibethinus* L.) (DG), is biomedical compatible, and is a water soluble polysaccharide consisting of pectin and starch (Hokputsa et al., 2004; Pongsamart and Panmaung, 1998). Toxicity studies showed safety for using high dose or long term of DG in mice and rats (Pongsamart, Sukrong and Tawatsin, 2001; Pongsamart, Tawatsin and Sukrong, 2002). DG was evaluated its antimicrobial property against bacteria and yeasts by agar diffusion test. Several strains of bacteria inhibited by DG were *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Lactobacillus pentosus*, *Escherichia*

*coli*, *Staphylococcus aureus* and *Proteus vulgaris* while 2 strains of yeasts, *Saccharomyces cervisiae* and *Candida albicans*, were not inhibited (Nantawanit, 2001; Lipipun, Nantawanit and Pongsamart, 2002). DG can be used to prepare several types of wound dressings such as films, gels and freeze-dry products. DG film and gel wound dressings were examined promotion of wound healing in pig skin. Data showed faster and better wound healing process than traditional treatment: povidone iodine. DG film and freeze-dry patch wound dressings were investigated in dog skin wounds. DG products also gave more advantages for healing process (Nakchat, 2002; Siripokasupkul, 2004).

However, there is no study showing DG as wound dressing in the hydrogel form. Our preliminary study using the freeze-thaw process found that, the DG solution couldn't be prepared as hydrogel. But combining DG with PVA, the hydrogels should be more biocompatible and might have better physical properties.

Purposes of the present study were:

1. To develop the DG/PVA hydrogel dressings by physical technique: freeze-thaw technique

2. To investigate the freeze-thaw process parameters

3. To evaluate physicochemical properties, stability and antimicrobial properties of DG/PVA hydrogels.

# CHAPTER II

# LITERATURE REVIEW

## 1. Wound care principles and wound dressings

### 1.1 The physiological stages of wound healing

Healing can be separated into three phases which occur in overlap between phases (Doughty, 1992: 36). The phases of wound healing are: inflammation, proliferation or granulation and remodeling or maturation. Figure 1 shows the overlap phases of normal wound healing.

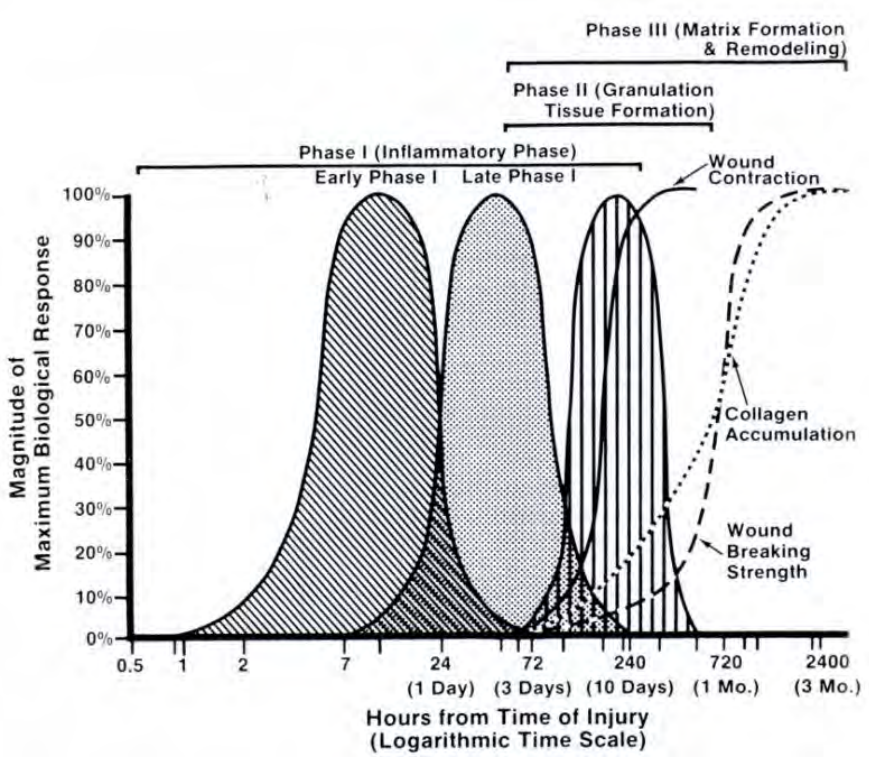



Figure 1. The three phases of normal wound healing (Daly, 1995: 33)

#### 1.1.1 Defensive or inflammatory phase

The inflammatory response is a series of local cellular and vascular responses which are triggered when the body is injured, or invaded by antigen. Other

functions of the inflammatory response are to rid itself of microorganisms, foreign matter, and dead tissue. It quickly changes in skin color, temperature, pain, swelling and may include a loss of function. The acute inflammatory phase generally lasts for 24 to 48 hrs and is usually completed within 7 days, though sub-acute may continue for approximately 2 weeks. This phase is immediate reaction to injure. The main events are hemostasis and inflammation (Bracciano, 2008: 50).

#### 1.1.1.1 Hemostasis

The basic, initial hemostatic responses to an injury due to vasoconstriction and clotting (Bracciano, 2008: 50). Injury occurring, the epidermis and dermis are disrupted and the cutaneous vasculature is severed, causing blood cells leak into the wound. Platelets aggregate and degranulate due to the contact with damaged collagen and tissue debris. Fibrin is deposited and polymerized, as well as continued platelets aggregation, then thrombus is formed. Hemostasis occurs due to conjunction of thrombus forming and vasoconstriction of the trauma vessels (Mast, 1992: 346).

#### 1.1.1.2 Inflammation

In this event, vasocongestion and the leakage of fluid occur because the release of vasoactive substances into the wound (Doughty, 1992: 37). Both *neutrophils* and monocytes come to the wound. *Macrophage* changed from monocytes produce growth factors that is affected on healing process. Generally, macrophages are phagocytosed and eliminate pathogens (Bale and Jones, 1997: 6).

### 1.1.2 Proliferative or fibroblastic phase

The proliferative phase is the second phase, the wound is filled with new connective tissues and epithelial cells. The main elements of this phase are granulation, epithelialization, and contraction. This phase overlaps the defensive phase and continues until complete healing process (Bracciano, 2008: 51).

#### 1.1.2.1 Granulation

New connective tissue (scar) formation is found in this step. This phase consists of neoangiogenesis and synthesis of various connective tissue substances.

*Neoangiogenesis* is stimulated by the hypoxic condition that results from disruption of vascular pathways. Proliferation of capillary occurred due to oxygen gradient between peripheral vascularity and hypoxic center. Hypoxic condition stimulates angiogenesis factors releasing by macrophages. These factors are attractants for endothelial cells to come into wound (Doughty, 1992: 39).

*Collagen synthesis* occurs concurrently with neoangiogenesis. Fibroblasts are important for synthesis of collagen and other connective tissues (Doughty, 1992: 39). Fibroblasts produce a network of collagen surrounding the the wound and also produce proteoglycans which improve flexibility of fiber. Fibronectin forms the network of tissue by holding both collagen and cell together (Bale and Jones, 1997: 7).

#### 1.1.2.2 Contraction

Granulation phase occurs concurrently with contraction in open wound. The tissue and skin surrounding the wound edge are mobilized and pulled together. Contraction phase does not appear in suture wound. Healing rate is faster because decreasing the amount of scar tissue (collagen) required (Doughty, 1992: 40). Decreasing of wound area occurs during contraction. This process is able to close the wound with or without prior epithelialization (Daly, 1995: 38).

#### 1.1.2.3 Epithelialization

Epithelialization is the natural act of healing tissue in which epithelium grows over a wound. It is the final part of proliferation phase; the epithelial cells move from the wound edges to resurface the wound. In small wounds, epithelialization occurs in the same time of collagen synthesis. However, in open wounds, epithelialization is slower than small wound because epithelium cannot move

across dry bed or necrotic tissue (Doughty, 1992: 40). In deep wound, regeneration starts at the margins of the wound. However superficial wound, epitheliums regenerate from hair follicles. Epithelium migration occurs until other cells are met (Bale and Jones, 1997: 7-8).

### 1.1.3 Remodeling or maturation phase

This phase consists of collagen synthesis and collagen lysis. It usually appears a scar with high tensile strength in this phase. An imbalance between the collagen formation and breakdown affects on wound healing. For instance, hypertrophic scarring and keloid are expected to be caused by higher collagen formation than breakdown. In addition, wound breakdown may occur by the decreasing of collagen formation due to hypoxic condition or malnutrition (Doughty, 1992: 40-41). This phase starts approximately 2 weeks after wound occurred and duration up to one year or longer time (Bracciano, 2008: 53).

## 1.2 Wound infection

Wound infection can occur at all phase of the healing process and all type of wound (Bale and Jones, 1997: 21). Healing rate of infected wound is not as fast as normal wounds. Infection means the contamination of pathogens that cannot be controlled by body responses. Infection usually inhibits healing process by destroying tissue and promoting excessive inflammation (Wiseman, et al., 1992: 562-563). Sources of wound infection include patient's normal flora and pathogens from environment or hand or cloth. (Ryan, 2004: 820). Normal cause of wound infections is *Staphylococcus aureus*, however the infections caused by gram-negative organism increasing. Anaerobic gram-negative wound infections have been found increasingly and the higher incidence of infection is found in immunocompromised patients (Ryan, 2004: 821).

## 1.3 Wound dressings

All effective wound management depends on decreasing or control factors such as pressure, improvement of systemic supports such as nutritional and fluid and

selection of appropriate topical treatment. The topical therapy of the wounds is the selection and application of an optimal dressing (Doughty, 1992: 46-50). There are two types of dressings: wet and dry dressings. It has been reported that wet dressing improves healing process with moist environment than dry dressing (Winter, 1962, cited in Kim et al., 2008).

### 1.3.1 Wound environment controlling by dressing

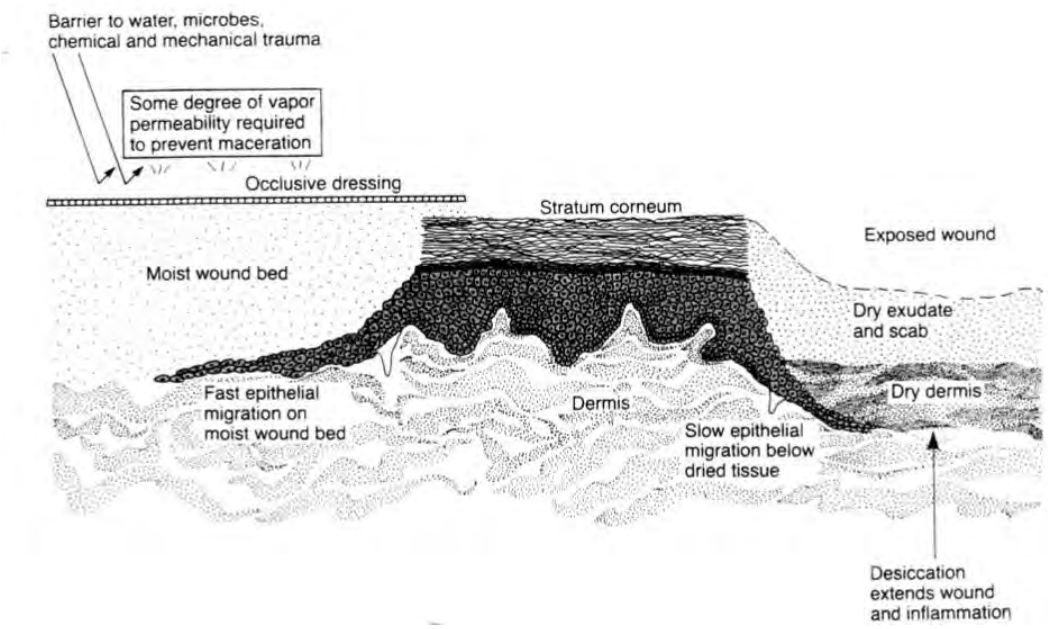



Figure 2. Wound healing process under an occlusive dressing (Winter, 1963: 91-92, cited in Wiseman et al., 1992: 565)

Wiseman, et al. (1992: 563) described that "dressing design for the 2nd and 3rd phases of wound healing is based mainly on the hydration and oxygen tension within the wound". Occlusion wound dressings are able to transmit gases and vapor from a wound surface to environment. Exposed wounds are able to inflame and necrotic more than occluded wounds in initial stage of healing. Collagen synthesis and epithelium migration are enhanced by occlusive condition. Occlusive dressings decrease tissue desiccation and further damage by maintaining wet environment. Epithelialization in humid condition is faster than in arid ones because epithelial cells are difficult to migrate below eschar (Figure 2). However, a moist condition that

improves healing may enhance growth of pathogens. Thus, wound with infection and has high exudate is the contraindication of occlusive dressings. Wound pH may be a way to inhibit pathogen growth under occlusive condition. Low pH (5.8 to 6.6) may be optimal and may have a positive affection on epithelialization.

### 1.3.2 Types of wound dressings

#### 1.3.2.1 Gauze dressings

Gauze dressings can be used effectively for absorbing exudates, debridement and filling in space. In addition, they can be used for delivery topical treatment to the wound. Gauze dressings are not suitable for dry wounds with necrotic tissue (Doughty, 1992: 53, 57).

#### 1.3.2.2 Transparent film dressings

Transparent adhesive dressings are the first available wet dressings. These dressings are semipermeable membranes allowing water vapor pass through. Moreover, they allow atmospheric oxygen to diffuse into the wound but prevent bacterial contamination. These dressings are not use in exudative wounds due to they have no absorbable property. These dressings are available in the market such as OpSite, Tegaderm, AcuDerm (Doughty, 1992: 51-52) and Bioclusive (Feedar, 1995: 164).

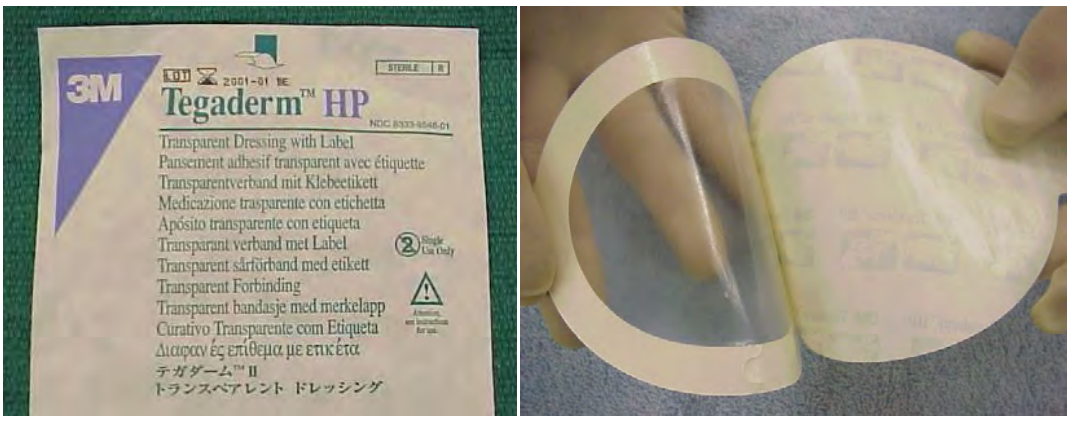



Figure 3. Tegaderm™ transparent film dressing (http://jan.ucc.nau.edu/~daa/woundproducts /products.html)

### 1.3.2.3 Hydrocolloid dressings

The hydrocolloid dressings are adhesive patch containing hydroactive particles. Most hydrocolloid dressings are occlusive because they have a repellent side. The contraindications of hydrocolloid dressings are infection wounds and wounds with high exudates. Examples of available products in the market are DuoDerm, Intrasite, Tegasorb (Doughty, 1992: 52-53), Restore, Comfeel and Cutinova hydro (Feedar, 1995: 167).


Figure 4. DuoDERM hydrocolloid dressing (http://jan.ucc.nau.edu/~daa/woundproducts/products.html)

### 1.3.2.4 Foam dressings

These foam dressings are no adhesion patch that has absorption capacity on the wound side and a repellent hydrophobic surface on the other side. They have low capacity of permeability but no total occlusion. Contraindications of these dressings are wounds with dry eschar and no exudates. Examples of products are Allevyn, Lyofoam (Doughty, 1992: 52-53), NU-DERM and Flexzan (Feedar, 1995: 165).


Figure 5. Flexzan foam dressing (http://jan.ucc.nau.edu/~daa/woundproducts/ products.html)

#### 1.3.2.5 Hydrogel dressings

Hydrogel dressings are available in 3 forms: sheet dressing, amorphous and impregnated gauze. All gel dressings help to maintain wet environment. Examples of gel dressings are Vigilon, ElastoGel, Intrasite Gel, Geliperm (Doughty, 1992: 52-53), NU-GEL, Clear site and Aquasorb (Feedar, 1995: 165).


Figure 6. Vigilon hydrogel dressing (http://www.delasco.com/pcat/1/Wound_Care /Vigilon/dlmiv002/)

## 2 Hydrogel

Hydrogel is insoluble-polymeric network, able to swell without dissolving, absorb and hold water within structure. Hydrogels are usually clarified as two-

component systems, one part is hydrophilic, three-dimensional network and other part is water (Bouwstra and Junginger, 1993: 441). Borders of the hydrogel are not clear. Polymers combined with water form a water-like solid. They have many interest properties because of the high amount of water in structures (Stoy, 1999: 91). Hydrogel network is usual made from hydrophilic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, polyacrylic acid (Varshney, 2007: 343). Hydrogels are used in many routes of medicinal applications including wound dressing, drug delivery application, transdermal system, dental using, injectable polymer, implant, ophthalmic using and stimuli-responsive using (Lopérgolo, Lugão and Catalani, 2003: 6217).

Hydrogels are used as wound dressing because they maintain moist environment, promote autolytic debridement in necrotic or slough wounds, give moisture to dehydrated wound, absorb exudates and no pain (Jones and Vaughan, 2005) due to touch of the soft patch with the nerve tips. Furthermore, hydrogel dressings are bacterial barrier, adherent to skin without stick and transparent for following the healing process (Lugao and Malmonge, 2001).

Hydrogels can be separated by water content into four groups including low, medium, high and superabsorbent hydrogels. Low water content hydrogel absorbs 20-40 % (by weight) of water, medium and high water content hydrogels absorb 40-75 % and 75-98 % of water, respectively. Moreover, superabsorbent hydrogel absorbs 98-99.95 % of water (Stoy, 1999: 94).

### 2.1 Preparation of hydrogel

There are several methods for preparation of hydrogel dressings using hydrophilic polymers include chemical cross-link using chemical agents such as borax, boric acid, formaldehyde, glutaraldehyde, etc., irradiation and physical cross-link by freeze-thaw process (Varshney, 2007: 344).

### 2.1.1 Chemical cross-link technique

Chemical technique is traditional method uses chemical agents for cross-link. Crosslink agents such as glutaraldehyde, formaldehyde, boric acid and epichlorohydrin are added during the hydrogel forming process. The chemical cross-link method usually leave residual chemical agents in the hydrogel network and have side effect (Li, Wang and Wu, 1998: 118).

Example study of hydrogel prepared by chemical cross-link:

Kunal, et al. (2006) clarified hydrogel from corn starch and PVA with glutaraldehyde. “The obtained hydrogel membrane could be tried as artificial skin and medicaments could be delivered directly to the site of action”.

### 2.1.2 Radiation technique

#### 2.1.2.1 Gamma radiation

Radiation was used to preparation of hydrogel dressings due to many advantages. The technique combines sterilization and cross-link together in one step and did not use initiators (Lugao and Malmonge, 2001).

Example study of hydrogel prepared by gamma radiation:

Varshney (2007: 343) studied PVA-polysaccharide hydrogel wound dressing. The hydrogel membranes were transparent, good mechanical properties, biocompatible, and economical dressings. These dressings are now available in India under different trade names.

#### 2.1.2.2 Electron beam radiation

Example study of hydrogel prepared by electron beam radiation:

PEO/PVA hydrogel were studied for wound dressing (Yoshii et al., 1999). PVA was added in PEO hydrogel dressing to increase mechanical strength.

The obtained hydrogel resulted in faster healing process compared with the gause dressing with a dry environment.

#### 2.1.2.3 Ultraviolet radiation

Example study of hydrogel prepared by UV radiation:

Lopérgolo et al. (2003: 44) studied preparation of polyvinyl pyrrolidone by UV cross-link. PVP hydrogel was formed by using low pressure Hg lamp (λ= 254 nm). The hydrogel was similar compared to hydrogels preparedd by high-energy radiation.

### 2.1.3 Physical crosslink technique

This technique is used in hydrogel preparation process without chemical agents and organic solvents (Xiao and Yang, 2006). The hydrogels obtained from freeze-thaw process are safe for application due to no toxicity. Furthermore, the physical cross-link hydrogel has optimal mechanical strength (Hassan, Ward and Peppas, 2000).

Example studies of hydrogels prepared by freezing-thawing technique:

"PVA-sodium alginate gel matrix based wound dressing system containing nitrofurazone" clarified by Kim et al. (2008). The obtained hydrogel resulted in more swelling and good mechanical properties compared to PVA hydrogel without sodium alginate. It did not clarify that neither PVA and sodium alginate mixed system nor nitrofurazone affected on better healing process.

Kokabi, et al. (2007) studied the addition of clay in PVA hydrogel wound dressings. The obtained hydrogel had better mechanical properties than PVA hydrogel without clay. In addition, the PVA-clay hydrogel could act as wound barrier against *Pseudomonas auroginosa*.

## 2.2 Hydrogel-based materials

### 2.2.1 Durian fruit-hulls polysaccharide gel (DG)

Polysaccharide gel extracted from Durian fruit-hulls was first extracted and characterized by Pongsamart and Panmaung in 1998. DG consisted of carbon, hydrogen and oxygen in atomic ratio of 2.88: 5.33: 3.09, while nitrogen and sulfur were not found. Five sugars found in DG were glucose, fructose, rhamnose, arabinose and 52.5 % galacturonic acid which was the major sugar. Moisture and ash contents were 5.71±1.06% and 7.73±2.11%, respectively, while fiber was not found. Powder of DG was soluble in water to viscous, acid solution and degraded at 203.1 °C. X-ray diffraction pattern showed that DG powder was amorphous. An average molecular weight of DG was 500 – 1400 kDa (Gerddit, 2002).

DG was regarded as a safety polysaccharide gel extract. Pongsamart, Sukrong, and Tawatsin (2001) illustrated toxicity of high oral dose DG (2 g/kg) in mice and rats. The data obtained showed that neither lethality nor severe toxicity was found in treated mice and rats and the toxic liver injury had not occurred.

In addition Pongsamart, Tawatsin, and Sukrong (2002) clarified that no toxicity occurred in treated mice for long-term oral consumption of DG (0.25 or 0.5 g/kg/day for 60 and 100 day). This result suggests that liver injury did not appear in treated mice same to the research in 2001.

DG was used to form several type of preparation improved wound healing. For instance, dressing film and fiber dressing patch (freeze-drying product) were investigated in female dog skin (Siripokasupkul, 2002). Open wounds covered with both DG dressing preparation healed faster than wounds treated with 1% povidone iodine, and 1% povidone iodine and covered with commercial dressing film –Opsite® Flexigrid. Furthermore, both DG dressing represented the properties of ideal wound dressing by maintaining moisture environment, improving healing process, reducing the inflammation and tissue reaction. Moreover, Nakchart (2002) demonstrated effect of DG dressing film and gel on full-thickness wound healing in pig skin. Wounds treated with DG dressing film clarified rapid wound closure and

smaller wound area than wounds applying 1% povidone iodine and covered with DG dressing film, DG dressing gel, and 1% povidone iodine, respectively.

Antimicrobial property of DG against microorganism was investigated by Nantawanit in 2001. Agar diffusion method was used, DG could inhibit growth activity against 7 strains of bacteria i.e. *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Lactobacillus pentosus*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris*.

### 2.2.2 Polyvinyl alcohol (PVA)

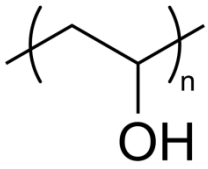



Figure 7. Chemical structure of polyvinyl alcohol (PVA) (http//en.wikipedia.or/wiki/file:Pva.png)

Figure 7 shows chemical structure of PVA. PVA is synthesized by polymerizing by vinyl acetate monomer following by hydrolysis. Thus PVA consisted of vinyl acetate and vinyl alcohol. Degree of hydrolysis shows percentage of vinyl alcohol in polymer. PVA has hydrophilic property and can be dissolved in water (Mallapragada and Schroeder, 2000: 31).

PVA has several advantages for medical application such as its biocompatibility, safety, stability. It is widely used in medical products such as films and coatings (Yeo et al., 2000, cited in Kim et al., 2008). But PVA hydrogels have many disadvantages such as low strength. Thus different methods have been discovered for improve its strength, ductility and temperature stability (Varshney, 2007: 344). PVA hydrogel prepared by each method has different characters. PVA hydrogels prepared from irradiation are easy to fracture at low strength below 1 MPa. PVA hydrogels formed by repeated freeze-thaw process are high strength but melt at 55 °C (Yoshii et al, 1995).

## 2.3 Mechanical characterization

Mechanical properties are investigated the ability of materials which can absorb force before fracture. Mechanical properties such as strength, flexibility, elongation and hardness are examined by tension or compression test (Hibbeler, 2004: 405).

### 2.3.1 The stress-strain diagram

Hibbeler (2004: 407-409) described that the data from a tension or compression test can be calculated and plot into stress-strain curve. The most stress-strain curves of materials showed linear relation. The constant value of slope from stress-strain curve is modulus of elasticity or Young's modulus. Young's modulus shows stiffness or hardness of material (Hibbeler, 2004: 414).

### 2.3.2 Stress–strain behavior

Hibbeler (2004: 411-413) classified materials into ductile or brittle groups using the stress-strain characters. For ductile materials, they can elongate more before fracture in contrast to brittle material.

## 2.4 Antimicrobial activity assay

"Two usual antimicrobial activity methods are in common use: the tube dilution method and the agar diffusion method" (Alcamo, 1997: 712).

### 2.4.1 Dilution Test

The tube dilution method examines the smallest amount of antimicrobial agent used to inhibit bacteria growth. This amount is called as the minimum inhibitory concentration (MIC) (Alcamo, 1997: 713). "It investigates the MIC by using serial dilutions of the antimicrobial agent in broth that span a clinically significant range of concentrations. The bacterial inoculums is adjusted to a concentration of $10^5$ to $10^6$ bacteria/ml and added to the broth. After incubation overnight, the tubes are examined for turbidity produced by bacterial growth. The first tube in which visible growth is absent (clear) is the MIC for that organism" (Ryan, 2004: 216).

### 2.4.2 Diffusion Test (Kirby-Bauer test)

The Kirby-Bauer diffusion test is used to examine the susceptibility of bacteria to antimicrobial agent (Nester et al., 2004: 518). It is an antimicrobial susceptibility method of bacteria onto the surface of Mueller-Hinton agar. Then antimicrobial discs are covered to the agar surface. Inhibitions of bacteria result in the inhibition zone (clear zone) surrounding the antimicrobial discs (Figure 8A). Diameter of the clear area correlates the solubility properties of antimicrobial agent. The agar diffusion test method is optimal for use with fast growing bacteria. It is not suitable for fungi, anaerobes or slow growing bacteria examination (Atlas, 1997: 472-473). Another diffusion method is gradient strip which shows elliptical zones corresponding to the MIC. The E test method (Figure 8B) investigates inhibition of antimicrobial agent against slow-growing, fastidious and anaerobic bacteria (Ryan and Ray, 2004: 217).

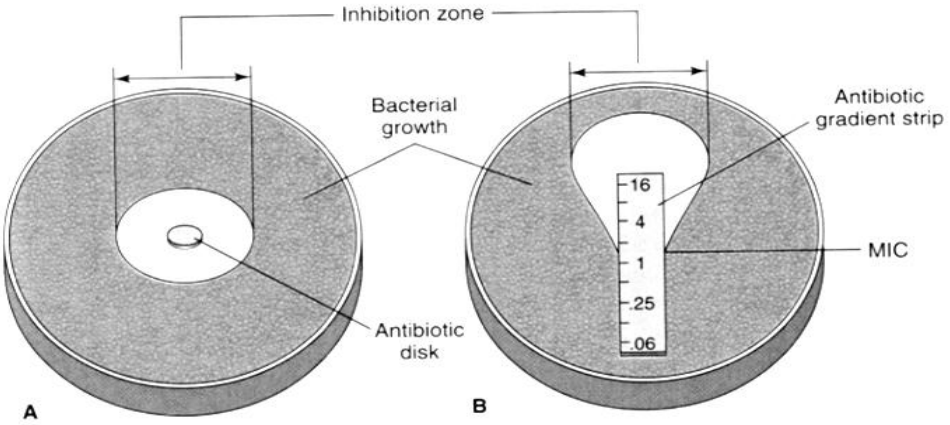



Figure 8. A: Agar Diffusion Test (Kirby-Bauer test), B: the E test method (Ryan, 2004: 217)

# CHAPTER III

# EXPERIMENTAL

## Materials

### Hydrogel-based materials

Agar

Carrageenan

Deionized Water

Konjac

Polysaccharide Gel Extract from Durian Fruit-Hulls I (Lot no. M3)

Polysaccharide Gel Extract from Durian Fruit-Hulls II (Lot no. M11)

Polyvinyl Alcohol MW. 27,000 and degree of hydrolysis 98 – 98.8 % (Lot no. 410956/1 13400, Fluka, Switzerland)

Polyvinyl Alcohol MW. 72,000 and degree of hydrolysis >98 % (Lot no. S4690938 730, Merck, Germany)

Polyvinyl Alcohol MW. 115,000 and degree of hydrolysis 86.5 – 89 % (Lot no. 33247902, BDH, England)

Polyvinyl pyrrolidone K30 (PVP K30)

Polyvinyl pyrrolidone K90 (PVP K90)

### Chemicals

Absolute Ethanol

Glutaraldehyde 25 %w/w (B/no. AH510211, Unilab)

Methylparaben

Propylene Glycol USP XXII (Lot no. 9051101860, Srichand united dispensary co., Ltd., Thailand)

Propylparaben

Sodium Chloride (Lot no. F2C273, APS Chemical Limited, Australia)

**Microbial agents and agar**

*Escherichia coli* ATCC 2738 (Lot no. 2738) and *Staphylococcus aureus* ATCC 6538 (Lot no. 2763) from Department of Medical Sciences, Ministry of Public Health, Thailand

Mueller Hinton Agar (Lot no. 225250, Difco ™, Difco, Becton Dickinson and company, France)

**Equipments**

Analytical balance (AG204, Mettler Toledo, Switzerland)
Analytical balance (PB3002, Mettler Toledo, Switzerland)
Critical Point Dryer (Balzers Union CPD 020, Liechtenstein)
Differential Scanning Calorimetry (DSC) (DSC822$^e$, Mettler Toledo, Switzerland)
Freezer (FC-27, Sharp)
Fourier Transform Infrared Spectrometer (Spectrum one, Perkin-Elmer)
Gammacell® (220 Excel, MDS Nordion, Canada)
Hot air oven (Memmert, Germany)
LLOYD instrument (model LR 10K, UK)
Magnetic stirrer (Model M6, Schott, Germany)
pH meter (Model 210A+, Thermo Orion, Germany)
Refrigerator (Hitachi, Japan)
Scanning Electron Microscope (JSM-5410 LV, JEOL, Japan)
Texture Analyzer (TA.XT Plus, Stable Micro System Ltd., UK)
Vernier Calipers (150 x 1/50mm, China)

**Miscellaneous**

Aluminium foil (MMP Packing, Thailand)
Beaker (Pyrex, USA)
Cylinder (Pyrex, USA)
Parafilm® (Pechiney Plastic Packaging, USA)

Petri dish (Pyrex, USA)
Universal pH Paper (pH-Fix 0-14, Lot no. 92110)
Universal pH Paper (pH-Fix 4.5-10.0, Lot no. 92120)

## Methods

### 1. Preformulation study

#### 1.1 Screening for hydrogel forming materials

##### 1.1.1 Synthetic polymers

Polyvinyl alcohol (PVA; MW. 27,000, 72,000 and 115,000) and polyvinyl pyrrolidone (PVP; PVP K30 and PVP K 90) were selected for studying the possibility of hydrogel formation. PVA hydrogels were prepared by dissolving the polymer in deionized water (DI) and heated for 1 hour to achieve 10 %w/w solution. PVP hydrogels were prepared by dissolving the polymers in deionized water to make a 20 %w/w solution.

##### 1.1.2 Natural polysaccharides

Natural polysaccharides used in this study were agar, carageenan, konjac and polysaccharide gel extracted from Durian fruit-hulls (DG). Each polysaccharide was dissolved in deionized water to prepare a 2 %w/w polysaccharide solution.

#### 1.2 Screening the methods used for hydrogel preparation

##### 1.2.1 Radiation cross-link

#### 1.2.2.1 Ultraviolet light (UV)

A mixture solution of 50 parts of synthetic polymer and 50 parts of natural polysaccharide was poured into 20 ml petri dish. Then the mixture was exposed directly to UV radiation at wavelength of 254 and 366 nm for 3-12 minutes.

#### 1.2.2.2 Microwave

A mixture solution of 50 parts of synthetic polymer and 50 parts of natural polysaccharide was poured into 20 ml petri dish. The mixture was then exposed directly to microwave irradiation at low, medium and high power for 1-3 minutes.

### 1.2.2 Chemical cross-link

Fifty parts of 10 %w/w PVA solution were mixed with 50 parts of 2 %w/w DG. Glutaraldehyde 0.5-3 ml and 0.1 M $H_2SO_4$ solution 2 ml were added into 10 g of DG/PVA mixture solution. The final mixture solution was poured into petri dish and incubated for 24 hrs.

### 1.2.3 Physical cross-link

Fifty parts of synthetic polymer solution were mixed with 50 parts of 2 %w/w DG solution and poured into 20 ml petri dish, then the mixture solution was frozen at -20 °C for 18 hrs and thawed at 30 °C for 6 hrs, 1-3 consecutive cycles.

## 1.3 Identification of hydrogel forming materials

Extraction of polysaccharide gel from Durian fruit-hulls (DG) was performed based on the method previously described by Pongsamart and Panmuang (1998). DG

powder and Polyvinyl alcohol (PVA) MW. 72,000 powder were selected for further study and identified by following method.

### 1.3.1 Scanning Electron Microscopy (SEM)

DG and PVA powders were fixed on the stage and sputter coated with gold. Then, samples were determined at 15 kV on JEOL model JSM-5410W SEM machine.

### 1.3.2 Differential Scanning Calorimetry (DSC)

Thermal analysis was performed by DSC using Mettler Toledo DSC822$^e$. All hydrogel forming material powder samples of 1-3 mg were heated from 25 to 400 °C under $N_2$ atmosphere (60 ml/min) with a heating rate of 10 °C/min (Yang et al., 2008).

### 1.3.3 Fourier Transformed Infrared Spectrometry (FTIR)

FTIR of DG and PVA powders were measured by Fourier Transformed Infrared Spectrometer (Spectrum one, Perkin-Elmer) by KBr pellet method.

## 2. Preparation of hydrogels by freeze-thaw processes

From preformulation data, hydrogels made from DG and PVA by freeze-thaw process were selected for further study on the factors influencing hydrogel preparation. Factors such as freeze-thaw duration, mixing temperature and thickness were selected for preliminary study. Then the suitable condition was used for further study on other processing parameters.

## 2.1 Preliminary study of freeze-thaw processing parameters

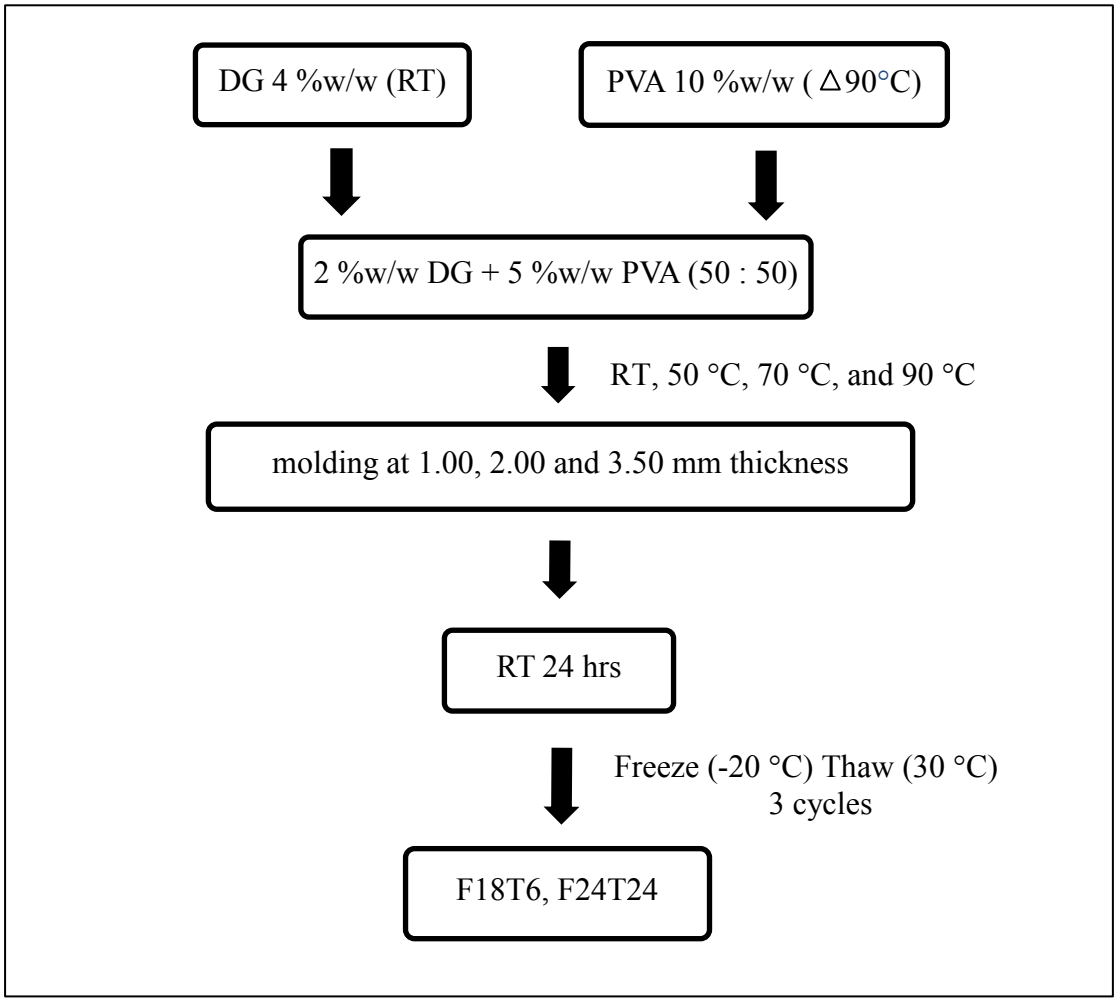



Figure 9. A schematic diagram for preparing DG/PVA (2 : 5) hydrogels by freeze-thaw method

In this study, hydrogels were made from 2 %w/w DG and 5 %w/w PVA (DG/PVA, 2 : 5) using following freeze-thaw conditions: freezing at -20 °C for 18 hrs and thawing at 30 °C for 6 hrs (F18T6); and freezing for 24 hrs and thawing for 24 hrs (F24T24). PVA powder was dissolved in deionized water and heated up to 90 °C for 60 min to achieve complete dissolution. DG powder was completely swollen in deionized water at room temperature (RT). The PVA 10% w/w and the DG 4% w/w solutions were mixed with a ratio of 50 : 50 at various temperatures of RT, 50 °C, 70

°C and 90 °C. The mixing solution was poured into a plastic mould and sealed. Then, the sample was kept at room temperature for 24 hours. The viscous solution was frozen at -20 °C and followed by thawing at 30 °C to form a hydrogel membrane. Paraben concentrate was used as preservative in concentration of 1% w/w. Only PVA 5% w/w solution was prepared by the same method above to perform the control hydrogel (Figure 9).

Two conditions, F18T6 and F24T24, were used in this freeze-thaw research. For F18T6, the viscous solution was frozen 18 hrs and thawed 6 hrs for 3 consecutive cycles. This condition corresponded to the study of Kim et al. (2008). For F24T24, the solution was frozen 24 hrs and thawed 24 hrs for 3 cycles following the research of Kokabi, et al. in 2007. The obtained hydrogels with the thickness of 1.00, 2.00 and 3.50 mm were characterized for their water content, water absorption capacity and gel fraction.

### 2.2 Preparation of 3.50 mm DG/PVA hydrogels by F24T24

From the preliminary results, F24T24 for 3 repeated cycles, the mixing temperature at room temperature and the thickness of 3.50 ± 0.35 mm were the optimal factors and selected for further study for variation of DG concentrations and freeze-thaw cycles.

PVA and DG solutions were prepared and mixed at room temperature as the same method as 2.1. Different concentrations of DG solutions at 4%, 6%, and 7% w/w were used. The DG/PVA mixture solutions were frozen 24 hrs and thawed 24 hrs (F24T24) for 3, 4 and 5 consecutive cycles (Figure 10).

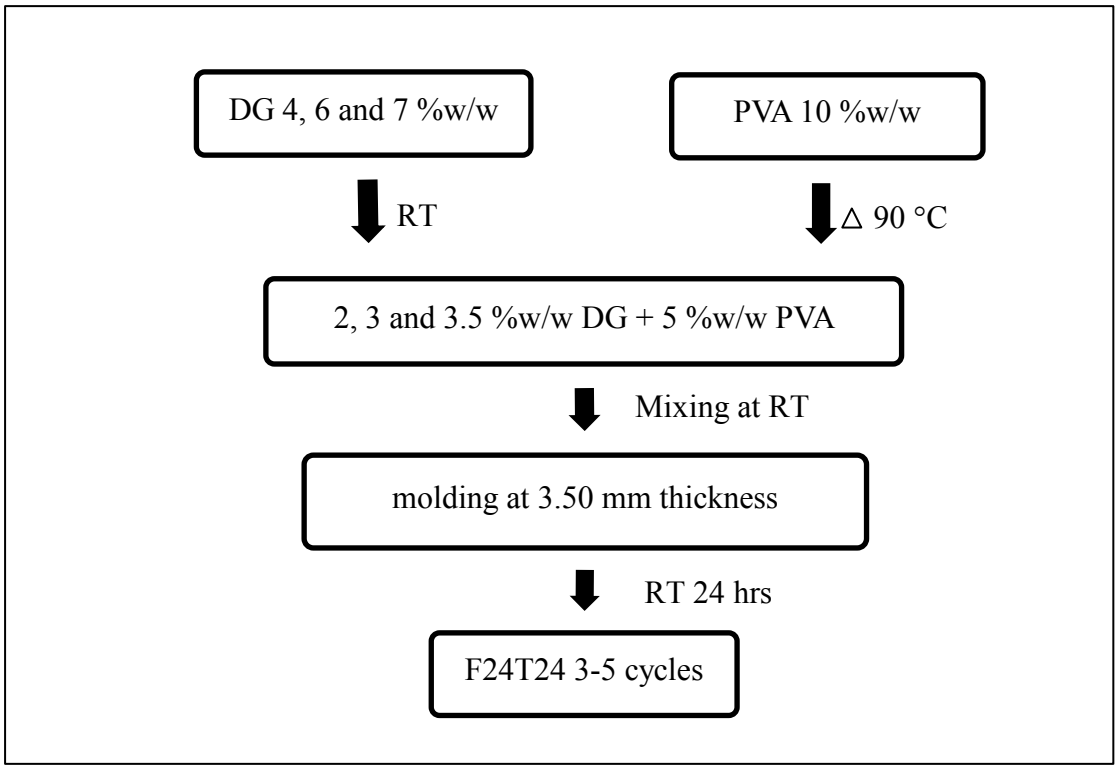



Figure 10. A schematic diagram for preparing 3.50 mm DG/PVA hydrogels by freeze-thaw method of F24T24 at different concentrations of DG and 3-5 cycles

## 3. Physicochemical characterization

### 3.1 Water content

The hydrogel membrane was cut into a size of 1.5 x 5.0 $cm^2$. Each piece of preweighed hydrogel ($W_0$) was dried at 50 °C in oven until having constant weight ($W_d$). Water content in the hydrogel was calculated by equation (1).

$$\text{Water content (\%)} = \frac{W_o - W_d}{W_o} \times 100 \quad (1)$$

### 3.2 Water absorption capacity

The hydrogel membrane was cut into a size of 1.5 x 5.0 $cm^2$ and soaked in excess of deionized water at room temperature for 4 days. The swollen membane was gently wiped using filter paper to remove excess surface water and weighed at various time points. The water uptake was calculated by equation (2) (Razzak et al, 2001; Salmawi, 2007; Varshney, 2007).

$$\text{Water absorption (\%)} = \frac{W_s - W_o}{W_o} \times 100 \quad (2)$$

Where $W_s$ is the weight of the swollen hydrogels.

$W_0$ is the initial weight of hydrogels (before soaking in water).

### 3.3 Gel fraction

The hydrogel membrane was cut into 2 pieces of identical size of 1.5 x 5.0 $cm^2$. One piece was dried at 50 °C in oven until having constant weight (Kim et al., 2008). The other piece was soaked in excess deionized water for 4 days (Kokabi et al., 2007), then the soaked hydrogel membrane was dried with the same condition until having constant weight. Gel fraction was calculated by equation (3).

$$\text{Gel fraction (\%)} = \frac{W_e}{W_o} \times 100 \quad (3)$$

Where :

$W_e$ is the dried weight correlated to one gram of initial hydrogel after rinsing in deionized water.

$W_0$ is the dried weight correlated to one gram of initial hydrogel before rinsing in deionized water.

### 3.4 Water vapor transmission rate (WVTR)

The water vapor transmission rate of the hydrogel was measured by recording the weight loss of a WVTR bottle containing water which covered with hydrogel. The WVTR bottle had a diameter of 35 mm and contained 25 ml of pure water. The hydrogel with a diameter of 40 mm and a thickness of 3.50 ± 0.35 mm was used to replace a cap of the WVTR bottle (Figure 11A) (Kokabi et al., 2007; Razzak et al, 2001; Salmawi, 2007). The WVTR bottle was placed in an oven at 35 ± 2 °C and 75 %RH for 24 hrs (Figure 11B). The WVTR was calculated using the following equation (4)

$$\text{WVTR} = \frac{(W_i - W_t)}{A \times 24} \times 10^6 \quad g/m^2/hr \qquad (4)$$

Where :

$W_i$ and $W_t$ are the weights of the WVTR bottle and hydrogel membrane before and after placing in an oven for 24 hrs, respectively.

A is the area of WVTR bottle month ($mm^2$).

A  B

Figure 11. WVTR bottle containing water and covered with hydrogel membrane (A), The humidity controlled chamber for the WVTR bottle (B)

### 3.5 Water evaporation rate (WER)

The evaporation rate of the moisture in hydrogel was investigated similar to WVTR. Weight loss of an empty bottle covered with hydrated hydrogel membrane at 35 ± 2 °C and 75 %RH for 24 hrs was monitored (Figures 12A-B).

A  B

Figure 12. WER bottle covered with hydrogel (A), The humidity controlled chamber for the WER bottle (B)

### 3.6 Scanning electron microscopy (SEM)

Electron photomicrographs of hydrogel membranes, unswollen and swollen, were taken with a scanning electron microscope (JEOL model JSM-5410W SEM machine). For unswollen hydrogel, the membrane sample was cut into small pieces and dehydrated before testing by using Critical Point Dryer (CPD). In brief, each piece of hydrogel was soaked and swirled in absolute ethanol for 10 min in triplicate. The sample was put in CPD to remove absolute ethanol by liquid carbondioxide under critical point. Then liquid carbondioxide changed into gaseous state under ambient condition, the sample was immediately dried with no collapsed in surface or its structure. The hydrogel surface and its cross-section were determined at the power of 15 kV.

For swollen hydrogel, the membrane was immersed in deionized water for 13 hrs. Then sample was cut into small pieces and dehydrated before testing by using CPD. The surface and the cross-section of the hydrogel membrane were determined at the same power as stated earlier.

### 3.7 Differential Scanning Calorimetry (DSC)

The DSC analysis was performed using Mettler Toledo DSC822$^e$. Dry hydrogels 1-3 mg were heated from 25 to 400 °C under $N_2$ atmosphere (60 ml/min) with a heating rate of 10 °C/min (Yang et al., 2008).

### 3.8 Fourier Transformed Infrared Spectrometry (FTIR)

FTIR of DG powder, PVA powder and hydrogel membranes were measured by Fourier Transformed Infrared Spectrometer (Spectrum one, Perkin-Elmer). Powder was prepared by KBr pellet method before testing while hydrogel membrane was examined by Attenuated Total Reflextance (ATR).

### 3.9 Mechanical properties

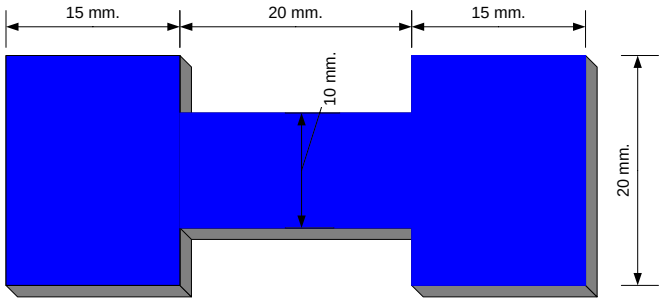



Figure 13. The specific hydrogel shape appropriate for mechanical testing

For unswollen hydrogel, the membrane was cut into specific shape as shown in Figure 13 (2 cm wide at the end and 1 cm wide in the middle). The mechanical data

were measured using LLOYD instrument model LR 10K with a constant crosshead speed of 20 mm/min at room temperature (Kim et al., 2008).

For swollen hydrogel, the hydrogel was immersed in deionized water for 13 hrs. Then the membrane sample was cut into the specific shape and measured by the same instrument and condition above.

### 3.10Adhesion property

The degree of adhesion of the hydrogel membrane was measured by Texture Analyzer (TA.XT *plus*, UK). The membrane with a thickness of 3.5 ± 0.35 mm was in contact with the aluminum plate size 1.0 x 1.0 $cm^2$ with a gross weight 100 g for 30 sec. The experiment was done at room temperature with a constant pulling speed of 0.02 mm/sec (Razzak et al, 2001).

## 4. Stability study

The PVA hydrogel and the DG/PVA hydrogel contained PVA 5% w/w and DG 3.5% w/w prepared by freezing at -20 °C 24 hours and thawing at 30 °C 24 hours for 3 consecutively cycles were kept at 4 °C, 30 °C 75 %RH and 40 °C 75 %RH for 3 months. Physical properties of the hydrogels were characterized at 1, 2 and 3 months compared with the initial properties.

## 5. Microbial testing of hydrogels

The hydrogel samples used for microbial testing were sterilized before testing by gamma irradiation at dose rate 0.13 kGy for 192.31 min (total dose 25 kGy)

### 5.1 Antimicrobial activity test

Antimicrobial property was investigated using agar diffusion test described by Nantawanit (2001). In brief, plate with internal diameter of 100 mm containing 25 ml of Mueller Hinton Agar (MHA) was inoculated with 0.1 ml suspension of microorganism ($10^8$ CFU/ml) by spread plate technique. The DG/PVA hydrogel with a thickness of 3.50 ± 0.35 mm and surface area 4 $cm^2$ (2.0 x 2.0 $cm^2$) was placed on the inoculated agar surface. The PVA hydrogel was used as control. The plates were incubated at 37 °C overnight. Microbial growth under the DG/PVA hydrogel was observed in comparison with the growth of microbial under the PVA hydrogel. Inhibitory zone was indicated by the observation of clear area surrounding DG/PVA hydrogel on surface of the medium.

### 5.2 Microbial penetration test

#### 5.2.1 Survival of bacteria under the hydrogel

This test was performed to prove that bacteria were able to stay under the hydrogel in hypoxic condition. Each steriled DG/PVA hydrogel with a thickness of 3.50 ± 0.35 mm was cut into a size of 2.0 x 2.0 $cm^2$. Suspension of microorganism ($10^8$ CFU/ml) 10 µl was dropped on the MHA surface prepared by the same method as 5.1 and covered with hydrogel sample. The steriled PVA hydrogel was used as control. Plates were incubated at 37 °C overnight. Bacterial growth between hydrogel and MHA was investigated.

#### 5.2.2 Bacteria penetration through the hydrogel

The microbial penetration test was performed to estimate the resistance of hydrogel dressing against microbe transmission from environment to the top surface of the wound. Each steriled DG/PVA hydrogel with a thickness of 3.50 ± 0.35 mm was cut into a size of 2.0 x 2.0 $cm^2$ (Razzak et al, 2001; Salmawi, 2007). The sample

was put on the MHA plate prepared by the same method as 5.1. Suspension of microorganism ($10^8$ CFU/ml) 10 µl was dropped on the top surface of the sample. The sterilized PVA hydrogel was used as control. Plates were incubated at 37 °C overnight. Bacteria passed through hydrogel were monitored daily by observing the colony of bacteria directly below hydrogel on MHA medium (Kokabi et al., 2007).

**6. Statistical analysis**

The results were analyzed by One-way ANOVA and Independent-samples T-Test. Test of normality and homogeneity of variances were performed.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 1. Preformulation study

### 1.1 Screening of materials and methods for hydrogel preparation

To study the materials which had potential to form hydrogel, synthetic polymers used in this study were PVA with MW. 27,000, 72,000 and 115,000, PVP K30 and PVP K90. Natural polysaccharides selected were agar, carrageenan, konjac and DG.

All the mixture solutions of synthetic polymers and natural polysaccharides which contacted with UV and microwave radiation could not form hydrogel membrane. The mixture solutions contacted with UV at both wavelengths for 3-12 minutes did not show any change. The samples which passed high power microwave radiation were boiled but were hot when passing medium and low power radiation.

For chemical cross-linking technique, only PVA and DG were studied. DG/PVA and PVA hydrogels could be formed by chemical crosslink. But the membranes had foul odor due to sulfuric acid and glutaraldehyde (GA). High concentration of GA formed rigid membrane. After the reaction was finished, the hydrogels were rinsed for several times to wash off the chemical residues.

The samples of PVP and PVP/DG could not form a hydrogel membrane by freeze-thaw at 1-3 cycles. Whereas the samples of PVA and PVA/DG were able to form a hydrogel membrane by freeze-thaw process, except PVA MW. of 115,000 due to its low degree of hydrolysis.

From data obtained, hydrogel-base materials selected for further study were PVA MW. 72,000 and DG. Physical crosslink by freeze-thaw technique was the suitable process used to prepare hydrogel membrane in this study.

### 1.2 Identification of hydrogel forming materials

Polysaccharide gel extracted from Durian fruit-hulls (DG) was isolated from dried fruit-hulls of durian (*Durio zibethinus L.*) previously described by Pongsamart and Panmuang (1998) and Hokputsa, et al. (2004). In brief, ground fruit-rind was suspended in hot water, the pH of mixture was adjusted to 4.5 and boiled for 20 min. The hot mixture was filtered through paper filter and filtrate was collected. Clear filtrate was evaporated, poured into 3 volumes of 75% ethanol and vigorously stirred. Precipitated gel was collected and washed with ethanol. The dried precipitate was ground and sieved to obtain powder. The two lots of DG powder, L-I and L-II, used in this study are shown in Figures 14A and 14B, respectively. DG powder L-I and DG powder L-II were used in antimicrobial study and preparation study.

Polyvinyl alcohol MW. 72,000 was bought from Merck and used without any further purification. The physical appearance of PVA powder was white and fluffy (Figure 14C).

A B C

Figure 14. Photographs of hydrogel forming materials: DG powder L-I (A), DG powder L-II (B), PVA powder (C)

#### 1.2.1 Scanning Electron Microscopy (SEM)

Powder morphology of DG L-I, DG L-II and PVA under scanning electron microscope are shown in Figures 15A, 15B and 15C, respectively. Particle size of DG L-II was larger than DG L-I. In agreement with Pongsamart and

Panmaung (1998), fiber and round particles were observed. Whereas the particle shape of PVA powder was long fibers which coiled and aggregated.

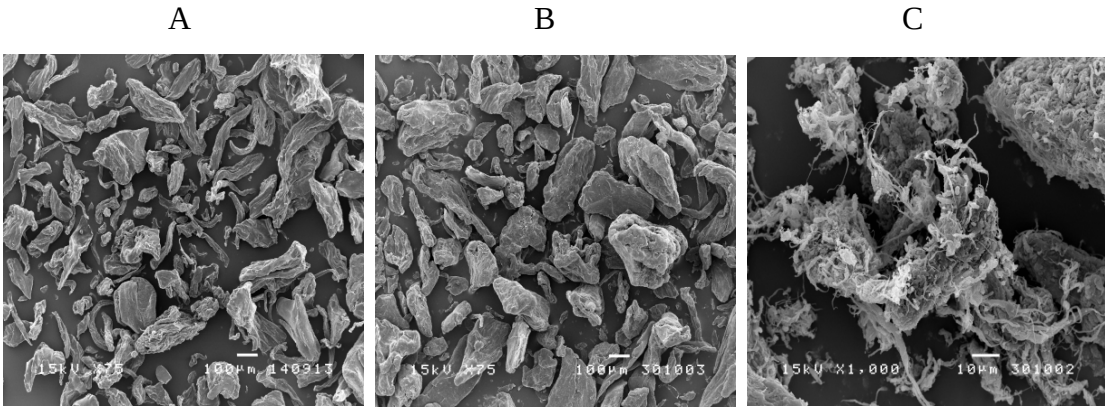



Figure 15. Scanning electron photomicrographs of hydrogel materials; A: DG powder L-I (x75), B: DG powder L-II (x75), and C: PVA powder (x1,000)

### 1.2.2 Differential Scanning Calorimetry (DSC)

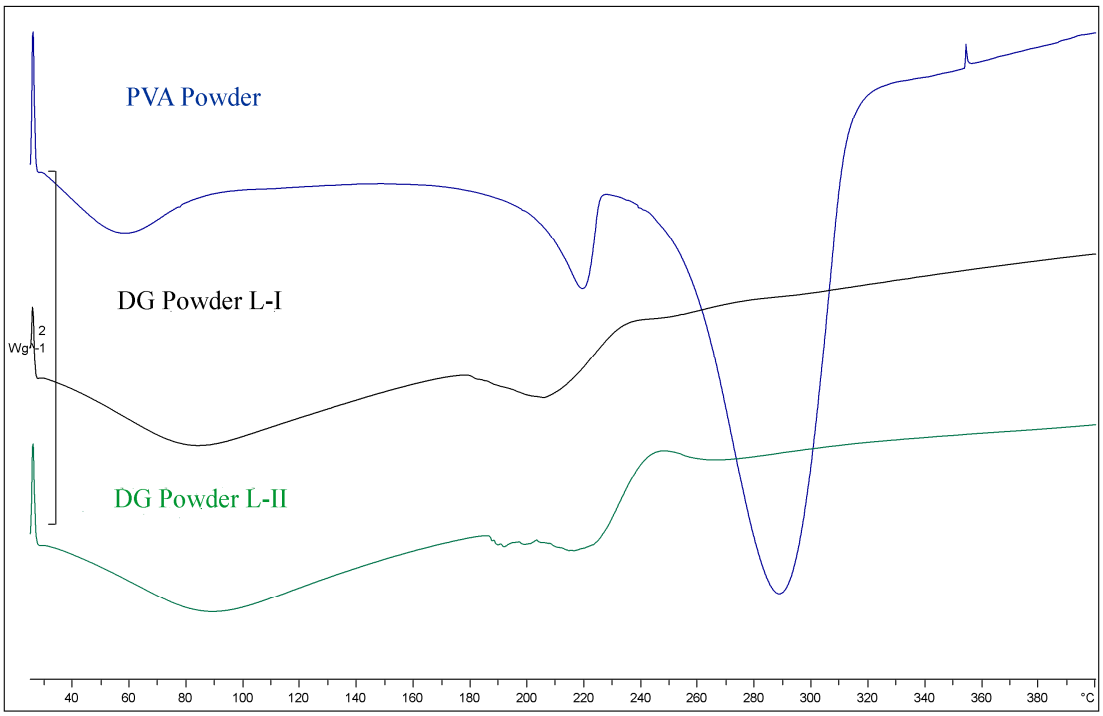



Figure 16. DSC thermograms of hydrogel forming materials

DSC thermograms of DG and PVA materials are shown in Figure 16. In PVA powder, three endothermic peaks were observed. Dehydration peak appeared at approximately 60 °C. The peak at about 220 °C was the melting temperature ($T_m$) of PVA, corresponding to Yang et al. (2008) who described $T_m$ at about 228.3 °C. Endotherm at 290 °C signified degradation of PVA powder.

Endothermic peaks of DG powder L-I and L-II were similar. Both peaks were broad. Dehydration peaks appeared at approximately 85 and 90 °C in DG L-I and DG L-II, respectively. The peak at about 200 °C might be degradation temperature. From the DSC thermograms, DG L-I and L-II showed no difference between the 2 lots of DG.

### 1.2.3 Fourier Transformed Infrared Spectrometry (FTIR)

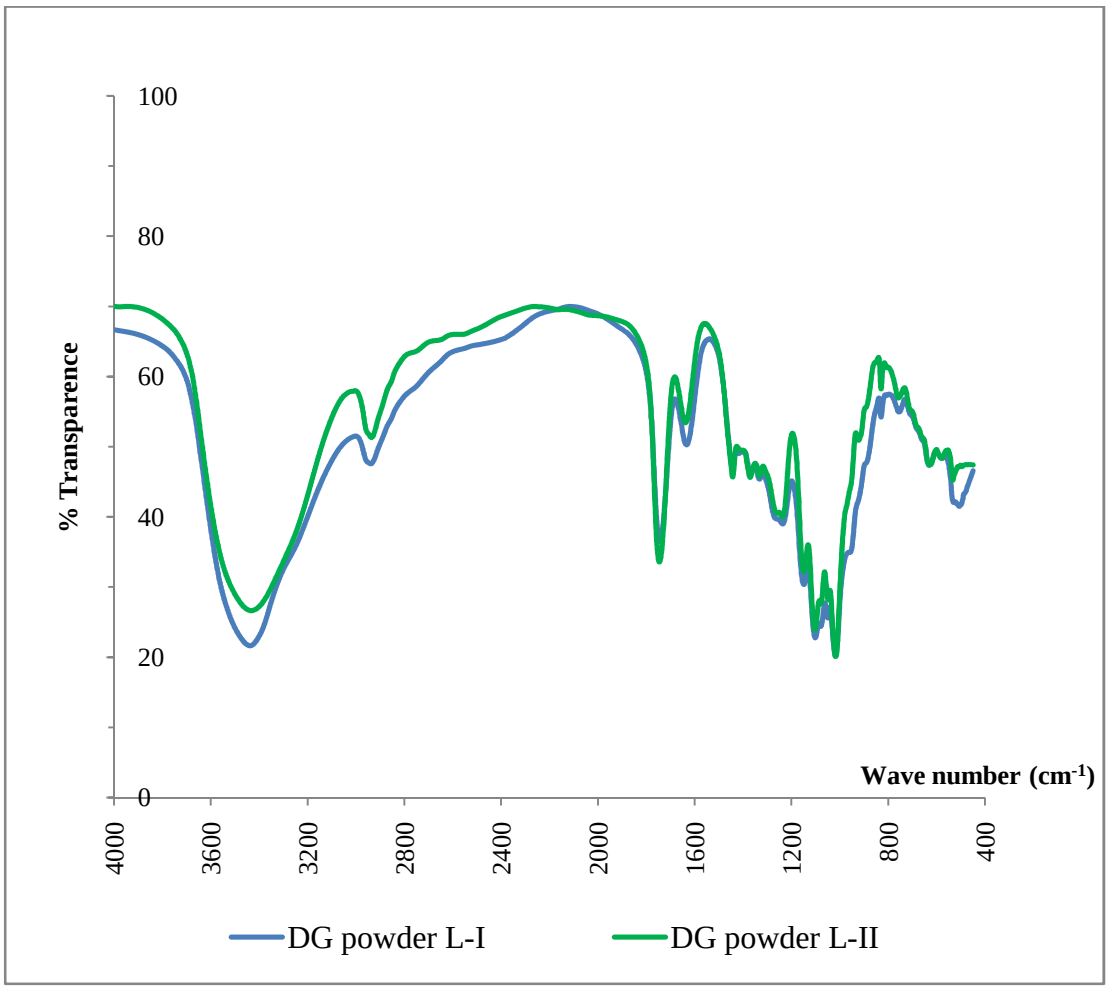



Figure 17. Fourier transformed infrared spectrums of DG powder L-I and L-II

FTIR was used to characterize the presence of specific chemical groups in DG L-I and DG L-II samples. The functional groups exhibited important absorption bands from FTIR measurements as shown in Figure 17. Characteristic alkyl (R-$CH_2$) stretching modes appeared at about ν = 2,936 and 2,939 $cm^{-1}$. The hydroxyl group contribution was observed with absorption ranging from ν = 3,435 $cm^{-1}$. Also, strong bond from carbonyl group associated with aldehyde group was verified (C=O at ν ≈ 1,745 $cm^{-1}$). This result indicated that DG L-I and DG L-II were chemically similar.

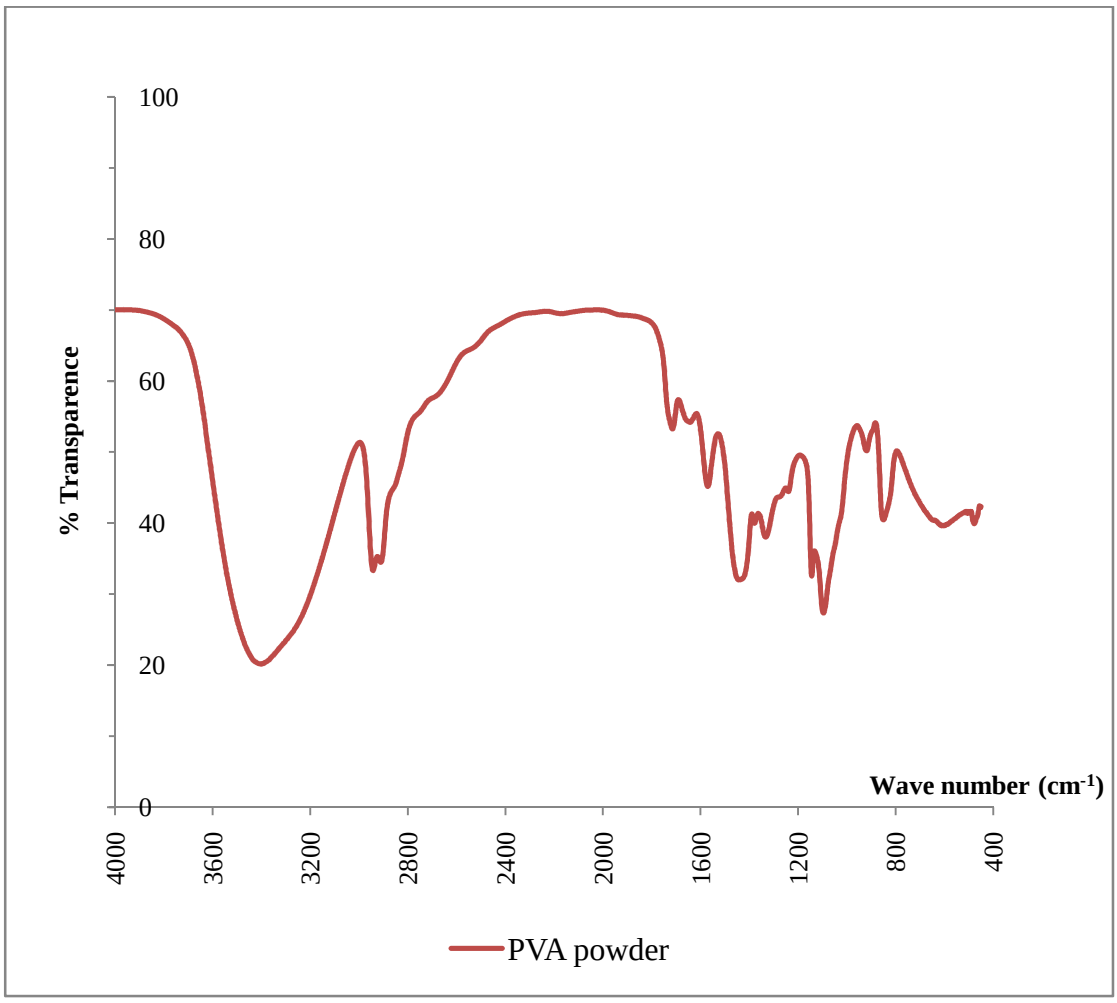



Figure 18. Fourier transform infrared spectrum of PVA powder

The chemical and composition characteristic of PVA powder was analyzed by FTIR spectroscopy. Figure 18 represents the FTIR spectrum of PVA powder. The bands with the peaks at ν = 478, 605 and 850 $cm^{-1}$ were assigned to the deformation vibration of free –OH groups and the peak observed at ν = 3,399 $cm^{-1}$ was assigned to

the stretching vibration of these groups. The ν = 1,239, 1,332 and 1,378 $cm^{-1}$ peaks were attributed to the characteristic $–CH_3$ bending. The peaks at ν = 2,910 and 2,942 $cm^{-1}$ were due to C-H stretching vibration.

## 2. Freeze-thaw process parameter study

### 2.1 Preliminary study of freeze-thaw process parameters

The DG/PVA (2 : 5) and PVA hydrogels could be formed after one freeze-thaw process. However, their strength, even after having 1 and 2 consecutive freeze-thaw cycles were not strong enough for handling and physical testing. Therefore, 3 cycles of freeze-thaw process was used to prepare a hydrogel membrane. There were 3 effects which were study in this experiment such as freeze-thaw duration, mixing temperature and thickness.

#### 2.1.1 Hydrogel appearances

The effect of freeze-thaw duration was studied. the DG/PVA hydrogels were transparent light-tan from DG powder, while PVA hydrogels were transparent and colorless. The appearances of DG/PVA and PVA hydrogels were similar in both conditions of freeze-thaw process, F18T6 and F24T24 (Figures 19A and 19B, respectively).

The second effect, mixing temperature, was investigated. Aappearances of DG/PVA hydrogels prepared at various temperatures were similar. Figures 20B-E show the DG/PVA hydrogels of 3.50 mm thickness prepared by F184T6 (3 cycles) and mixing at various temperatures of RT (DG-RT), 50 ºC (DG-50), 70 ºC (DG-70) and 90 ºC (DG-90), respectively. Figure 21 shows the DG/PVA hydrogels which were prepared by F24T24 (3 cycles). There was no effect of the mixing temperature on the hydrogel appearances.

The effect of thickness was considered, the DG/PVA and PVA hydrogels could be prepared with the minimum thickness of 1.00 mm but they were difficult to handle. The DG/PVA hydrogels with all thickness had transparent light-tan color,

while the PVA hydrogels with all thickness were transparent and colorless. Thus thickness did not affect their transparency and color of DG/PVA and PVA hydrogels.

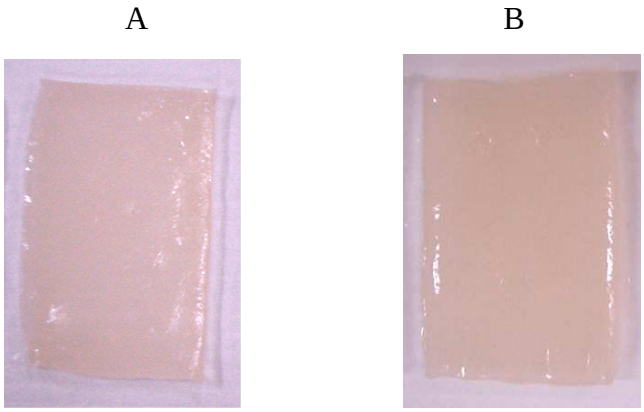



Figure 19. Photographs of DG-RT prepared by F18T6 (A) and F24T24 (B)

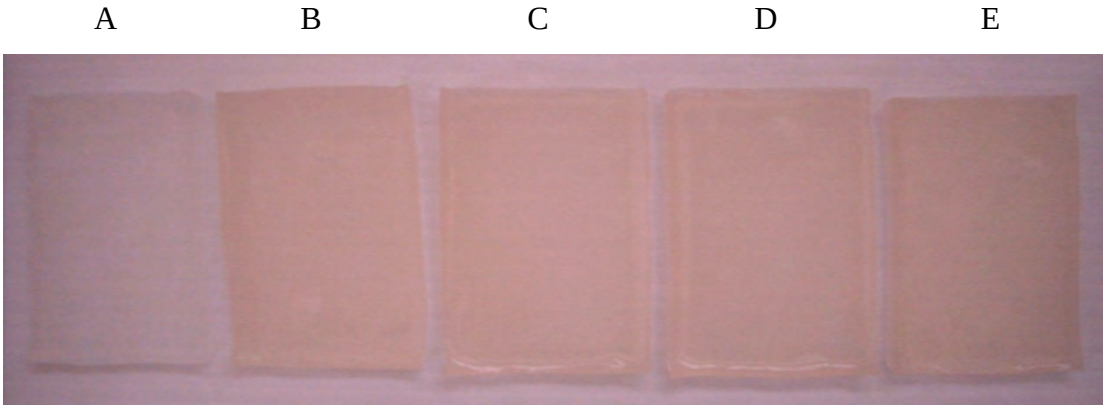



Figure 20. Photographs of the hydrogels prepared by F18T6 (3 cycles): PVA (A), DG-RT (B), DG-50 (C), DG-70 (D) and DG-90 (E)

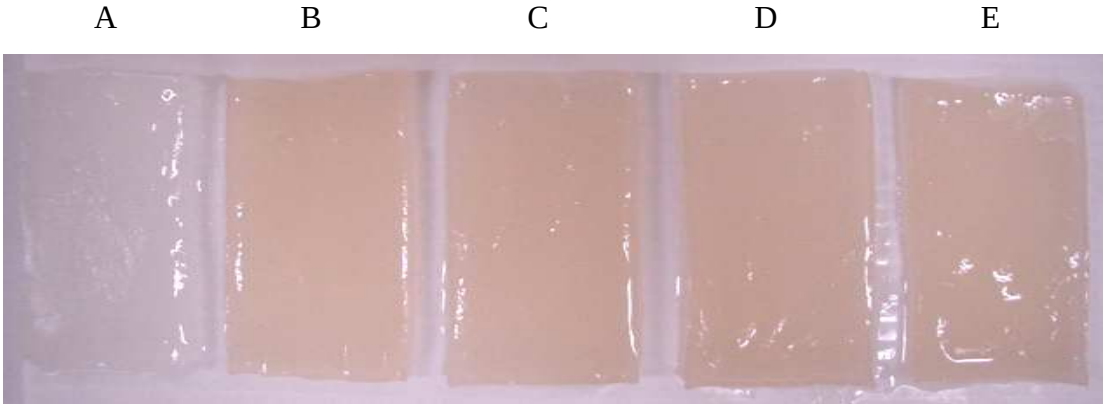



Figure 21. Photographs of the hydrogels prepared by F24T24 (3 cycles): PVA (A), DG-RT (B), DG-50 (C), DG-70 (D) and DG-90 (E)

### 2.1.2 Water content

Table 1. Water contents of hydrogels prepared by F18T6 (3 cycles) at various temperatures and thickness

| **Hydrogels (Mixing temperature)** | **Thickness (mm)** | | |
|---|---|---|---|
| | **1.00 ± 0.10** | **2.00 ± 0.20** | **3.50 ± 0.35** |
| DG-RT | 91.53 ± 0.55 | 92.03 ± 0.10 | 92.10 ± 0.02 |
| DG-50 | 91.41 ± 0.20 | 91.60 ± 0.06 | 91.39 ± 0.03 |
| DG-70 | 91.47 ± 0.27 | 91.07 ± 0.08 | 91.19 ± 0.15 |
| DG-90 | 91.26 ± 0.35 | 91.05 ± 0.15 | 91.11 ± 0.07 |
| PVA | 93.29 ± 0.12 | 93.40 ± 0.24 | 93.40 ± 0.12 |

Table 2. Water contents of hydrogels prepared by F24T24 (3 cycles) at various temperatures and thickness

| **Hydrogels (Mixing temperature)** | **Thickness (mm)** | | |
|---|---|---|---|
| | **1.00 ± 0.10** | **2.00 ± 0.20** | **3.50 ± 0.35** |
| DG-RT | 90.39 ± 0.16 | 90.60 ± 0.16 | 90.52 ± 0.24 |
| DG-50 | 89.98 ± 0.16 | 90.52 ± 0.17 | 90.39 ± 0.15 |
| DG-70 | 88.30 ± 0.20 | 90.16 ± 0.10 | 90.24 ± 0.04 |
| DG-90 | 89.44 ± 0.12 | 90.20 ± 0.18 | 90.43 ± 0.39 |
| PVA | 92.65 ± 0.02 | 92.08 ± 0.12 | 93.32 ± 0.12 |

The effect of freeze-thaw duration was studied. Water contents of the DG/PVA and PVA hydrogels prepared by both conditions were similar, slightly lower in F24T24 condition. While water contents of PVA hydrogels were slightly higher than the PVA hydrogels incorporated with DG (Table 1-2).

The second effect, mixing temperature, was investigated. Water contents of the DG/PVA hydrogels prepared by various mixing temperatures were similar and slightly higher when mixed at room temperature (Figures 22-23).

The effect of thickness was considered. Water contents of DG/PVA hydrogels with various thickness were not different. The values were higher than 90 %, except the DG/PVA hydrogel prepared by F24T24 with the thickness of 1 mm (Figures 24-25). Water contents of the PVA hydrogels with all thickness were also higher than 90 % and slightly higher than those of the DG/PVA hydrogels.

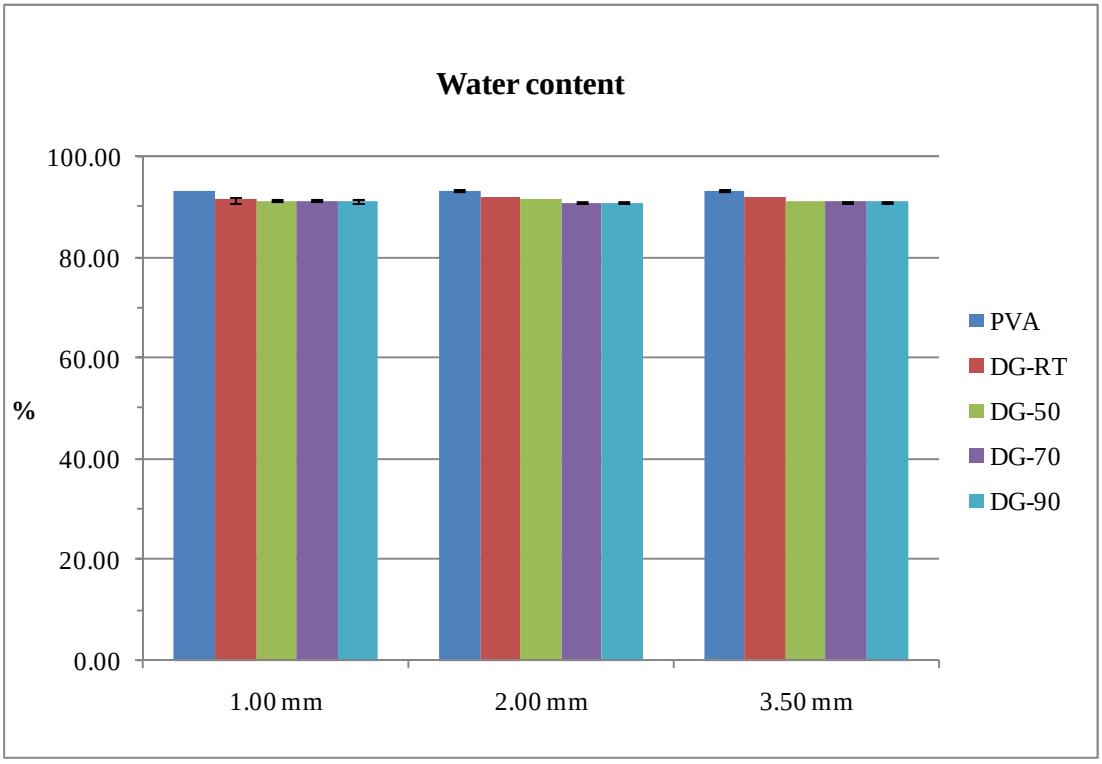



Figure 22. Water contents of hydrogels prepared by F18T6 (3 cycles) at various mixing temperatures

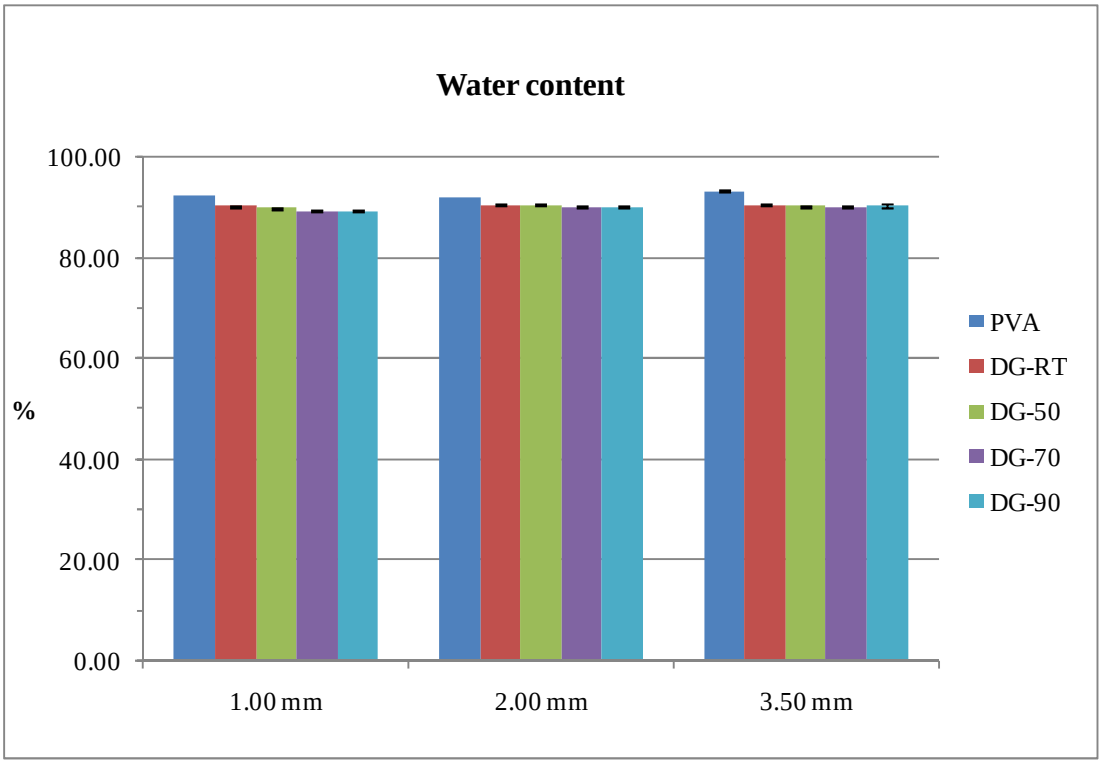



Figure 23. Water contents of hydrogels prepared by F24T24 (3 cycles) at various mixing temperatures

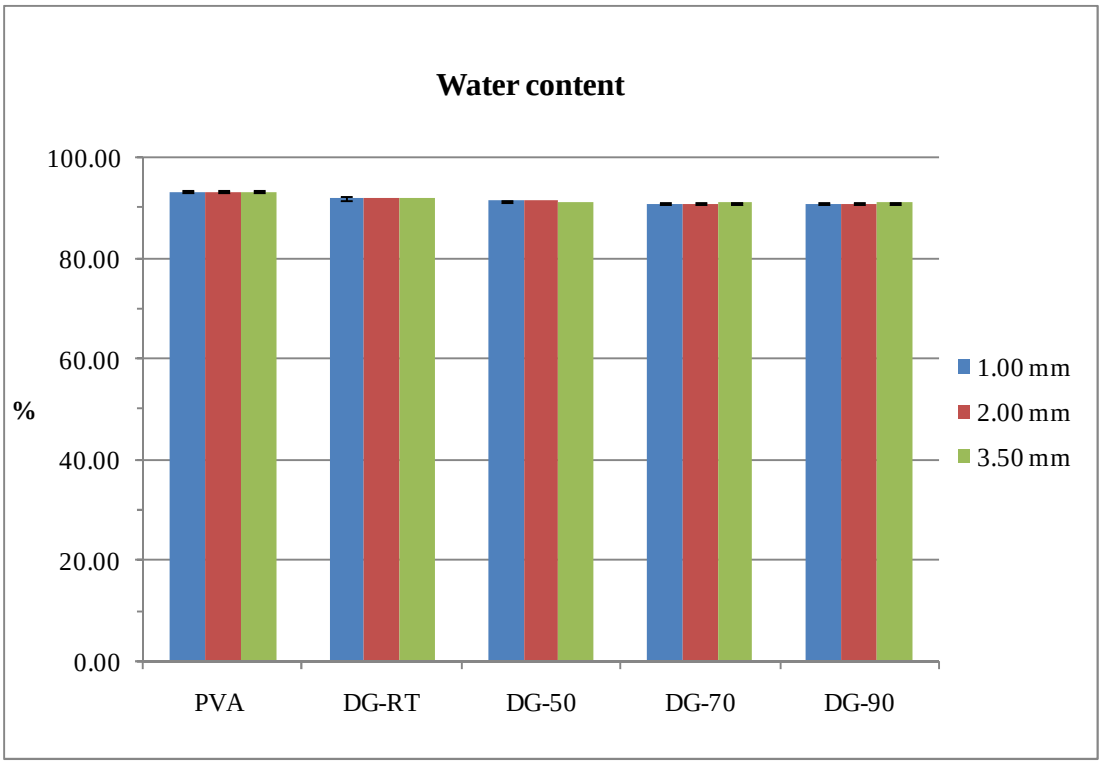



Figure 24. Water contents of the hydrogels prepared by F18T6 (3 cycles) with the thickness of 1.00, 2.00 and 3.50 mm

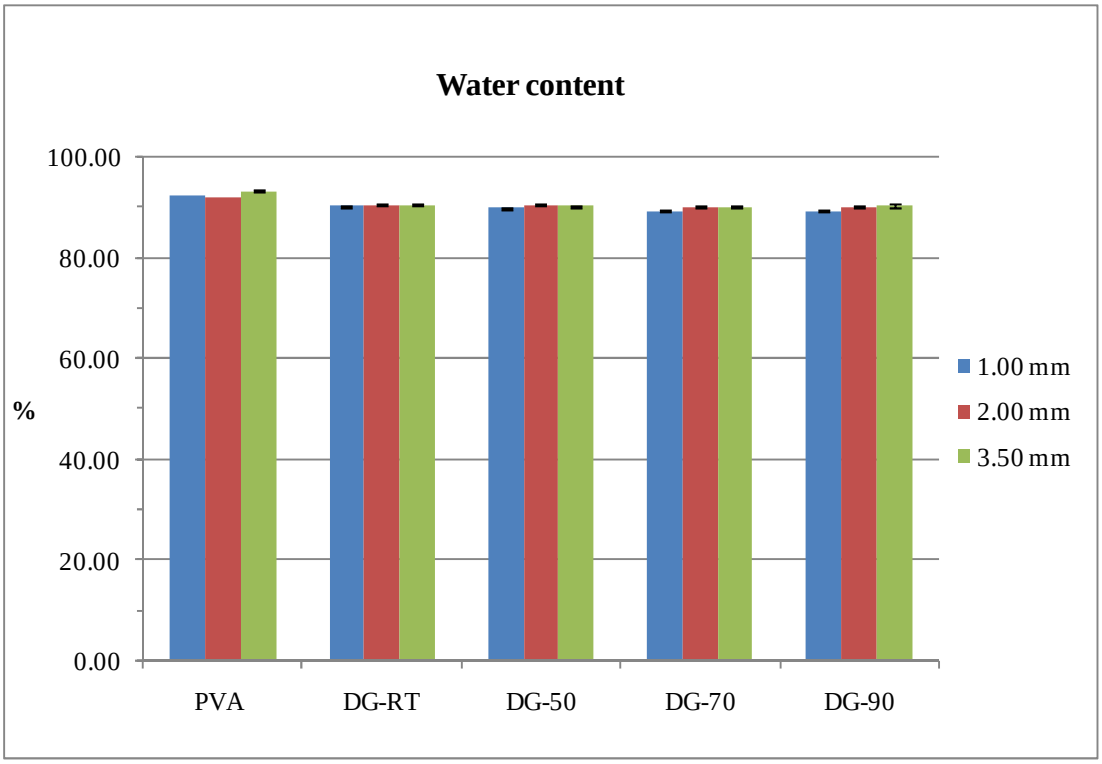



Figure 25. Water contents of the hydrogels prepared by F24T24 (3 cycles) with the thickness of 1.00, 2.00 and 3.50 mm

### 2.1.3 Water absorption capacity

The effect of freeze-thaw duration was studied. DG/PVA and PVA hydrogels prepared by F24T24 condition absorbed much more water than F18T6 condition. Data of the PVA hydrogels with thickness of 1.00 mm were not shown because their network structures were not strong enough for handling and testing (Table 3-4).

The second effect, mixing temperature, was investigated. The DG/PVA hydrogels prepared by mixing at room temperature showed higher water absorption than those prepared at higher mixing temperatures (Figures 26-27).

The effect of thickness was considered. The thickness of hydrogel was one of the factors that affected the water absorption capacity in the hydrogel structures. DG/PVA hydrogels with the thickness of 1.00 mm absorbed much more water and faster than the thickness of 2.00 or 3.50 mm except DG-70 and DG-90 prepared by F24T24. They also absorbed more in the 2-4 hours but their swelling

property were immediately decreased afterward (Figures 28-29). The thicker hydrogels could absorb less water than thinner hydrogels could do, but they were also strong after swelling and able to hold much water in their structures. While the thinner hydrogels absorbed large volume of water at initial and followed by the leakage of much water or hydrogel-base.

Table 3. Water absorption capacity of the hydrogels prepared by F18T6 (3 cycles) at various mixing temperatures and thickness

| **Hydrogels (Mixing temperature)** | **% Maximum absorption ± SD ($T_{max}$)** | | |
|---|---|---|---|
| | **1.00 ± 0.10 mm** | **2.00 ± 0.20 mm** | **3.50 ± 0.35 mm** |
| DG-RT | 126.04 ± 20.33 (2 hrs) | 65.05 ± 8.15 (2 hrs) | 43.89 ± 3.83 (4 hrs) |
| DG-50 | 75.59 ± 10.89 (2 hrs) | 57.41 ± 5.63 (2 hrs) | 41.43 ± 6.75 (4 hrs) |
| DG-70 | 52.39 ± 2.40 (2 hrs) | 43.45 ± 3.09 (2 hrs) | 41.14 ± 7.93 (4 hrs) |
| DG-90 | 47.73 ± 6.02 (2 hrs) | 42.58 ± 10.35 (2 hrs) | 38.90 ± 2.82 (2 hrs) |
| PVA | - | 20.72 ± 3.72 (2 hrs) | 15.80 ± 4.11 (4 hrs) |

Table 4. Water absorption capacity of the hydrogels and prepared by F24T24 (3 cycles) at various mixing temperatures and thickness

| **Hydrogels (Mixing temperature)** | **% Maximum absorption ± SD ($T_{max}$)** | | |
|---|---|---|---|
| | **1.00 ± 0.10 mm** | **2.00 ± 0.20 mm** | **3.50 ± 0.35 mm** |
| DG-RT | 128.03 ± 11.63 (4 hrs) | 95.16 ± 3.39 (2 hrs) | 67.56 ± 14.94 (4 hrs) |
| DG-50 | 87.69 ± 3.28 (2 hrs) | 85.98 ± 4.68 (2 hrs) | 55.68 ± 3.28 (4 hrs) |
| DG-70 | 74.74 ± 1.63 (4 hrs) | 86.07 ± 4.74 (2 hrs) | 59.99 ± 5.48 (6 hrs) |
| DG-90 | 72.57 ± 2.76 (2 hrs) | 85.68 ± 7.43 (2 hrs) | 61.97 ± 11.91 (4 hrs) |
| PVA | - | 29.24 ± 4.65 (4 hrs) | 31.24 ± 3.80 (12 hrs) |

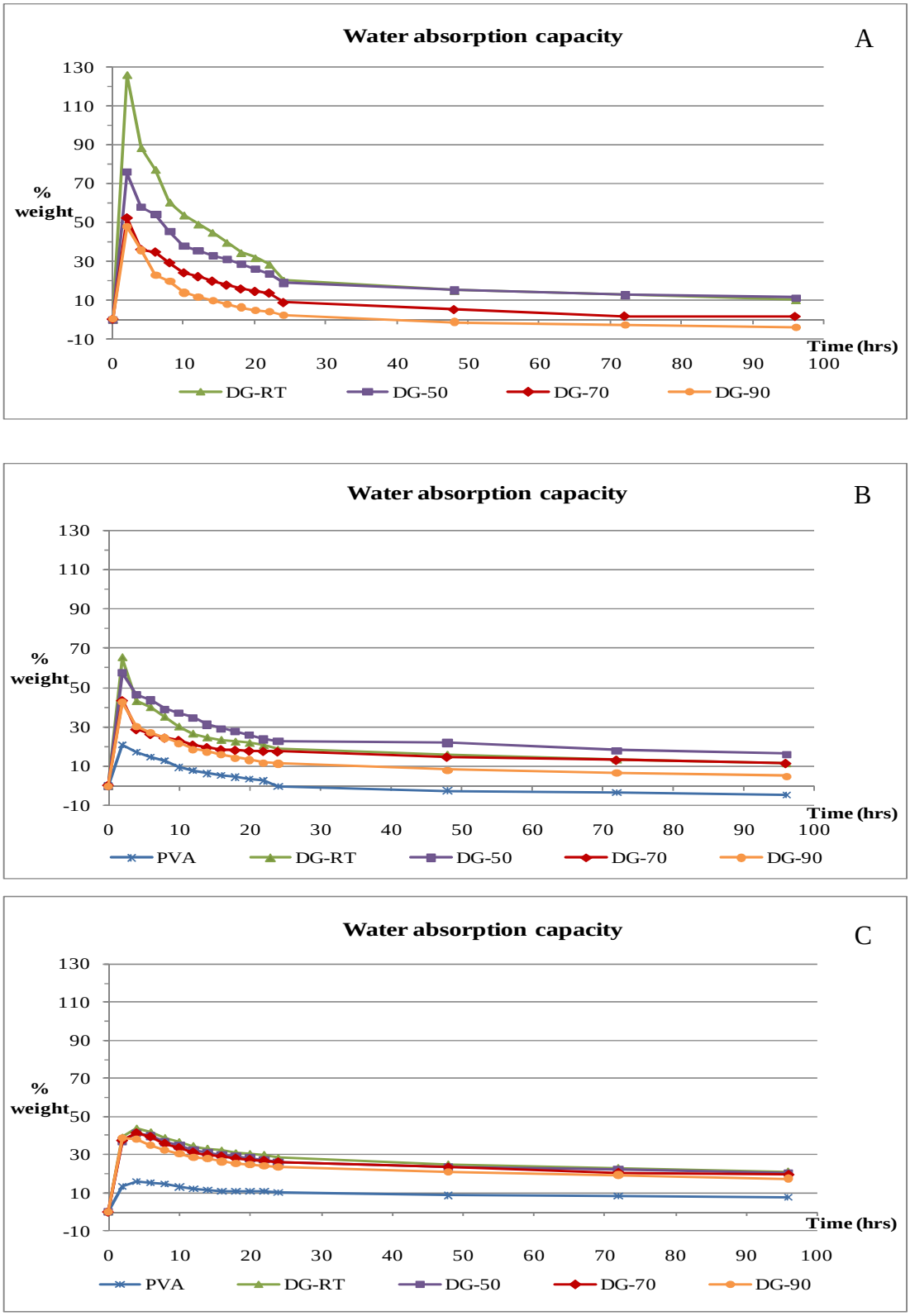



Figure 26. Water absorption capacity of hydrogels prepared by F18T6 (3 cycles), at various thickness (A) 1.00 ± 0.10 mm (B) 2.00 ± 0.20 mm (C) 3.50 ± 0.35 mm

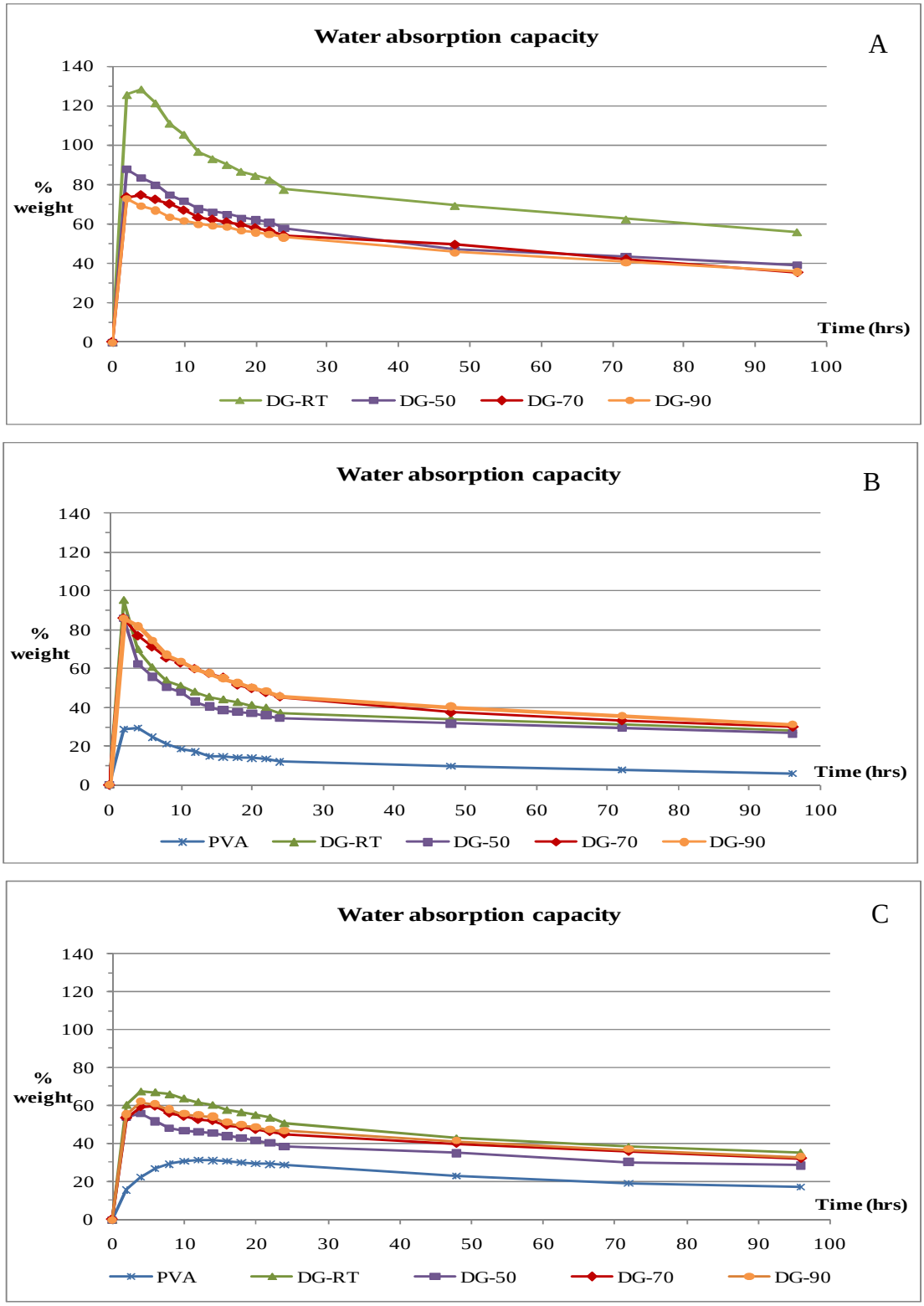



Figure 27. Water absorption capacity of hydrogels prepared by F24T24 (3 cycles), at various thickness (A) 1.00 ± 0.10 mm (B) 2.00 ± 0.20 mm (C) 3.50 ± 0.35 mm

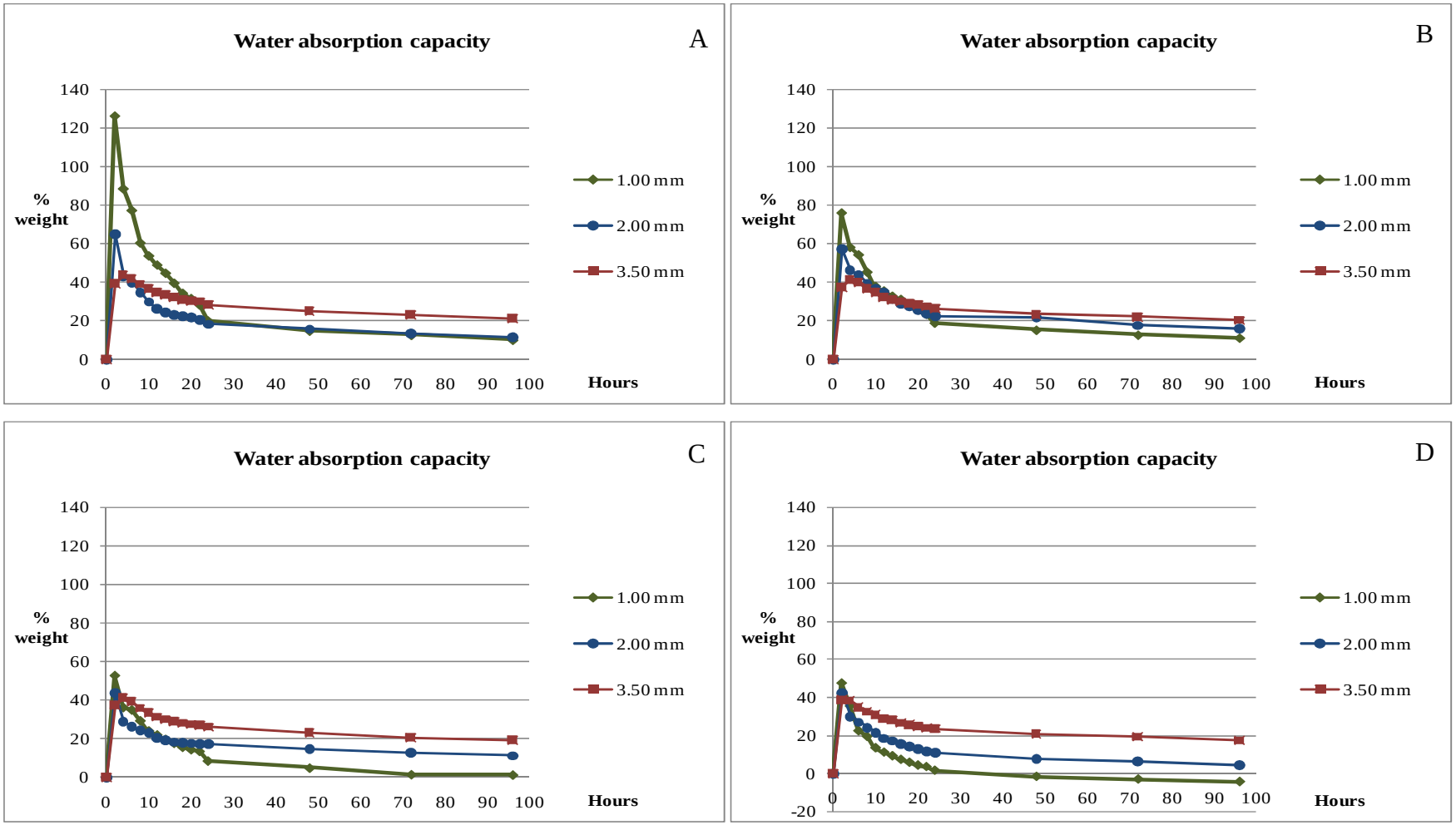



Figure 28. Water absorption capacity of the DG/PVA hydrogels prepared by F18T6 (3 cycles) with the thickness of 1.00, 2.00 and 3.50 mm: DG-RT (A), DG-50 (B), DG-70 (C) and DG-90 (D)

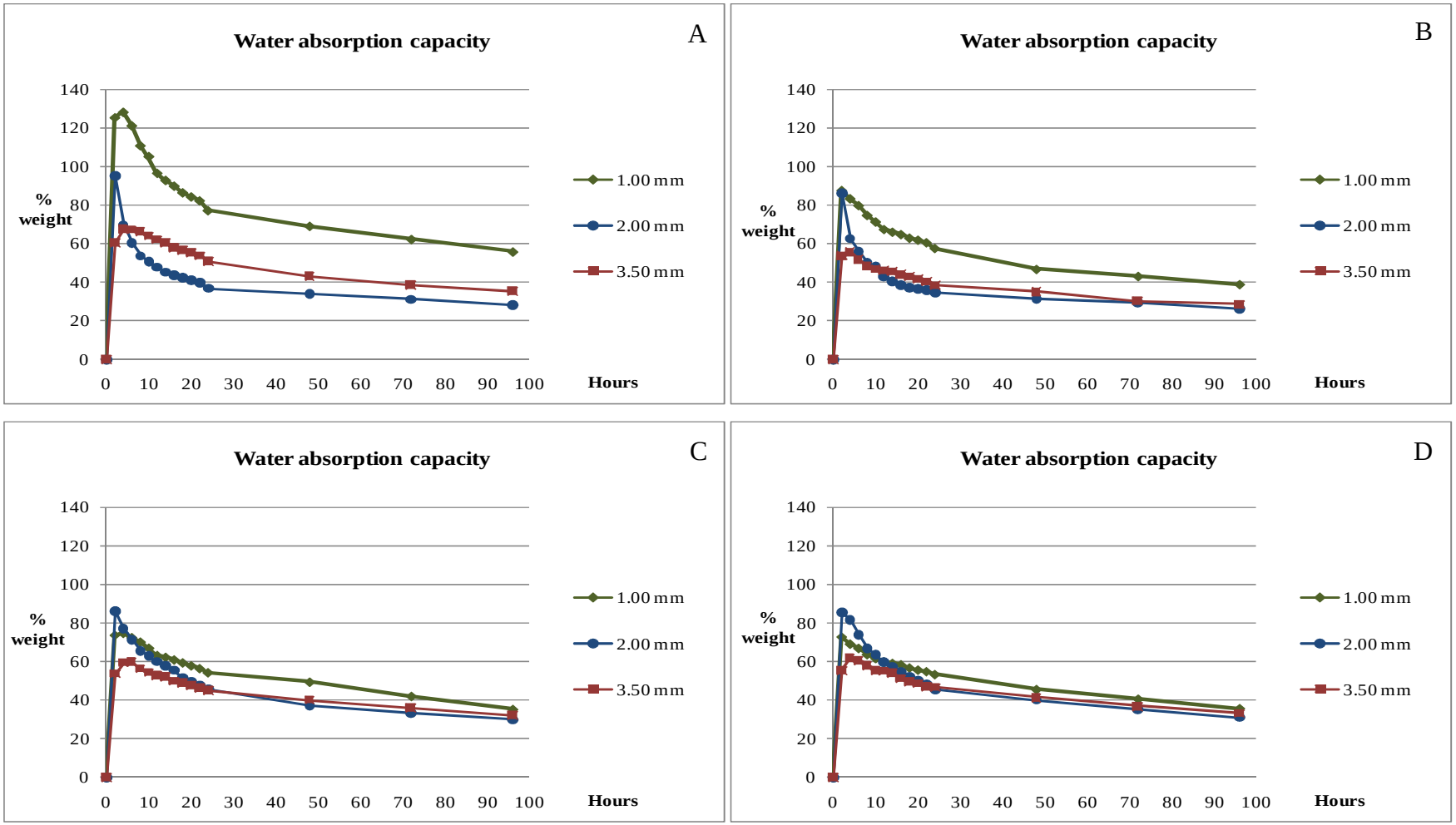



Figure 29. Water absorption of DG/PVA hydrogels prepared by F24T24 (3 cycles) with the thickness of 1.00, 2.00 and 3.50 mm: DG-RT (A), DG-50 (B), DG-70 (C) and DG-90 (D)

### 2.1.4 Gel fraction

The gel fraction was the weight ratio of dried hydrogel in swollen and unswollen conditions. It could be represented to an index of cross-linked degree (Kokabi et al., 2007)**.** A lot of unreacted polymers remained in low fraction network structure and leached into water after swelling. The effect of freeze-thaw duration was studied. Gel fractions of most PVA and DG/PVA hydrogels prepared by F24T24 condition were higher than F18T6 condition (Tables 5-6). Thus F24T24 condition gave hydrogel with better strength than F18T6 condition.

Table 5. Gel fractions of the hydrogels prepared by F18T6 (3 cycles) at various mixing temperature and thickness

| **Hydrogels (Mixing temperature)** | **Thickness (mm)** | | |
|---|---|---|---|
| | **1.00 ± 0.10** | **2.00 ± 0.20** | **3.50 ± 0.35** |
| DG-RT | 47.81 ± 2.20 | 47.81 ± 0.22 | 52.52 ± 1.78 |
| DG-50 | 56.98 ± 2.06 | 54.77 ± 0.59 | 56.99 ± 1.03 |
| DG-70 | 55.24 ± 2.81 | 53.15 ± 1.11 | 56.85 ± 1.22 |
| DG-90 | 57.95 ± 2.39 | 54.12 ± 0.62 | 60.21 ± 1.27 |
| PVA | 50.86 ± 1.12 | 53.96 ± 2.19 | 59.75 ± 3.66 |

Table 6. Gel fractions of the hydrogels prepared by F24T24 (3 cycles) at various mixing temperature and thickness

| **Hydrogels (Mixing temperature)** | **Thickness (mm)** | | |
|---|---|---|---|
| | **1.00 ± 0.10** | **2.00 ± 0.20** | **3.50 ± 0.35** |
| DG-RT | 58.16 ± 0.80 | 53.75 ± 0.87 | 51.17 ± 1.03 |
| DG-50 | 59.96 ± 1.49 | 57.30 ± 0.68 | 58.50 ± 0.72 |
| DG-70 | 61.52 ± 1.41 | 59.17 ± 1.63 | 60.28 ± 1.86 |
| DG-90 | 59.93 ± 2.33 | 48.77 ± 1.06 | 57.30 ± 1.99 |
| PVA | 68.94 ± 1.28 | 52.23 ± 1.17 | 55.98± 0.87 |

The second effect, mixing temperature, was investigated. Gel fraction of the DG/PVA hydrogel increased when mixing temperature was increased. From Figure 30, the DG/PVA hydrogels prepared by F18T6 and mixing at higher temperatures (50 °C, 70 °C and 90 °C) had higher gel fractions than mixing at room temperature. Figure 31 shows gel fraction of hydrogels prepared by F24T24. Gel fractions of DG/PVA hydrogels increased when the mixing temperatures were increased. Whereas when the mixing temperature over 70 °C, gel fraction of the DG/PVA hydrogel decreased. Thus high temperatures affected high crosslink in DG/PVA hydrogels. But mixing at very high temperature (90 °C) did not suitable for hydrogel incorporated with DG because high temperature might change stability of natural polysaccharide.

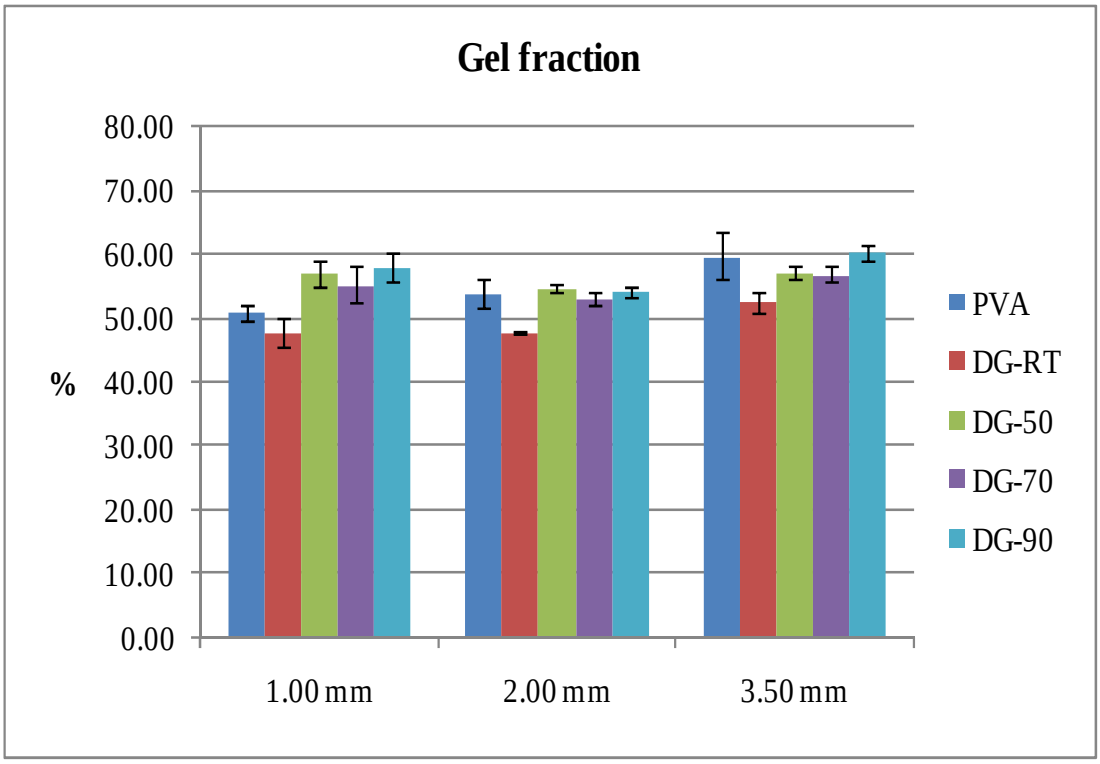



Figure 30. Gel fractions of the hydrogels preparing by F18T6 (3 cycles)

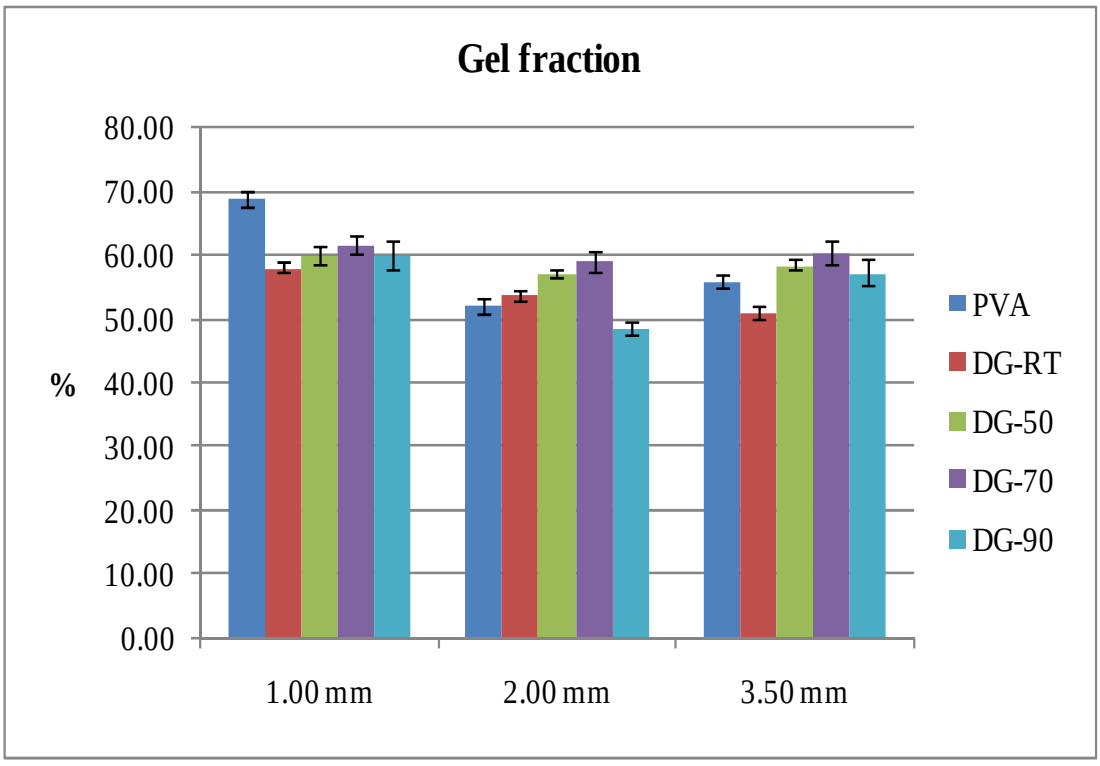



Figure 31. Gel fractions of the hydrogels preparing by F24T24 (3 cycles)

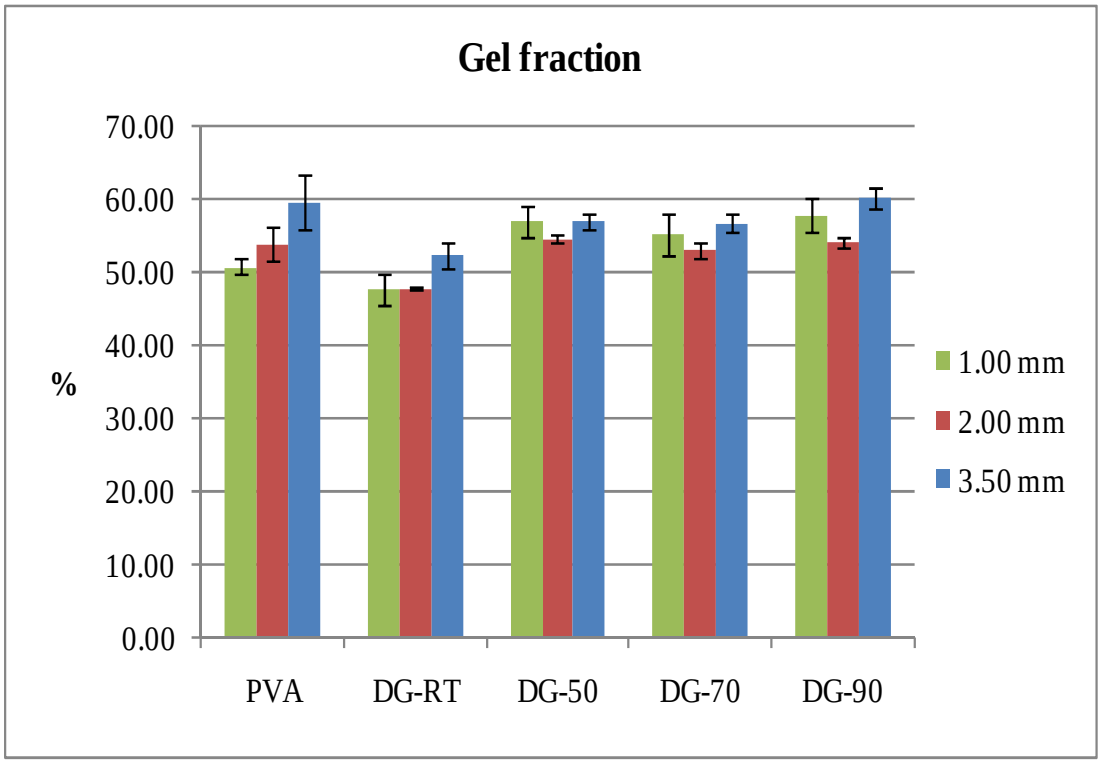



Figure 32. Gel fractions of the hydrogels prepared by F18T6 (3 cycles) with the thickness of 1.00, 2.00 and 3.50 mm

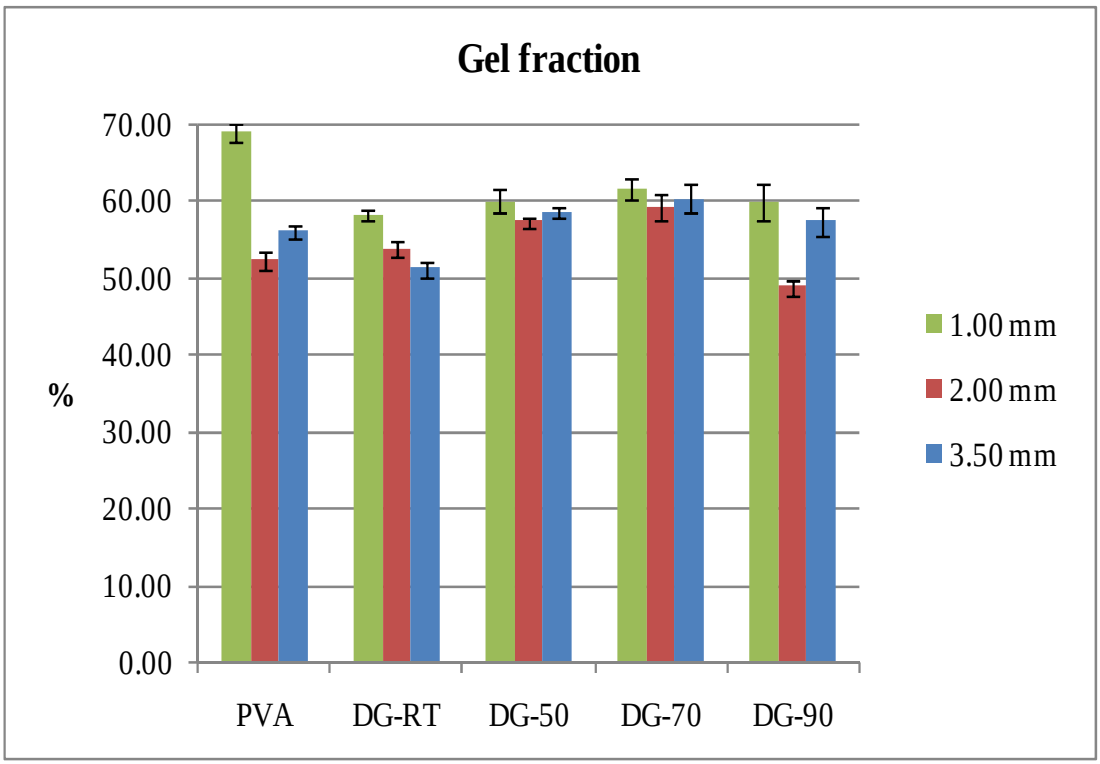



Figure 33. Gel fractions of the hydrogels prepared by F24T24 (3 cycles) with the thickness of 1.00, 2.00 and 3.50 mm

The effect of thickness was considered. Hydrogels with high thickness prepared by F18T6 showed high gel fraction (Figure 32). Hydrogels prepared by F24T24 showed gel fraction in contrast to F18T6. The most hydrogels with high thickness had lower gel fraction than thinner hydrogels (Figure 33).

From data obtained, it was found that DG/PVA (2 : 5) hydrogel prepared by F24T24 for 3 cycles with the thickness of 3.50 mm and mixed at room temperature was the best hydrogel. Because its property which has high water content supplying moisture to the wound environment for the healing process improvement. Furthermore, it could absorb much water and retain much more water than other hydrogels which represents potentially high absorption of wound exudates. After maximum absorption, it was more stable than thinner hydrogels which easy fracture. Then the DG/PVA hydrogel prepared by F24T24 with the thickness of 3.50 mm and mixed at room temperature was selected for further study.

### 2.2 Study of freeze-thaw process parameters

From the preliminary results, the DG/PVA hydrogel prepared by F24T24 with the thickness of 3.50 mm and mixed at room temperature was used in this study. Two process parameters were investigated.

#### 2.2.1 Effect of DG concentration

##### 2.2.1.1 Hydrogel appearances

The DG/PVA hydrogels with all concentrations of DG such as 2 %w/w (DG/PVA 2 : 5), 3 %w/w (DG/PVA 3 : 5) and 3.5 %w/w (DG/PVA 3.5 : 5) got transparent tan color from DG powder, while higher concentrations of DG showed darker tan color (Figure 34)

A B C

Figure 34. Photographs of the DG/PVA hydrogels prepared by F24T24 (5 cycles): DG/PVA (2 : 5) (A), DG/PVA (3 : 5) (B), DG/PVA (3.5 : 5) (C)

#### 2.2.1.2 Water contents

Water contents of most hydrogels were approximate 90 %. DG/PVA hydrogels with various amounts of DG had similar water contents. Water contents of the PVA hydrogels were more than 90 % and slightly higher than the DG/PVA hydrogels (Table 7).

Table 7. Water contents of the DG/PVA hydrogels with varying concentrations of DG (2 %, 3 % and 3.5 %w/w) prepared by F24T24 for 3-5 cycles

| **Hydrogels** | **Freeze-thaw cycle (F24T24)** | | |
|---|---|---|---|
| | **3 cycles** | **4 cycles** | **5 cycles** |
| DG/PVA (2 : 5) | 90.52 ± 0.24 | 91.47 ± 0.23 | 91.66 ± 0.02 |
| DG/PVA (3 : 5) | 90.26 ± 0.19 | 90.47 ± 0.03 | 89.95 ± 0.09 |
| DG/PVA (3.5 : 5) | 90.10 ± 0.26 | 90.08 ± 0.36 | 89.98 ± 0.42 |
| PVA | 93.32 ± 0.12 | 93.63 ± 0.10 | 93.73 ± 0.13 |

#### 2.2.1.3 Water absorption capacity

Water absorption of the DG/PVA hydrogels prepared by F24T24, 3 cycles with DG concentrations of 2, 3 and 3.5 %w/w were higher than the PVA hydrogels (Figure 35A). Maximum swelling of the DG/PVA (3.5 : 5) hydrogel appeared at 13 hrs after immersion in deionized water. Water absorption capacity of the DG/PVA (3.5 : 5) hydrogel was higher than the DG/PVA (3 : 5) and DG/PVA (2 : 5). After constant absorption, all hydrogels decreased in their weights. The erosion rate of a membrane weight was higher when the DG content was increased. It might be the release of unreacted polymer into water.

Water absorption of the hydrogels prepared by F24T24, 4 and 5 cycles were similar to the hydrogels prepared by 3 cycles (Figures 35B and 35C). Maximum swelling of the DG/PVA (3.5 : 5) hydrogel was the highest, it was higher than DG/PVA (3 : 5), DG/PVA (2 : 5) and PVA hydrogels, respectively.

In conclusion, DG concentration incorporated in hydrogels affected the maximum water absorption capacity of the hydrogels. Higher concentration of DG had better in hydrogel properties than lower concentration.

#### 2.2.1.4 Gel fraction

The DG/PVA hydrogels incorporated with different amount of DG showed various gel fractions. Gel fractions of DG/PVA hydrogels with high DG concentrations were higher (Figure 36). Thus addition of DG into PVA hydrogels increased their crosslink

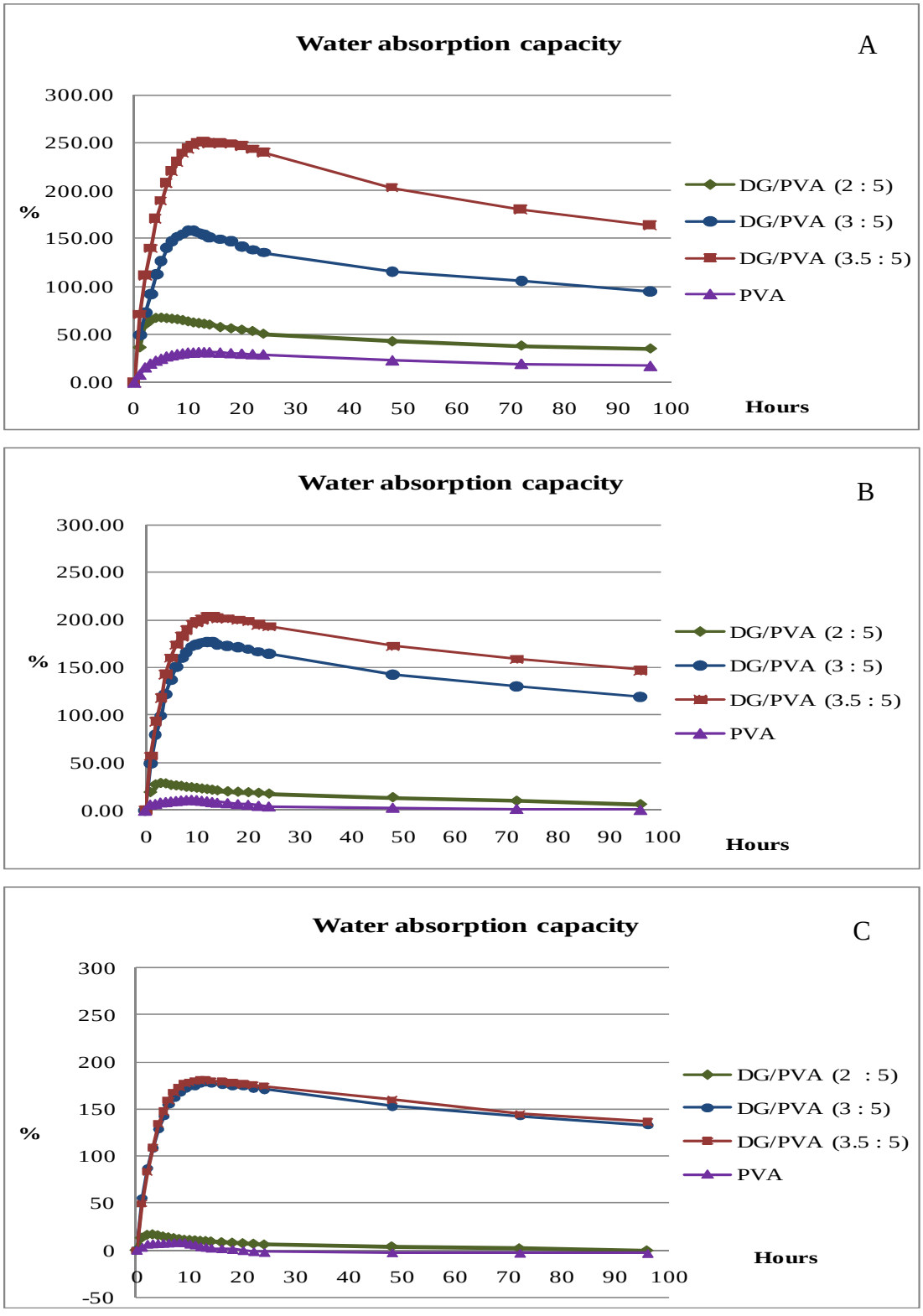



Figure 35. Water absorption capacity of hydrogels prepared by F24T24 for 3 cycles (A), 4 cycles (B) and 5 cycles (C)

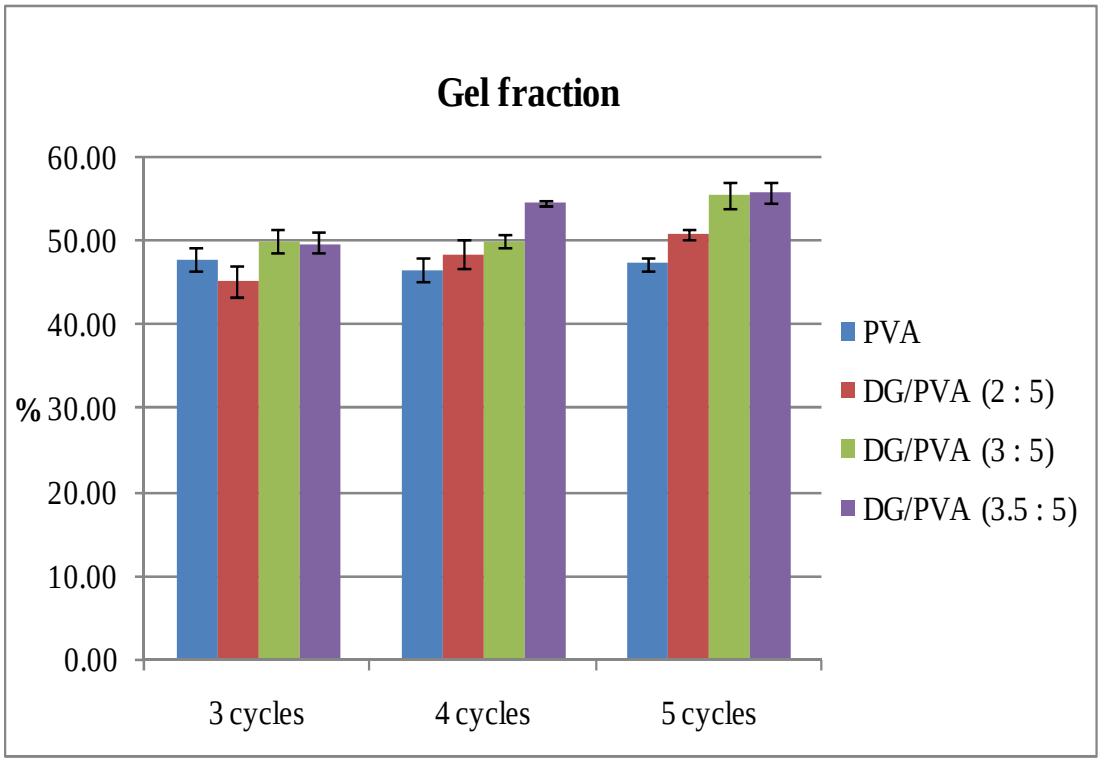



Figure 36. Gel fractions of the DG/PVA and PVA hydrogels with the thickness of 3.50 mm prepared by F24T24 for 3-5 cycles

### 2.2.1.5 Mechanical properties

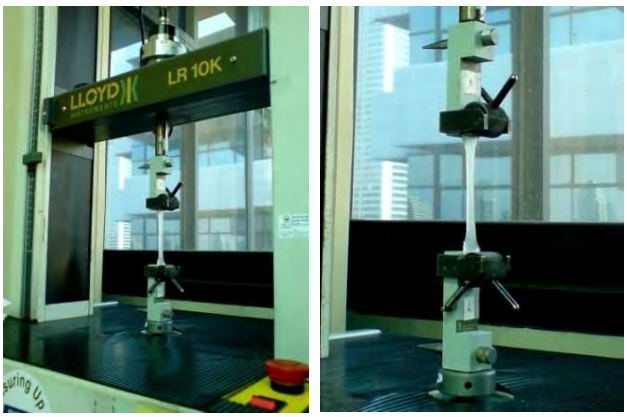



Figure 37. Mechanical properties of the PVA and DG/PVA hydrogels tested by LLOYD instrument

Mechanical properties of hydrogels were performed by LLOYD instrument (Figure 37). Concentration of DG incorporated in hydrogel network

affected strength of hydrogels. Statistical analysis showed that DG/PVA (3.5 : 5) hydrogels prepared by 3 and 4 cycles improved hydrogel strength compared to PVA hydrogel ($p<0.05$). Whereas hydrogel strength of the DG/PVA and PVA hydrogels prepared with 5 cycles were not different (Figure 38A).

Percentage elongation showed ductile capacity of the hydrogels (Figure 38B). In the group of hydrogels prepared by 3 cycles, the DG/PVA (3 : 5) and DG/PVA (3.5 : 5) hydrogels could expand more than PVA hydrogel ($p<0.05$), but DG/PVA (2 : 5) was not different. However, concentration of DG did not affect elongation property in hydrogels prepared by 4 and 5 cycles.

Young's modulus of the hydrogels is shown in Figure 38C. The lower Young's modulus signified spongy property of the material. The DG/PVA (2 : 5) and DG/PVA (3.5 : 5) hydrogels prepared by 3 cycles were stiffer than PVA hydrogel, whereas DG/PVA (2 : 5) and DG/PVA (3.5 : 5) hydrogels were not different. However, concentration of DG did not affect Young's modulus in hydrogels prepared by 4 and 5 cycles which were similar to elongation property.

In summary, the hydrogels prepared by 3 cycles, DG/PVA (2 : 5) hydrogel was stiffer than the PVA hydrogel. DG/PVA (3 : 5) hydrogel could expand more than the hydrogel without DG. While DG/PVA (3.5 : 5) hydrogel improved all mechanical properties such as strength, elongation and stiffness compared to the PVA hydrogel. In the group of hydrogel prepared by 4 cycles, only DG/PVA (3.5 : 5) hydrogel has higher strength than the PVA hydrogel. DG concentrations did not affect mechanical properties of the hydrogels prepared using 5 cycles

. **2.2.1.6 Scanning Electron Microscopy (SEM)**

The scanning electron photomicrographs (x 100) of the hydrogel surfaces are shown in Figure 39. Surface appearances of the DG/PVA hydrogels in all DG concentrations were different to the PVA hydrogels. The DG/PVA hydrogel showed folding porous structure while PVA hydrogel showed small pore and fibrous structure.

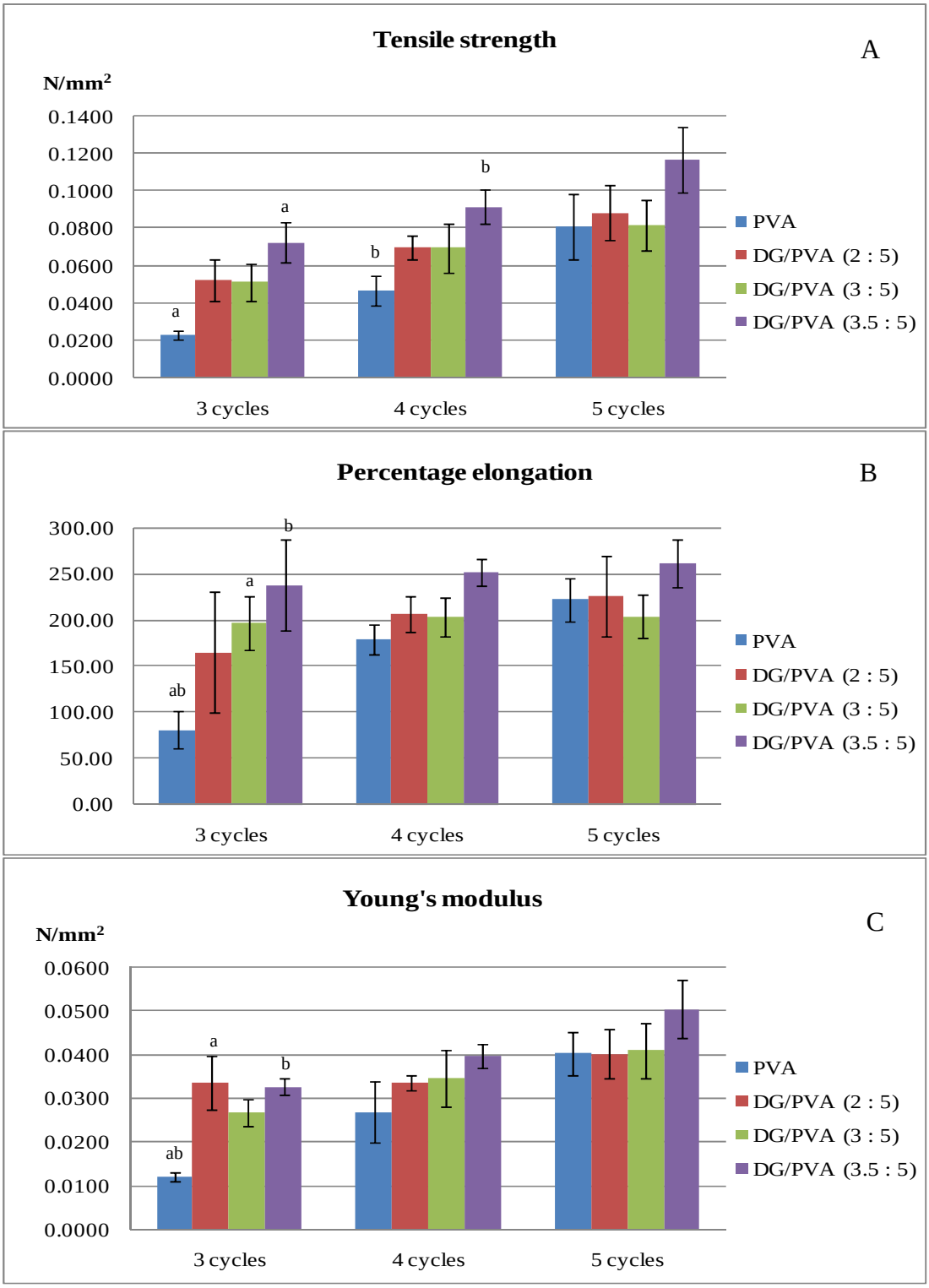



Figure 38. Mechanical properties of the DG/PVA and PVA hydrogels prepared by F24T24 for 3-5 cycles: Tensile strength (A), Percent elongation (B) and Young's modulus (C)

a and b are significant differences between groups ($p < 0.05$)

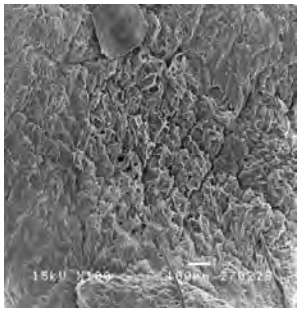
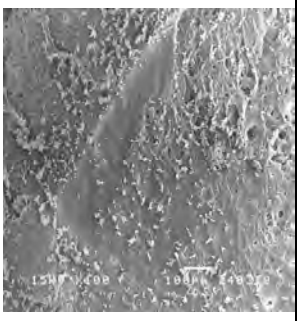
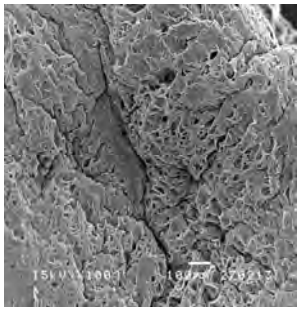
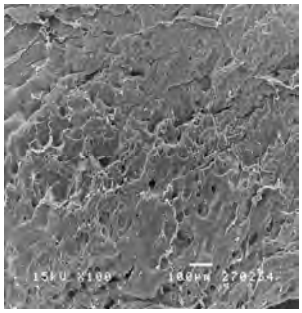
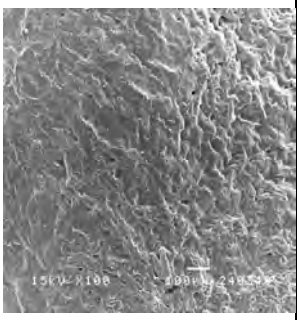
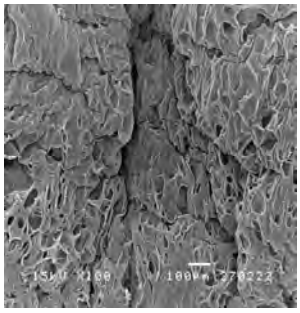
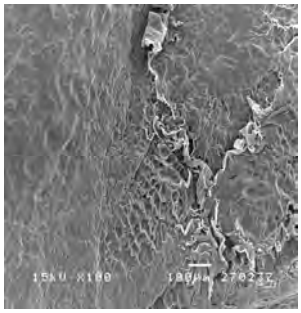
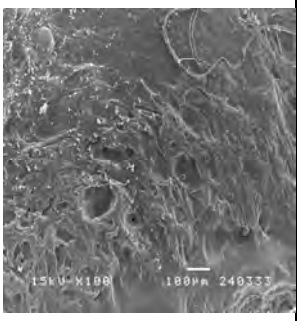
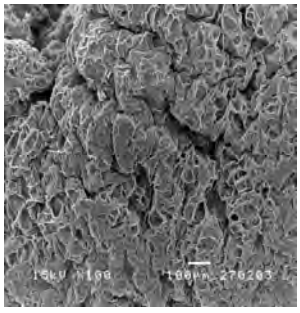
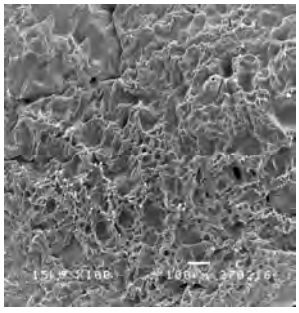
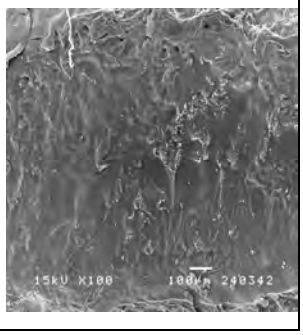
| Hydrogel | 3 cycles (x 100) | 4 cycles (x 100) | 5 cycles (x 100) |
|---|---|---|---|
| **PVA** |  |  |  |
| **DG/PVA (2 : 5)** |  |  |  |
| **DG/PVA (3 : 5)** |  |  |  |
| **DG/PVA (3.5 : 5)** |  |  |  |

Figure 39. Scanning electron photomicrographs of the DG/PVA and PVA hydrogels prepared by F24T24 for 3, 4 and 5 cycles

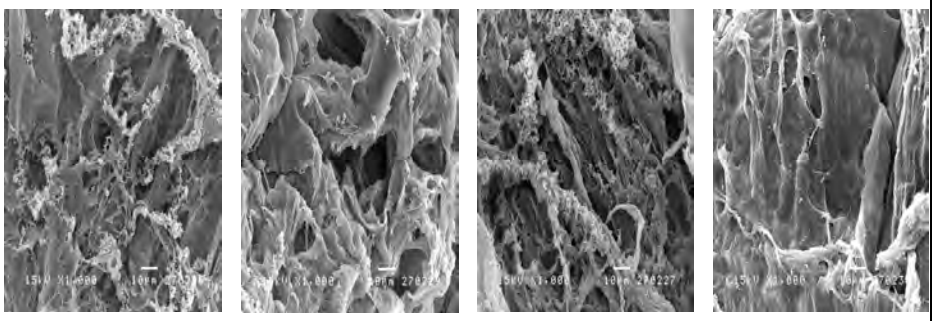
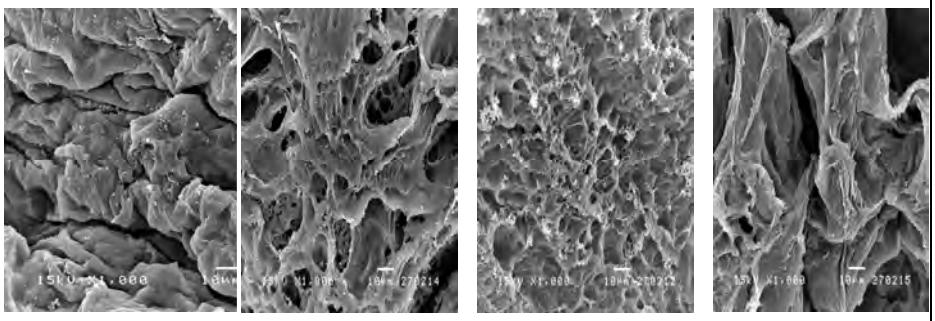
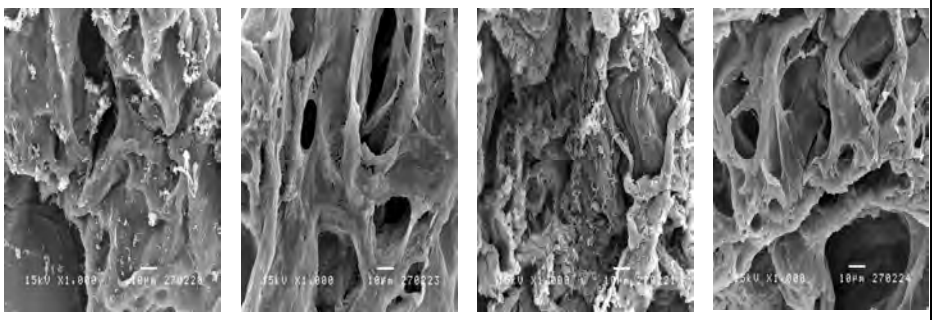
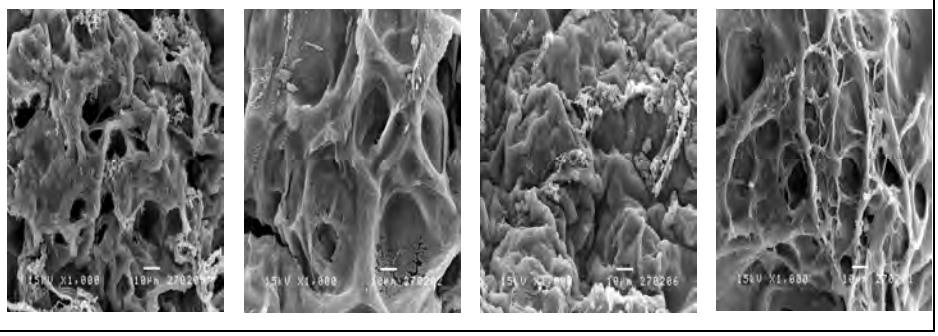
| Hydrogel | Surface (x 1000) | Surface swollen (x 1000) | Cross section (x 1000) | Cross section swollen (x 1000) |
|---|---|---|---|---|
| PVA |  | | | |
| DG/PVA (2 : 5) |  | | | |
| DG/PVA (3 : 5) |  | | | |
| DG/PVA (3.5 : 5) |  | | | |

Figure 40. Scanning electron photomicrographs of the hydrogels prepared by F24T24 for 3 cycles before and after swelling

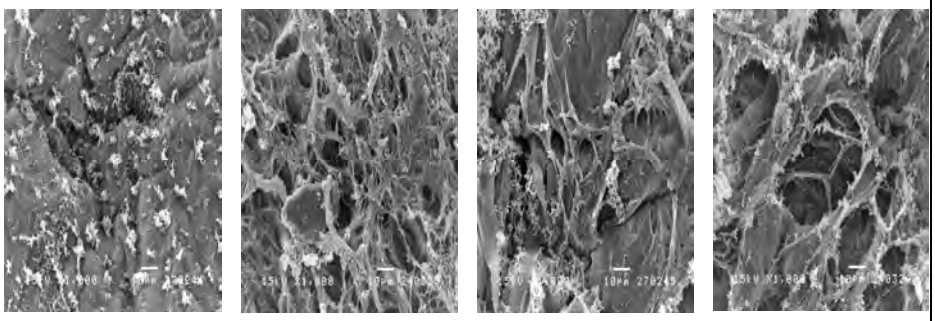
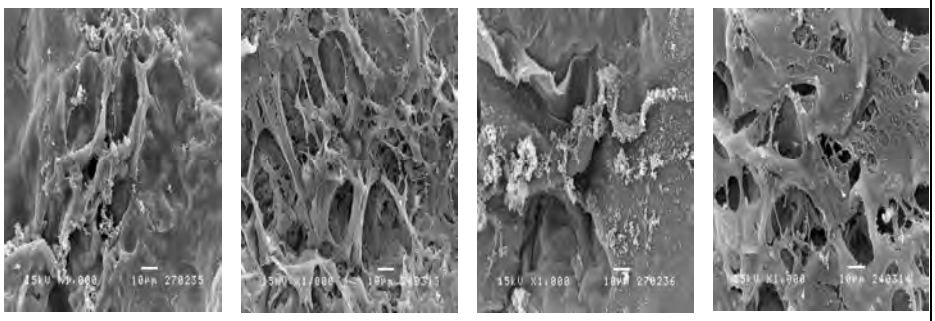
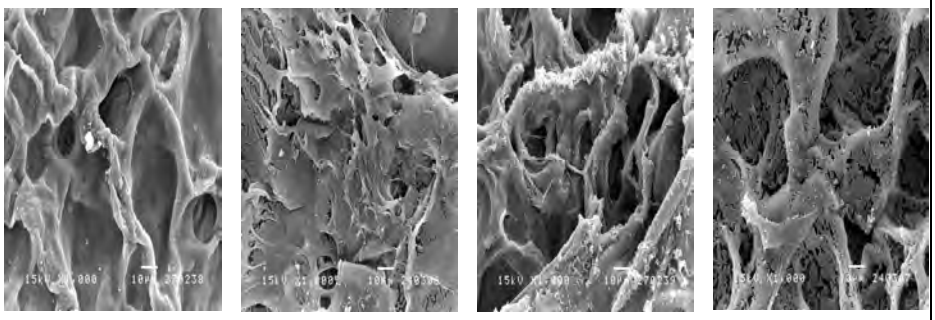
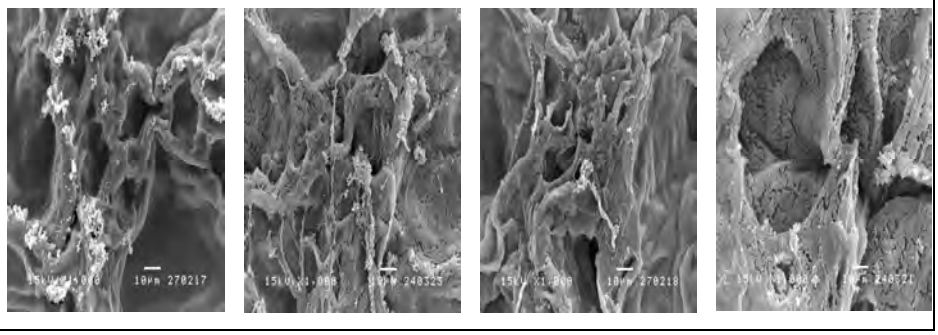
| Hydrogel | Surface (x 1000) | Surface swollen (x 1000) | Cross section (x 1000) | Cross section swollen (x 1000) |
|---|---|---|---|---|
| PVA |  | | | |
| DG/PVA (2 : 5) |  | | | |
| DG/PVA (3 : 5) |  | | | |
| DG/PVA (3.5 : 5) |  | | | |

Figure 41. Scanning electron photomicrographs of the hydrogels prepared by F24T24 for 4 cycles before and after swelling

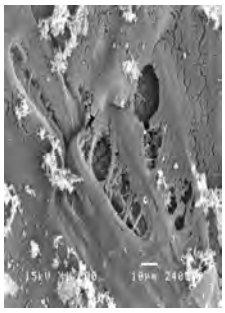
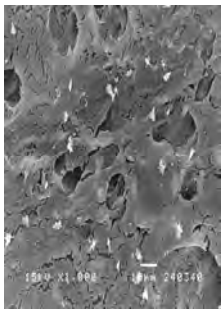
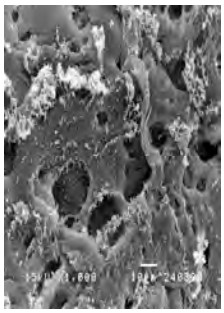
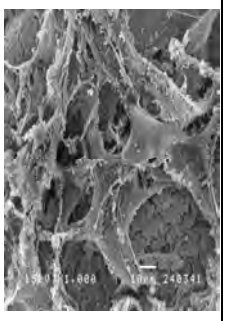
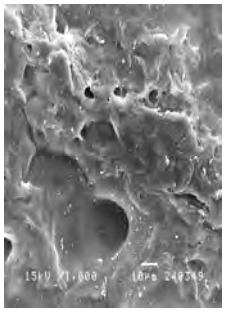
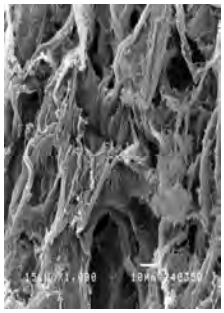
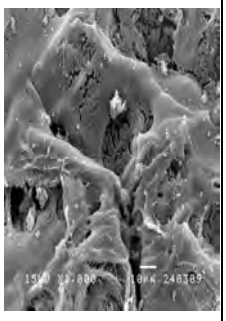
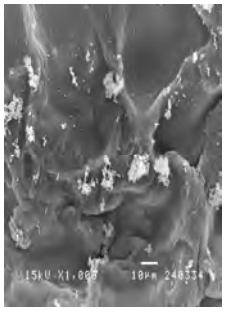
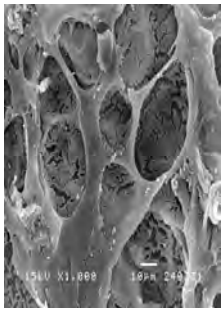
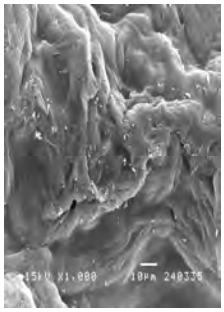
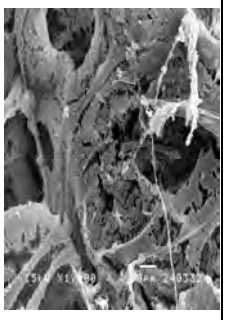
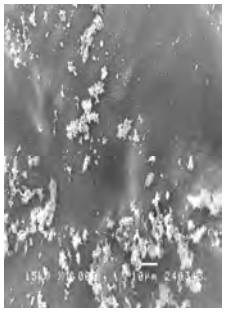
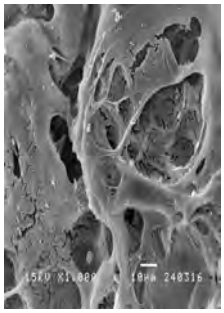
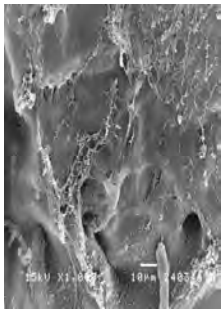
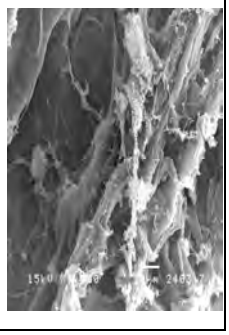
| Hydrogel | Surface (x 1000) | Surface swollen (x 1000) | Cross section (x 1000) | Cross section swollen (x 1000) |
|---|---|---|---|---|
| PVA |  |  |  |  |
| DG/PVA (2 : 5) |  |  |  |  |
| DG/PVA (3 : 5) |  |  |  |  |
| DG/PVA (3.5 : 5) |  |  |  |  |

Figure 42. Scanning electron photomicrographs of the hydrogels prepared by F24T24 for 5 cycles before and after swelling

From Figures 40-42, there were differences between surface and cross section of the DG/PVA hydrogels and the PVA hydrogel. The surface and cross section of the PVA hydrogel contain networks of large pores, while the DG/PVA hydrogel structures seemed to have PVA network covered with DG. The larger pore in structures of the DG/PVA and PVA hydrogels occurred after swelling with water for 13 hrs. However, the hydrogel network incorporated with high DG concentration expanded more than the network with lower DG concentration. It proved that DG incorporated in the hydrogel resulted in change of the neat structures of the PVA hydrogel.

In conclusion, DG concentration affected all physical properties of DG/PVA hydrogels. The hydrogel with high concentration of DG was transparent and dark tan color. It had high water content, gel fraction and also absorbed high amount of water. All mechanical properties were changed when DG incorporated in PVA hydrogel.

### 2.2.2 Effect of freeze-thaw cycle for hydrogel preparation

#### 2.2.2.1 Hydrogel appearances

The PVA hydrogel prepared by F24T24, 5 cycles was resulted in the hydrogels with more opaque than 4 and 3 cycles, respectively. Sizes of the PVA hydrogels prepared by 4 and 5 cycles were smaller than 3 cycles due to the contraction of the polymer. The DG/PVA (2 : 5) hydrogels prepared by 4 and 5 cycles were smaller same to PVA hydrogels, but DG/PVA (3 : 5) hydrogel DG/PVA (3.5 : 5) hydrogel did not change their size and shape (Figure 34). All types of the hydrogels prepared by F24T24, 3cycles was retained their initial forms.

#### 2.2.2.2 Water contents

Water contents of the DG/PVA (2 : 5) hydrogels prepared by freeze-thaw for 3-5 cycles were higher than 90 %. The DG/PVA (3 : 5) and DG/PVA (3.5 : 5) hydrogels contained water more than 90% when prepared by 3 and 4 cycles and were slightly decreased when freeze-thaw up to 5 cycles (Figure 43). Water contents of all PVA hydrogels were more than 90 % and higher than the DG/PVA hydrogels.

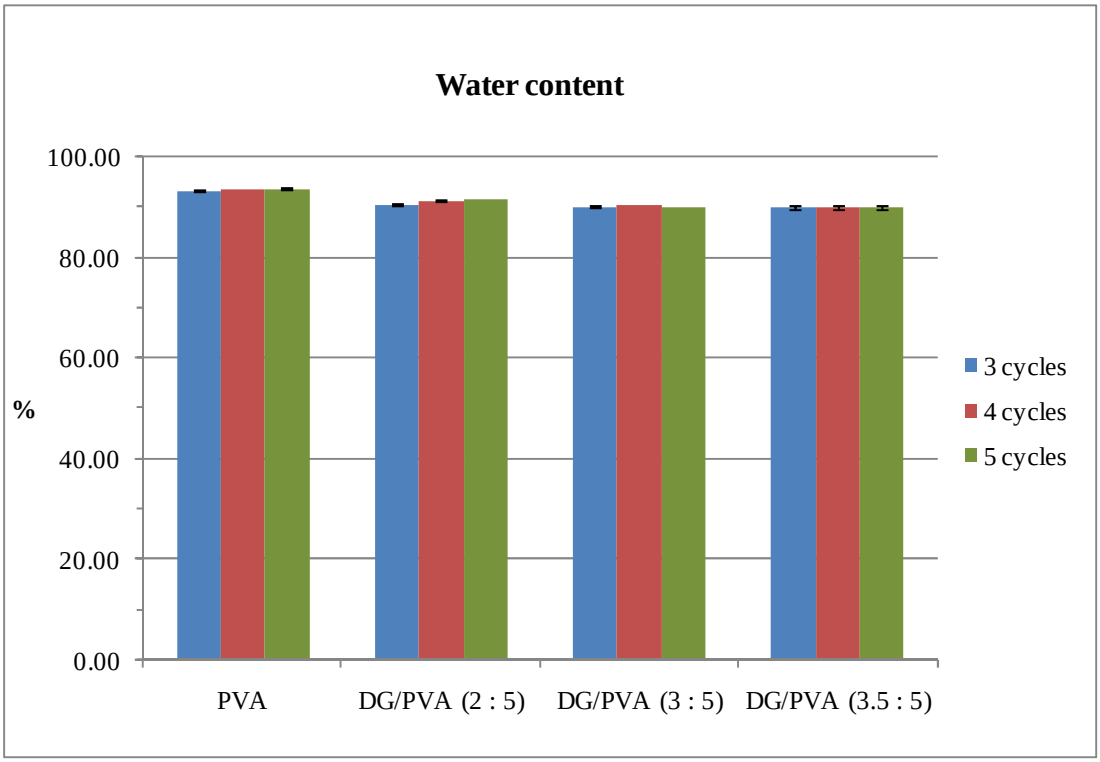



Figure 43. Water contents of the hydrogels prepared by F24T24 for 3-5 cycles

#### 2.2.2.3 Water absorption capacity

Maximum absorptions of the DG/PVA (3.5 : 5) , DG/PVA (2 : 5) and PVA hydrogels were similar when the hydrogels were prepared by F24T24, 3-5 cycles but minimum swelling of the DG/PVA (3 : 5) hydrogel was different (Figure 44). Water absorptions of the DG/PVA (3.5 : 5) , DG/PVA (2 : 5) and PVA hydrogels prepared by F24T24, 3 cycles were higher than 4 cycles and 5 cycles, respectively. While maximum water absorption of the DG/PVA (3 : 5) hydrogel prepared by 5 cycles was higher than 4 cycles and 3 cycles, respectively.

Increase the number of freeze-thaw cycles had affected on decrease the maximum water absorption except for the DG/PVA (3 : 5) hydrogels. The DG/PVA (3.5 : 5) hydrogel prepared by F24T24, 3 cycles was the highest of water absorption and was the most retaining weight at 4 days.

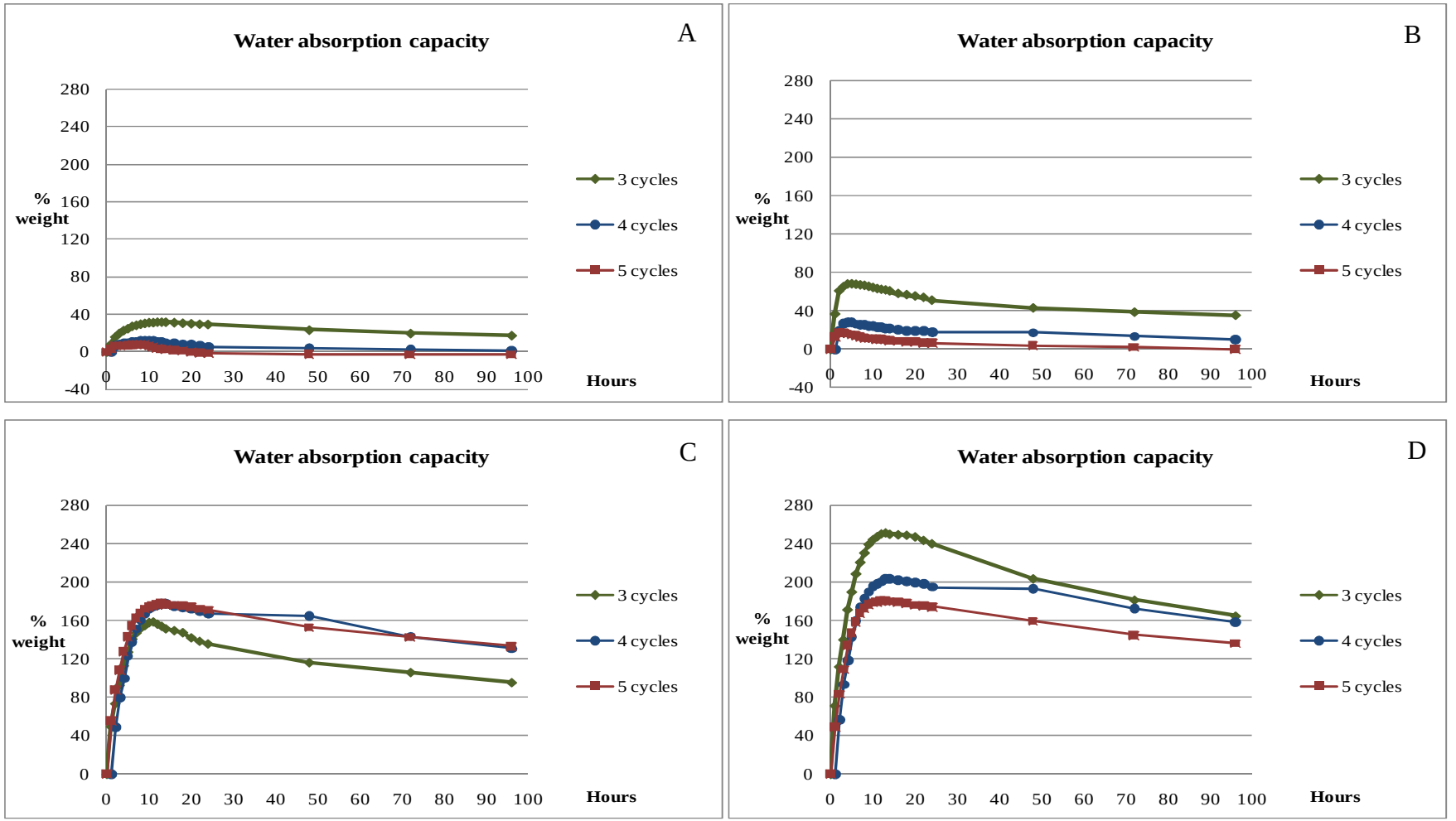



Figure 44. Water absorption of hydrogels prepared by F24T24: PVA (A), DG/PVA (2 ; 5) (B), DG/PVA (3 : 5) (C) and DG/PVA (3.5 : 5) (D)

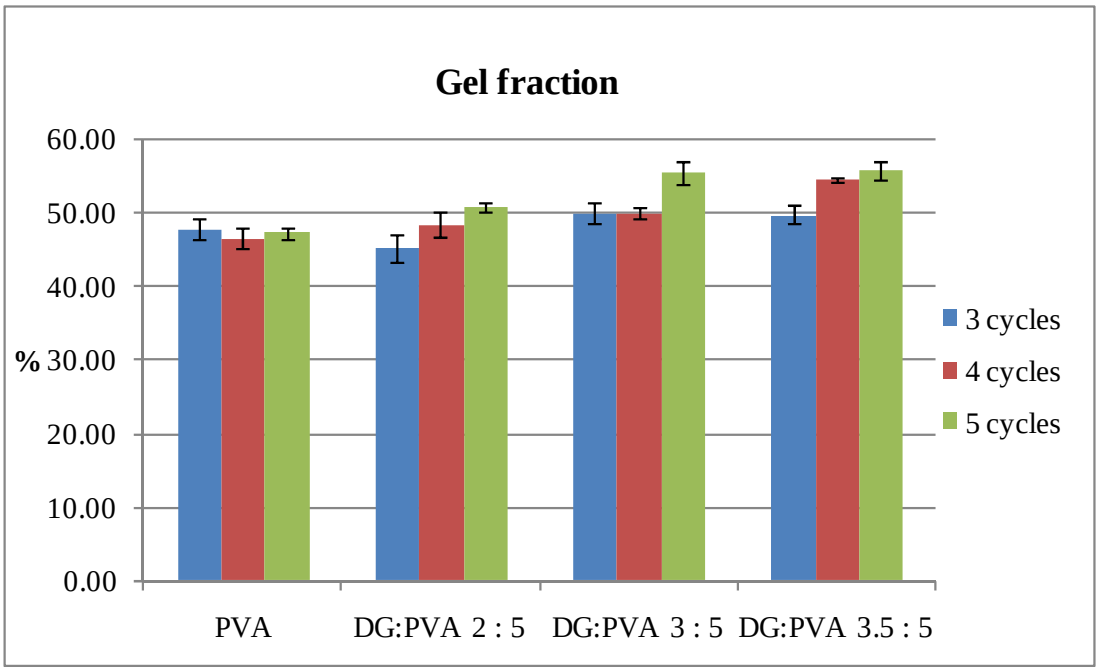



Figure 45. Gel fractions of the hydrogels prepared by F24T24 for 3-5 cycles

#### 2.2.2.4 Gel fraction

After soaking in deionized water for 4 days, gel fractions of the PVA hydrogels prepared by F24T24 for 3-5 cycles were similar (Figure 45). Gel fractions of the DG/PVA hydrogels were higher when number of cycles was increased. Thus the DG/PVA hydrogels prepared by higher freeze-thaw cycles were stronger and denser than lower freeze-thaw cycles.

#### 2.2.2.5 Mechanical properties

Tensile strengths (stress at maximum load) of the PVA hydrogels with and without DG were higher when the number of freeze-thaw cycles increased (Figure 46A). However, the PVA and DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 5 cycles were significantly more strength than 3 cycles ($p<0.05$).

When the number of cycle increased, the PVA hydogel prepared by 5 cycles was significantly ($p<0.05$) elongated more than the samples from 3 cycles before fracture. However, the number of freeze-thaw cycles for DG/PVA hydrogels did not affect the elongation values (Figure 46B).

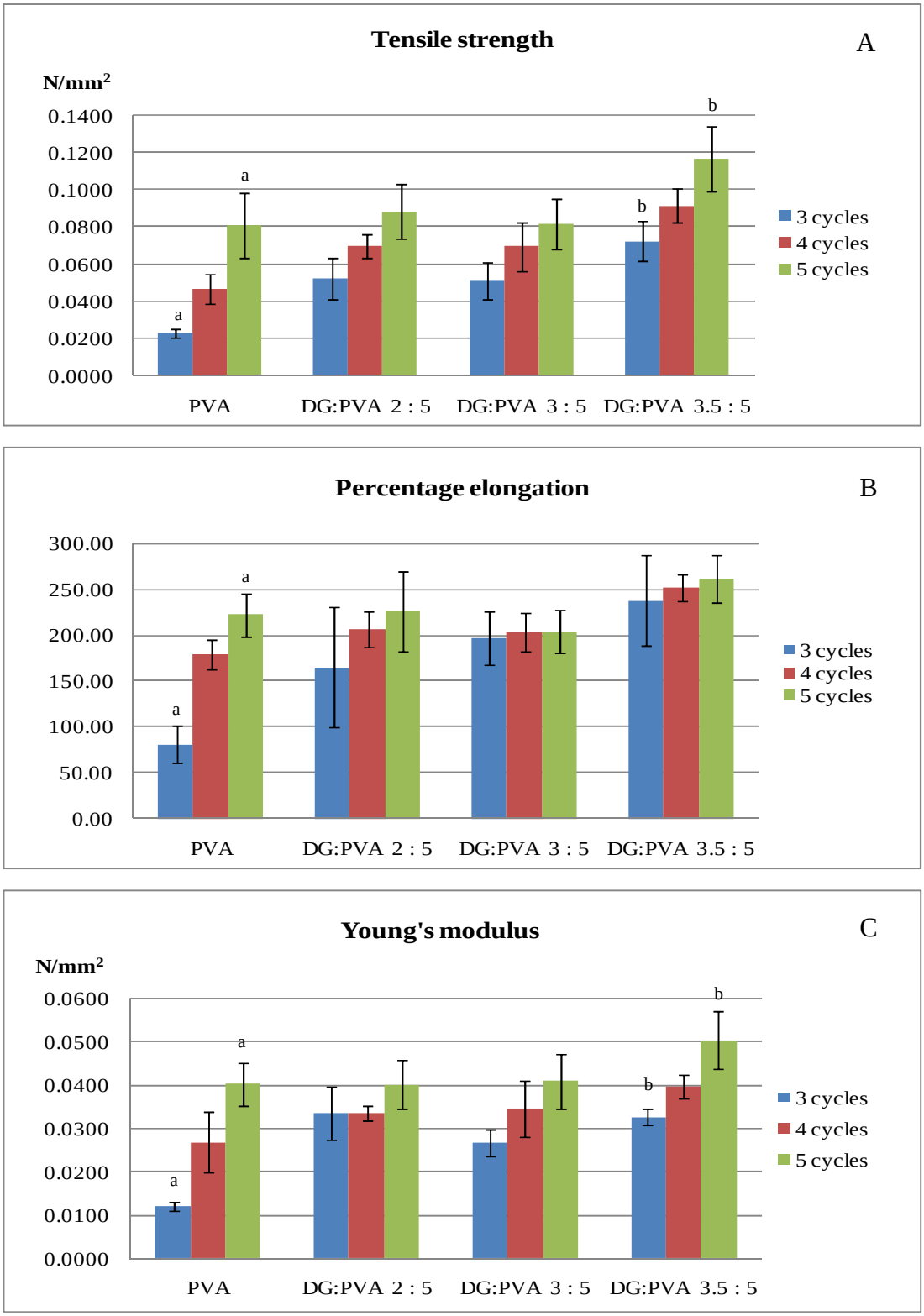



Figure 46. Mechanical propertie of the DG/PVA and PVA hydrogels prepared by F24T24 for 3-5 cycles: Tensile strength (A), Percent elomgation (B) and Young's modulus (C)

a and b are significant differences between groups ($p < 0.05$)

When the number of cycle increased, the hardness of the PVA and DG/PVA (3.5 : 5) hydrogels prepared by 5 cycles was significantly ($p<0.05$) harder than 3 cycles, similar to the property of tensile strength (Figure 46C).

According to the data obtained, this study found that the PVA hydrogel prepared by F24T24 for 5 cycles was more ductile, stiffer and stronger which was more suitable for handling than 3 cycles. In group of DG/PVA hydrogels, only DG/PVA (3.5 : 5) hydrogel prepared by 5 cycles was stronger and harder than 3 cycles.

#### 2.2.2.6 Scanning Electron Microscopy (SEM)

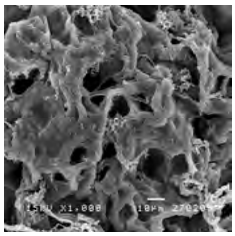
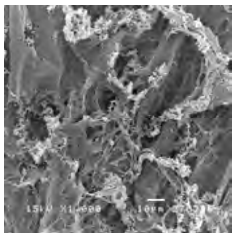
The DG/PVA and PVA hydrogels prepared by 3 freeze-thaw cycles had many networks, while the structures of 4 and 5 cycles were smoother (Figure 39). The larger pore structures of the DG/PVA and PVA hydrogels occurred after swelling in water for 13 hrs. All types of hydrogel prepared by 4 and 5 freeze-thaw cycles after swelling were fragile due to the denser structure from higher cycles of preparation (Figures 40-42). From the data obtained, the denser and smoother surface network occurred when the numbers of freeze-thaw cycles increased which affected the structures with more fracture after swelling in water.

From the study of freeze-thaw cycle, the DG/PVA hydrogels prepared by 3 cycles of freeze-thaw process had better properties than 4 and 5 cycles. DG/PVA hydrogels prepared by 3 cycles showed higher water absorption capacity than other cycles due to its lower gel fractions.

### 3. Study of hydrogel properties for biomedical application

DG/PVA (3.5 : 5) and PVA hydrogels with the thickness of 3.50 mm prepared by F24T24 (3 cycles) were discussed on their water/swelling behaviors, mechanical/structural behaviors and surface properties**.**

Table 8. Hydrogel properties of DG/PVA and PVA hydrogels

| **Hydrogel properties** | | **DG/PVA (3.5 : 5) hydrogel** | **PVA hydrogel** |
|---|---|---|---|
| **Water/ Swelling behaviors** | **Water content** | 90.10 ± 0.26 | 93.32 ± 0.12 |
| | **Water absorption** | 250.96 ± 33.92 | 31.24 ± 3.80 |
| | **Gel fraction** | 49.84 ± 1.19 | 47.80 ± 1.47 |
| | **WVTR** | 30.05 ± 5.71 | 31.84 ± 3.43 |
| | **WER** | 25.62 ± 3.15 | 16.62 ± 0.84 |
| **Surface properties** | **Adhesion** | 21.52 ± 3.05 | 18.15 ± 4.18 |
| | **SEM (surface x 1,000)** |  |  |
| **Mechanical /Structural behaviors** | **Tensile strength** | 0.0727 ± 0.0106 | 0.0230 ± 0.0026 |
| | **Elongation (%)** | 237.77 ± 49.72 | 81.39 ± 20.03 |
| | **Young's modulus** | 0.0327 ± 0.0019 | 0.0121 ± 0.0010 |
| | **Tensile strength (swell)** | 0.01705 ± 0.0046 | 0.0272 ± 0.0024 |
| | **Elongation (%) (swell)** | 63.65 ± 22.09 | 83.22 ± 6.76 |
| | **Young's modulus (swell)** | 0.0189 ± 0.0035 | 0.0141 ± 0.0018 |

From table 8, water contents of two hydrogels were higher than 90 %. Gel fractions of both hydrogels were similar, slightly lower in PVA hydrogel. Water absorption capacity of DG/PVA hydrogel was approximate 8 times higher than PVA hydrogel. When DG was incorporated in PVA hydrogel, WVTR decreased while WER increased but there were no differences between DG/PVA and PVA hydrogels. Thus DG 3.5 %w/w incorporated in PVA hydrogel improved the swelling property while also exhibited good water content compared to pure PVA hydrogel.

Figure 47. Measurement of the hydrogel adhesiveness by Texture Analyzer

Adhesion properties of the PVA and DG/PVA hydrogels to aluminum plate were measured by Texture Analyzer (Figure 47). Adhesiveness of the DG/PVA hydrogel seemed to tighter than the PVA hydrogel, whereas they were not statistical different ($p>0.05$). According to the data obtained, it was found that DG contained in hydrogel network did not change adhesive property of the neat PVA hydrogel. From scanning electron micrographs of DG/PVA and PVA hydrogels (Figures 39-40), there were differences between both surface structures. DG/PVA hydrogel surface structure might have network covered with DG but the difference of surface structure did not affect adhesion property of hydrogels.

All mechanical properties (strength, elongation, and stiffness) of DG/PVA hydrogel were better than PVA hydrogel (Table 8). DG incorporated in PVA hydrogel affected higher strength, higher elongation and stiffer than neat PVA hydrogel. After swelling in water for 13 hrs, all properties of PVA hydrogel were not different from unswelling hydrogel, whereas strength, ductility and stiffness of swelling DG/PVA

hydrogel were lower than unswelling hydrogel ($p<0.05$). This can be explained that water passed into hydrogel and changed their primary structure then DG was leached out from the structure.

DSC curves of DG/PVA and PVA hydrogels are shown in Figure 48. Three endothermic peaks of PVA hydrogel appeared at about 95, 212 and 280 °C. Peak at about 95 °C was predicted as residual water in the hydrogel. Melting point (212 °C) and degraded temperature (280 °C) were slightly lower than PVA powder. It might be expected that the decrease in temperature occurred due to the presence of paraben concentrate. In agreement with Yang et al. (2008) that the PVA molecules arrangement were changed when the PVA hydrogel incorporated with chitosan and glycerol. Furthermore, endothermic peaks of DG/PVA hydrogel did not show melting endotherm of PVA due to decrease of PVA content. Thus DG incorporated in PVA hydrogel modified the thermal characteristics of neat PVA hydrogel.

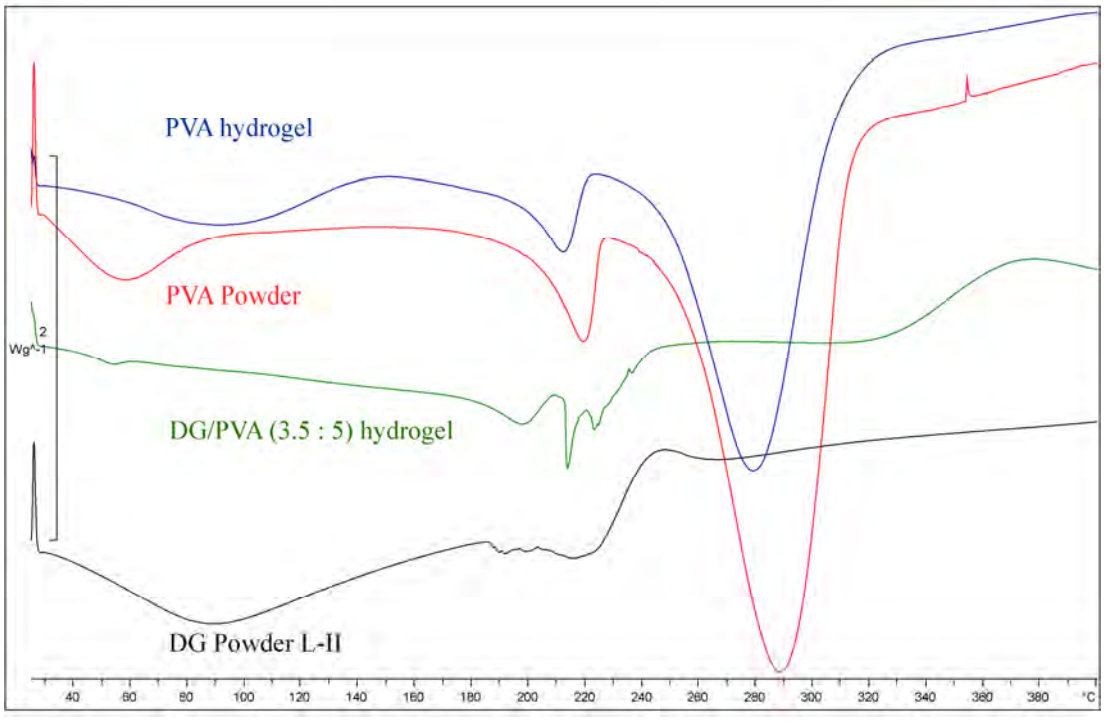



Figure 48. Thermal analysis of hydrogels and PVA and DG powders

In Figure 49, FTIR spectra of PVA hydrogel and PVA powder are shown. It clearly revealed the major peaks of PVA hydrogel associated with PVA power.It

could be observed hydrogen bonded band (ν = 3,256 $cm^{-1}$). Intramolecular and intermolecular hydrogen bondings were expected to occur among PVA chain.

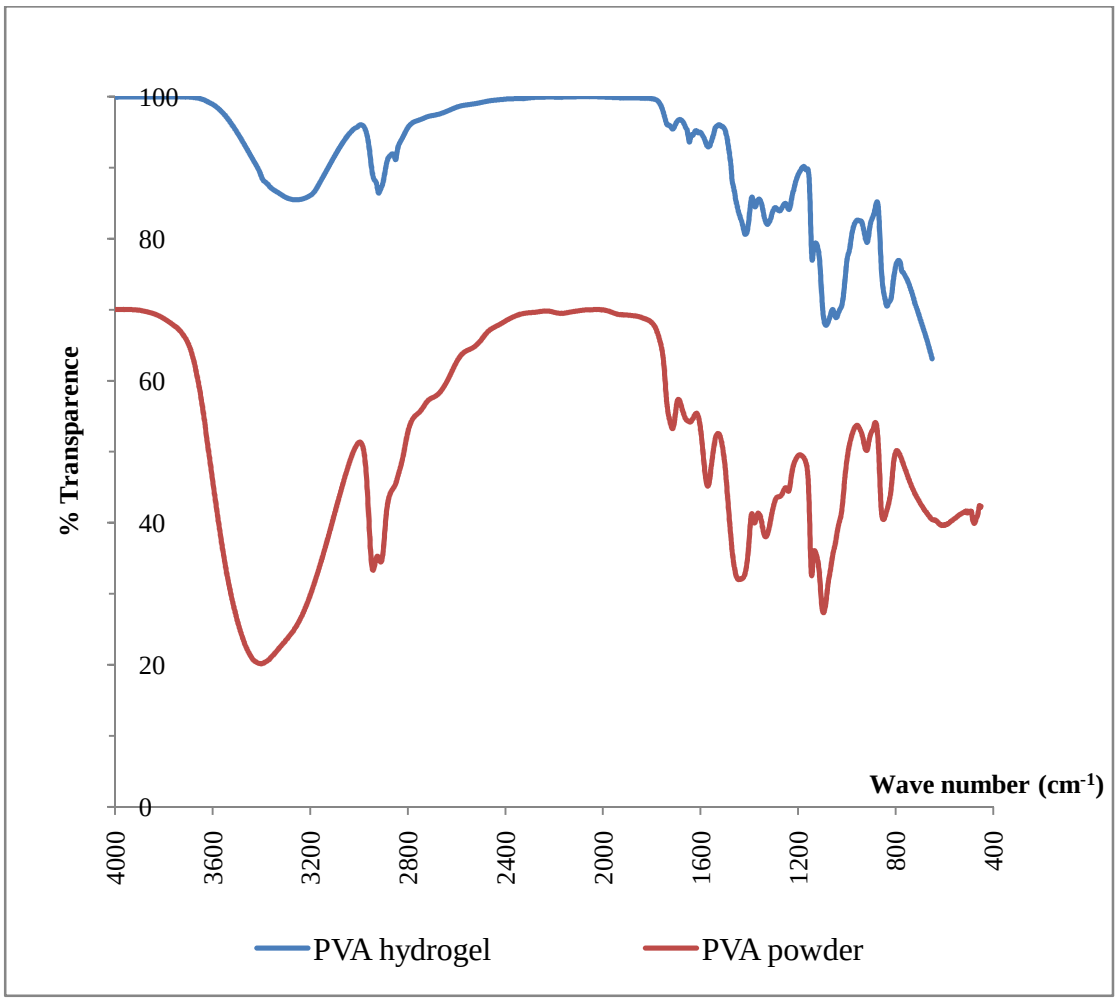



Figure 49. Fourier transformed infrared spectra of PVA hydrogel and PVA powder

On the FTIR spectrum of DG (Figure 50), a characteristic peak was found at 1,746 $cm^{-1}$, which was attributed to the carbonyl absorption. FTIR spectrum of PVA crosslinked by DG (DG/PVA 3.5 : 5 hydrogel) could be observed that two important peaks at ν = 2,851 and 2,919 $cm^{-1}$ of C-H stretching were related to aldehydes, a duplet absorption with peak attributed to the alkyl chain. By crosslink PVA with DG, the C=O stretching vibration peak (ν = 1,746 $cm^{-1}$) changed when compared to DG. This result indicated that the aldehyde groups of DG might be reacted with –OH groups of PVA chain.

In conclusion, DG incorporated in PVA hydrogel changed the mechanical properties of neat PVA hydrogel might be due to crosslink DG with PVA.

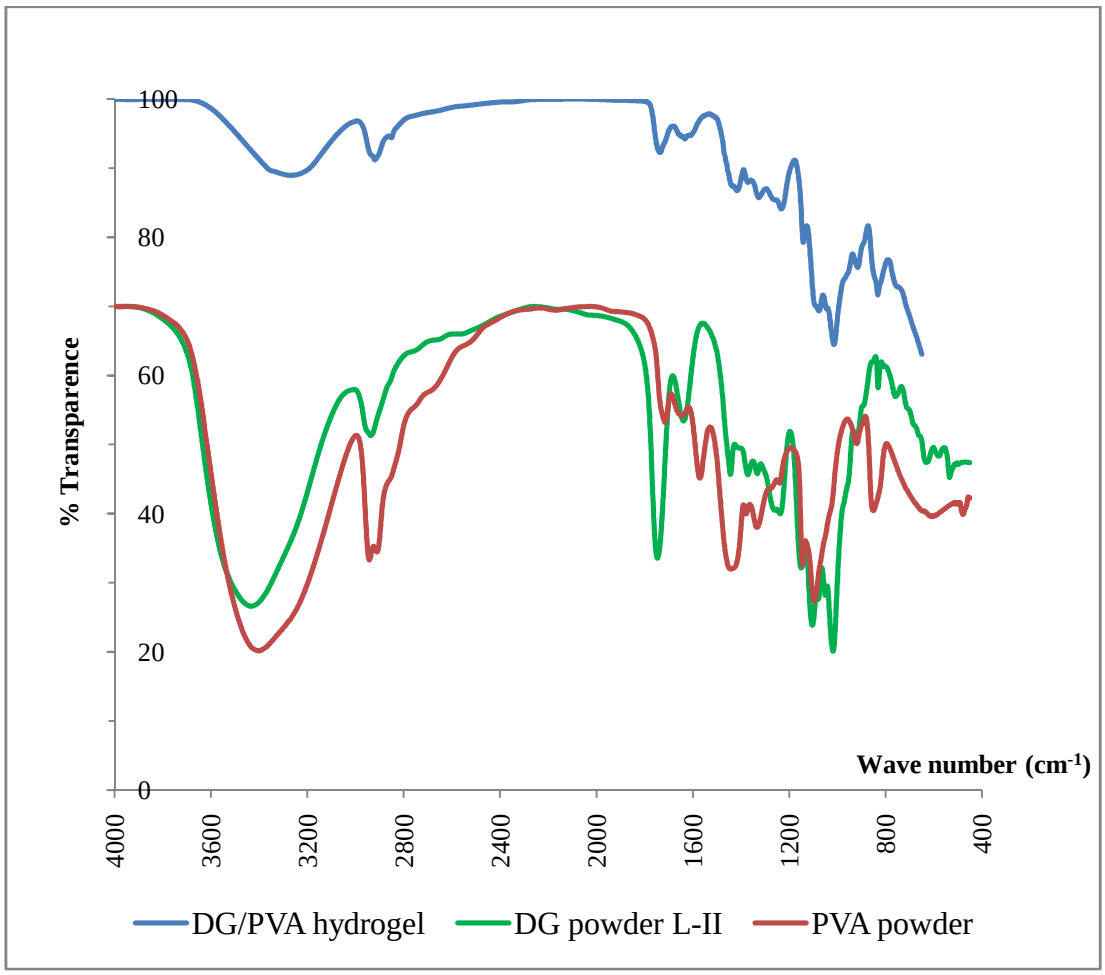



Figure 50. Fourier transformed infrared spectrums of DG powder, PVA powder and DG/PVA hydrogel

From the data obtained, the hydrogel prepared by F24T24, 3 cycles and incorporated with 3.5 %w/w DG got transparent tan color which suitable for observing healing process. Its high water content was preserved for moisture environment. The DG/PVA hydrogel prevented maceration by absorbing much water and allowing vapor through atmosphere. Furthermore, the hydrogel was strong and expandable enough for handling and applications. This hydrogel did not distrub healing process because it had not tight adhesive. Thus the DG/PVA (3.5 : 5) hydrogel and PVA hydrogel were selected for stability study.

## 4. Stability study

### 4.1 Hydrogel appearances

Physical characters of the PVA and DG/PVA hydrogels were different after storage for 3 months. The PVA hydrogels kept at 40°C 75 %RH and 30°C 75 %RH were more opaque than initial. Contraction of the PVA hydrogel occurred to the patch kept at 40°C and 30°C. Whereas the PVA hydrogel kept at 4°C did not change in shape from initial (Figure 51).

A B C

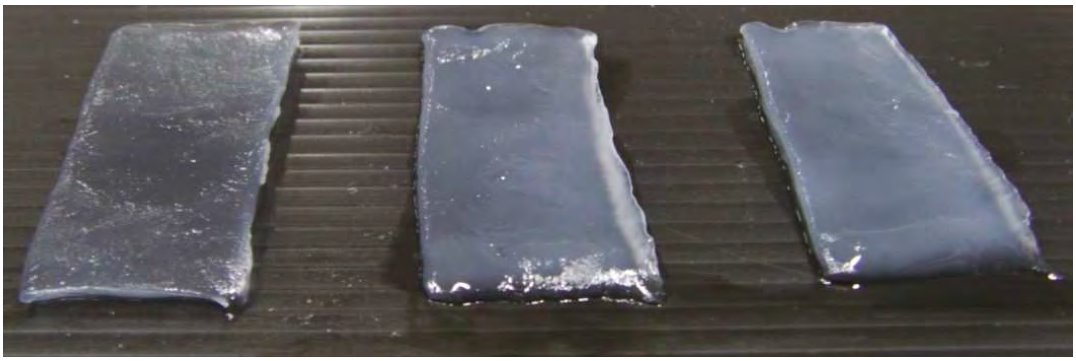


Figure 51. The PVA hydrogels kept 3 months at (A) 4°C, (B) 30°C 75 %RH, (C) 40°C 75 %RH

The DG/PVA hydrogels kept at 30°C 75 %RH and 40°C 75 %RH were changed in color and their shapes (Figure 52). At 30 °C and 40 °C, the DG/PVA hydrogels color was getting opaque and darker. The contraction occurred in DG/PVA hydrogels kept at 30 °C and 40 °C similar to PVA hydrogels. While the DG/PVA hydrogel kept at 4 °C was also transparent with no change in its color.

All the DG/PVA and PVA hydrogels was decreased in their thickness after storage for 1 month at all temperatures (Figure 53). The DG/PVA hydrogels and PVA hydrogels had similar characters, hydrogels kept at 4 °C was decreased in thickness less than keeping at higher temperatures, hydrogels kept at 40 °C were the thinnest. When keeping for long period, the decreasing of thickness was more obvious than keeping in early month. Whereas the DG/PVA hydrogels kept at all temperatures

were thicker than all PVA hydrogel. Thus hydrogels with DG retained their water and structure better than those without DG.

A B C D

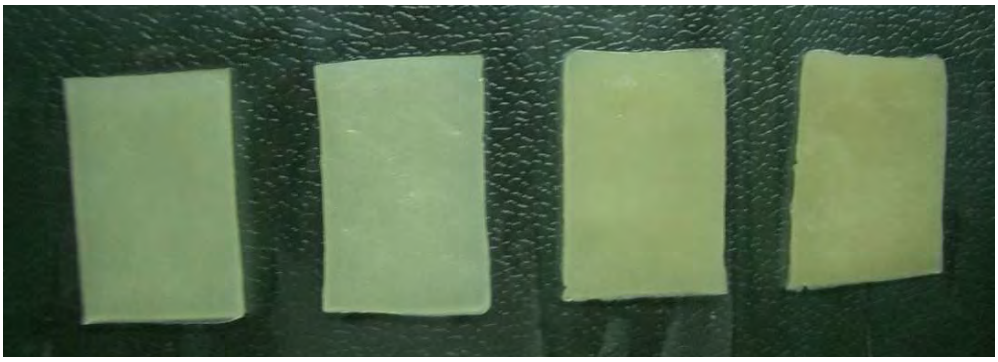


Figure 52. The DG/PVA hydrogels before and after storage for 3 months: initial DG/PVA hydrogel (A), DG/PVA hydrogels kept 3 months at (B) 4°C, (C) 30°C 75 %RH, (D) 40°C 75 %RH

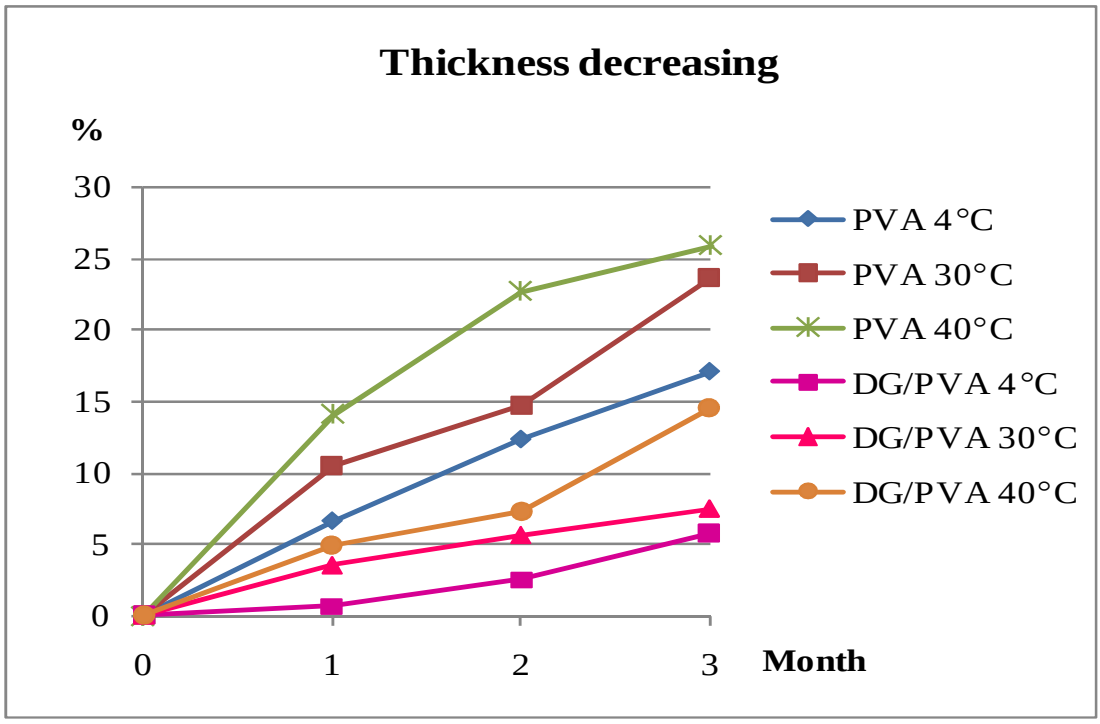



Figure 53. Percentage of thickness decreasing of the DG/PVA and PVA hydrogel after 3 months

Weight decreasing of DG/PVA and PVA hydrogels was correlated to the thickness (Figure 54). Both DG/PVA and PVA hydrogels kept at 40 °C lost their own weights more than keeping at lower temperature. When the time of keeping hydrogel was increased, the weight loss increased. While the DG/PVA hydrogels kept at all temperatures retained their weights more than the PVA hydrogels.

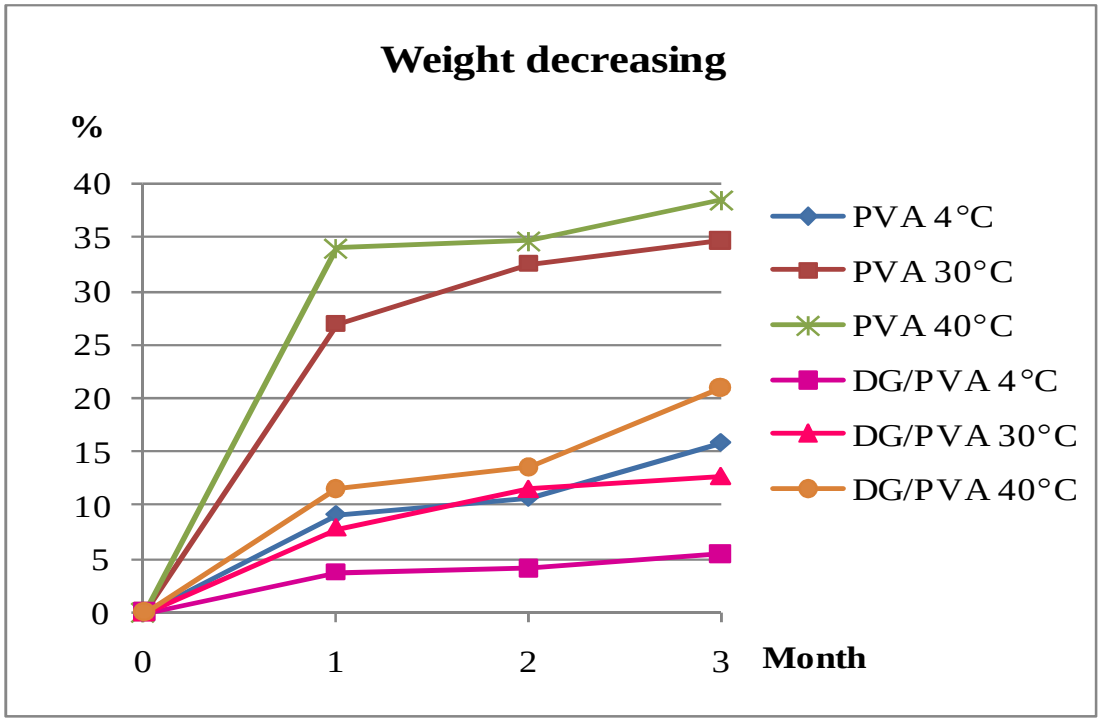



Figure 54. Percentage of weights decreasing of the DG/PVA and PVA hydrogel after 3 months

### 4.2 Water content

Water contents of the PVA hydrogels were shown in Table 9. The PVA hydrogels kept in all conditions had lower water contents than the initial hydrogel (Water content of initial PVA hydrogel was 93.32 ± 0.12 %w/w). The PVA hydrogels kept at 40 °C 75 %RH retained lower water than 30 °C 75 %RH and 4 °C. Thus storage at 4 °C could preserve water in the PVA hydrogel more than other temperatures.

Table 9. Percentage of water content of PVA hydrogels after 3 months

| **Storage conditions** | **Time of keeping (months)** | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| **4 °C** | 85.95 ± 0.87 | 88.90 ± 0.49 | 85.49 ± 0.60 |
| **30 °C 75 %RH** | 78.82 ± 3.47 | 76.18 ± 2.37 | 74.75 ± 1..16 |
| **40 °C 75 %RH** | 74.96 ± 1.67 | 72.90 ± 1.06 | 74.52 ± 2.00 |

Table 10. Percentage of water content of DG/PVA hydrogels after 3 months

| **Storage conditions** | **Time of keeping (months)** | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| **4 °C** | 87.97 ± 0.24 | 88.40 ± 0.30 | 88.03 ± 0.82 |
| **30 °C 75 %RH** | 85.75 ± 0.37 | 82.81 ± 0.62 | 83.72 ± 0.16 |
| **40 °C 75 %RH** | 85.73 ± 0.31 | 83.62 ± 0.75 | 77.80 ± 1.61 |

Water contents of the DG/PVA hydrogels were similar to the PVA hydrogels (Table 10). The DG/PVA hydrogels kept in all conditions had lower water contents than the initial hydrogel (Water content of initial DG/PVA hydrogel was 90.10 ± 0.26 %w/w). Water content of the DG/PVA hydrogel kept at 4 °C was higher than 30 °C 75 %RH and 40 °C 75 %RH.

From data obtained, it was found that all storage DG/PVA hydrogels had higher water content than PVA hydrogels. Thus DG incorporated in PVA hydrogel retained its water content better than the PVA hydrogel.

## 4.3 Water absorption capacity

### 4.3.1 Water absorption capacity of the PVA hydrogels

Figure 55 shows water absorption capacity of the PVA hydrogels after keeping in 3 conditions for 1 month. It was found that all PVA hydrogels absorbed less water than initial hydrogel. The PVA hydrogel kept at 4 °C had the lowest water absorption capacity and could not retain its initial weight. While hydrogels kept at 30 °C and 40 °C could absorb similar amount of water.

After keeping for 2 months, maximum swelling of PVA hydrogels keeping at all temperatures was higher than 1 month (Figure 56). The swelling sequence of hydrogels kept for 2 months similar to 1 month, PVA hydrogel kept in refrigerator lost its initial weight similar to 1 month. The maximum swelling of only the hydrogel kept at 40 °C 75 %RH was higher than initial.

Water absorption of the PVA hydrogels after 3 months was arranged in the same order to after 1 and 2 months (Figure 57). Maximum swelling of the PVA hydrogels keeping at all conditions was slightly higher than initial PVA hydrogel. Maximum absorption of the PVA hydrogel kept at 4 °C was slightly lower than 30 °C and 40 °C respectively.

In summary, water absorption capacity of the PVA hydrogels was changed after storage for 1 month. Water absorption in all conditions of keeping was decreased and then increased after 2 and 3 months. Maximum absorption of hydrogel kept at 40 °C was higher than 30 °C and 4°C, respectively.

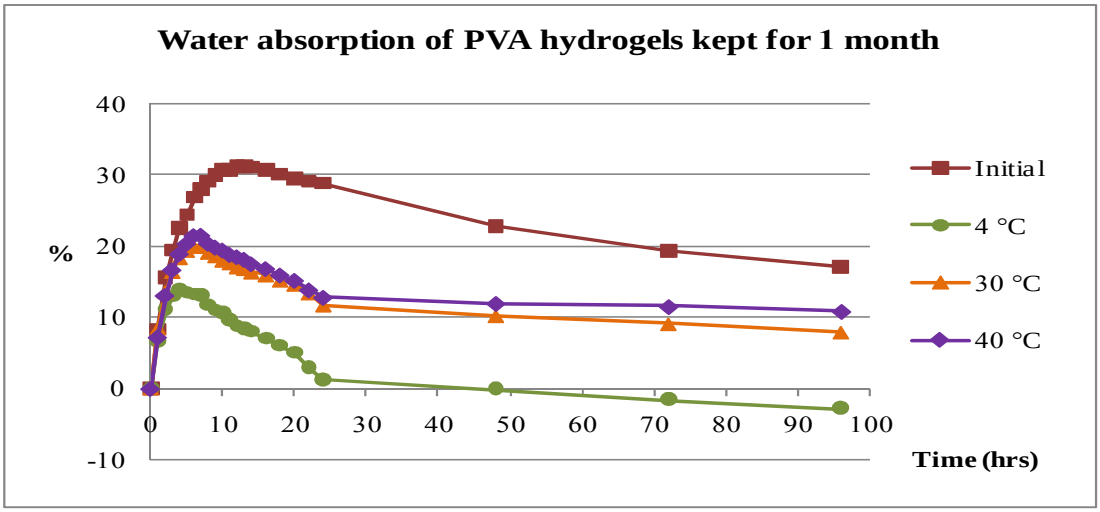



Figure 55. Water absorption capacity of the PVA hydrogels kept for 1 month

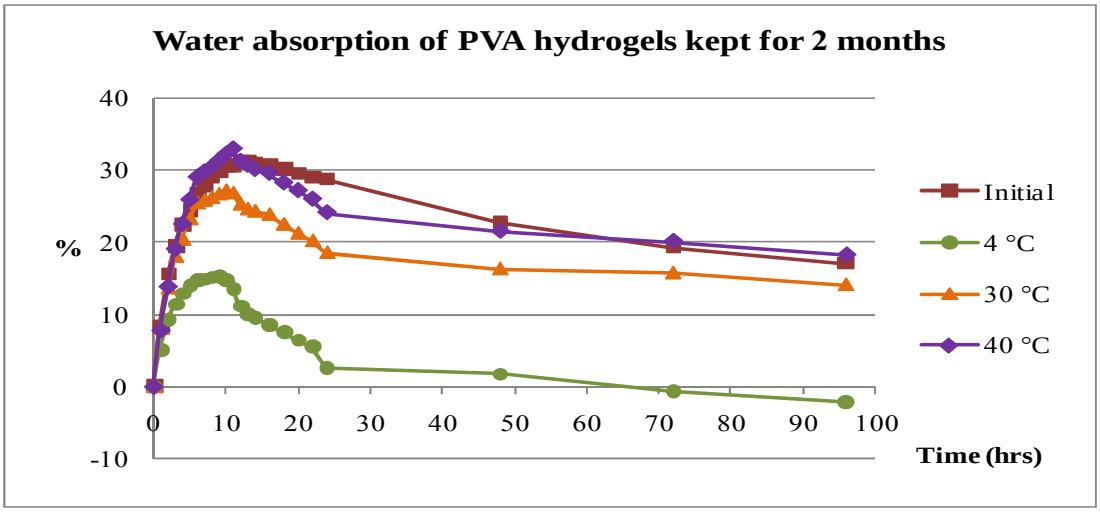



Figure 56. Water absorption capacity of the PVA hydrogels kept for 2 months

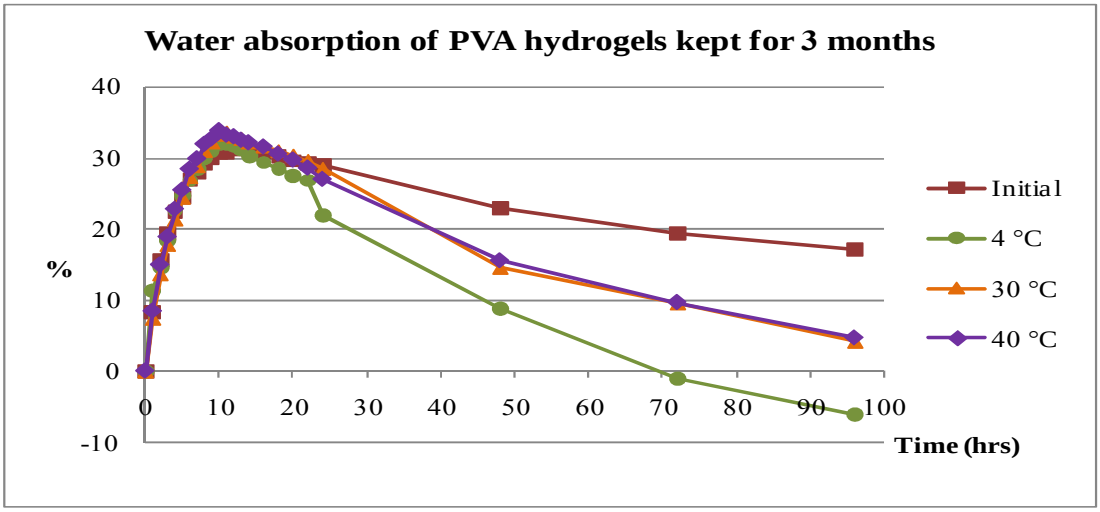



Figure 57. Water absorption capacity of the PVA hydrogels kept for 3 months

### 4.3.2 Water absorption capacity of the DG/PVA hydrogels

Water absorption of the DG/PVA hydrgel was changed after 1 month (Figure 58). All DG/PVA hydrogels had lower water absorption capacity than the initial DG/PVA hydrogel. The DG/PVA hydrogel kept at 4 °C could swell more than at 30 °C and 40 °C, respectively.

After 2 months, DG/PVA hydrogel kept at 4 °C also absorbed much more water than 30 °C and 40 °C, respectively. The DG/PVA hydrogels kept at 4 °C and 30 °C could absorb less water than 1 month, while hydrogel kept at 40 °C absorbed much more water than 1 month (Figure 59). But the water absorption of all hydrogels kept for 2 months was lower than the initial.

Water absorption capacity of the DG/PVA hydrogels after 3 months were similar to hydrogels kept for 2 months (Figure 60). All DG/PVA hydrogels absorbed less water than the initial hydrogel.

In conclusion, water absorption capacity of the DG/PVA hydrogels after 3 months differed to PVA hydrogels. All PVA hydrogels absorbed less water in 1 month, after that the absorption was increased. Maximum water absorptions of all PVA hydrogels kept for 3 months were slightly higher than the initial hydrogel. While absorption of DG/PVA hydrogels could be separated in 2 groups; first group, hydrogels kept at 4 °C and 30 °C, the decreasing of water absorption was occurred in all months. The second group, hydrogel kept at 40 °C, the decreasing of water absorption was occurred in the first month then its absorption capacity was increased. But all DG/PVA hydrogels after 3 months had lower water absorption capacity than the initial DG/PVA hydrogel.

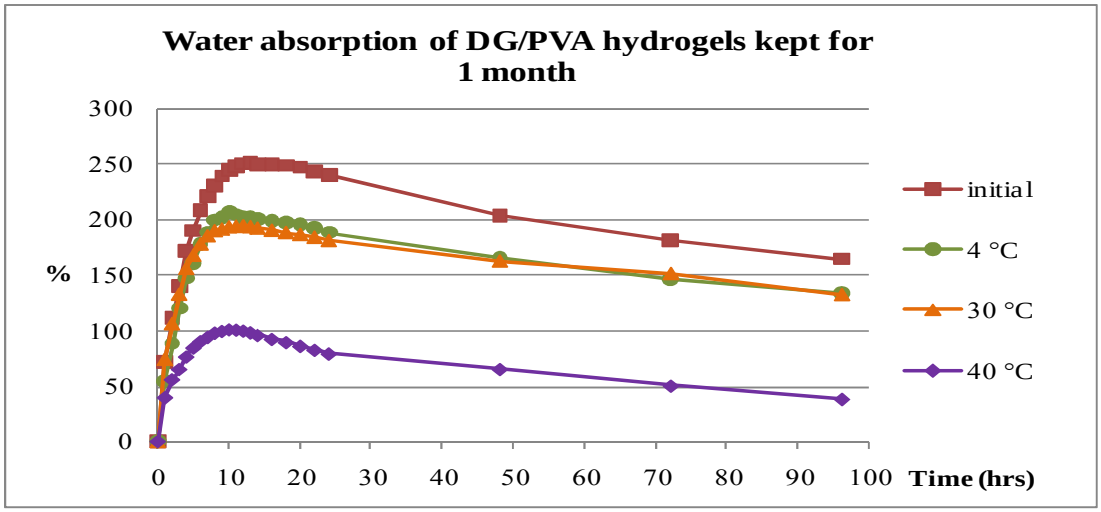



Figure 58. Water absorption capacity of the DG/PVA hydrogels kept for 1 month

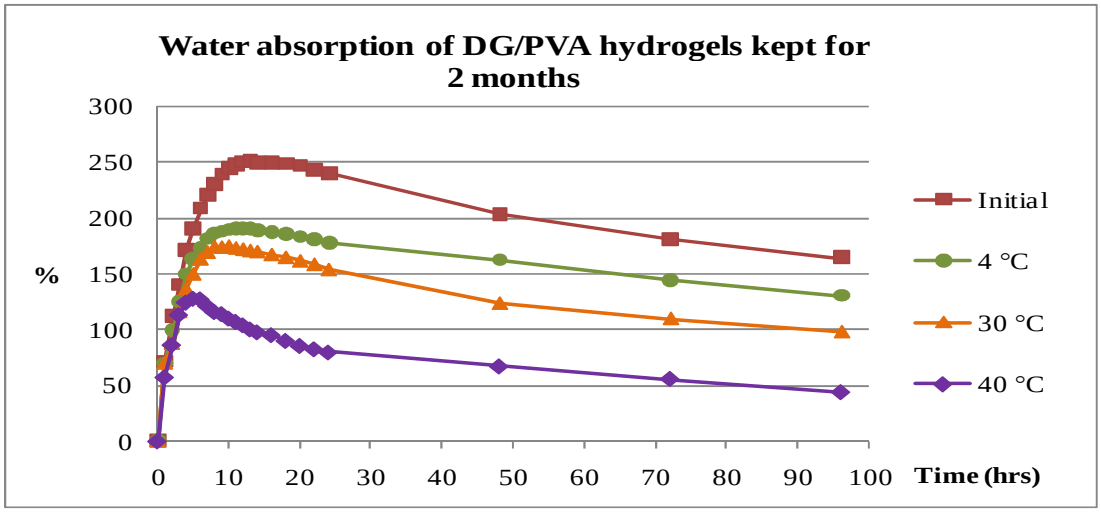



Figure 59. Water absorption capacity of the DG/PVA hydrogels kept for 2 months

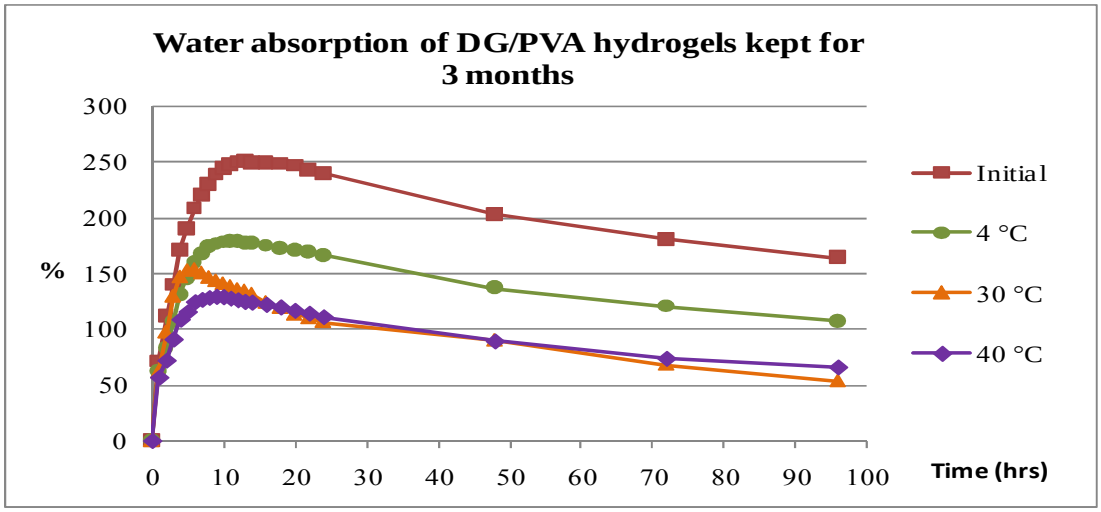



Figure 60. Water absorption capacity of the DG/PVA hydrogels kept for 3 months

### 4.4 Gel fraction

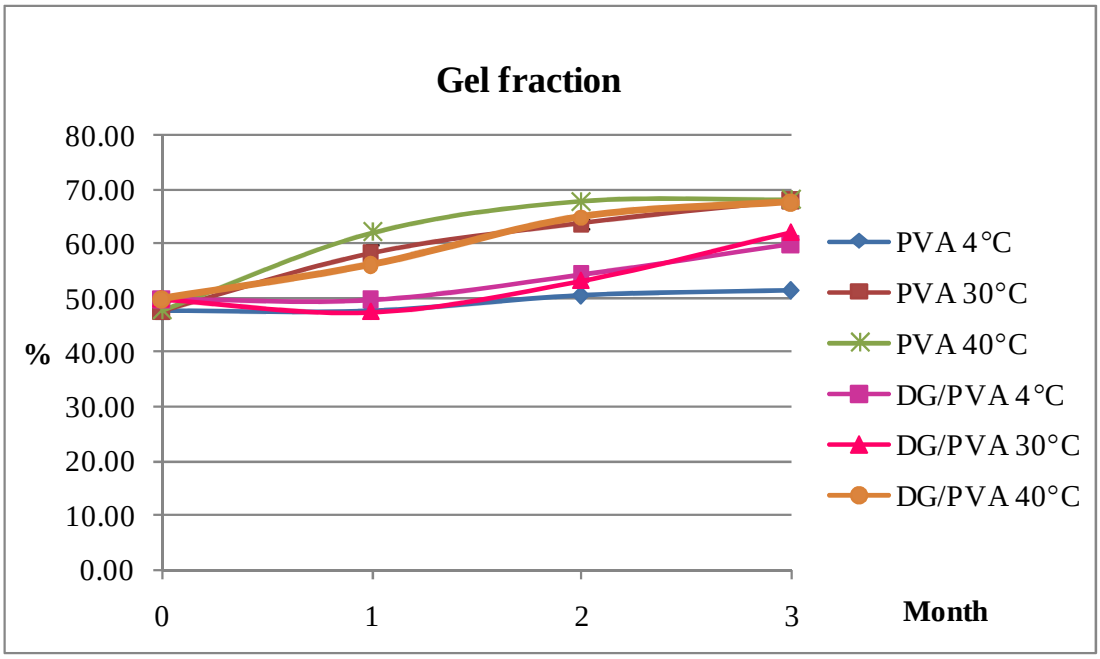



Figure 61. Gel fraction of the DG/PVA and PVA hydrogels kept for 1-3 months

Gel fractions of the PVA hydrogels are shown in Figure 61. After keeping in refrigerator, the gel fraction was slightly decreased in the first month then it was slightly increased more than the initial hydrogel. However, the PVA hydrogels kept at 30 °C and 40 °C were increased in their gel fraction after 1-3 months. After 3 months, the hydrogels with high gel fraction (hydrogels kept at 30 °C and 40 °C) were dense and the contraction of the patch occurred (Figure 51).

Gel fraction of the DG/PVA hydrogels kept for 1-3 months are shown in Figure 61. The DG/PVA hydrogels kept at 4 °C and 30 °C had slightly lower gel fraction than the initial DG/PVA hydrogel in the first month (initial gel fraction was 49.84 ± 1.19), after that gel fractions was increased higher than the initial. For hydrogel kept at 40 °C, gel fraction was increased after kept for 1-3 months.

In summary, temperature of storage affected gel fraction of both DG/PVA and PVA hydrogels. Lower temperature increased gel fraction less than higher

temperature, thus both hydrogels kept at 40 °C with higher change of gel fraction showed more contraction than at other temperatures.

### 4.5 Mechanical properties

Mechanical testing of hydrogels kept for 3 months also performed with the same method as previous testing. Tensile strengths of the PVA hydrogels kept at all temperatures were increased after keeping only 1 month. For the statistical point of view, there were strong differences between initial PVA hydrogel and PVA hydrogels after keeping 3 months ($p<0.05$). All preserved hydrogels were stronger than initial hydrogel. Figure 62 illustrates that hydrogel kept at 4 °C was less strength significantly than hydrogels keeping at 30 °C and 40 °C.

The DG/PVA hydrogels was changed in their strength after keeping for 3 months. Only the DG/PVA hydrogel kept at 40 °C was significantly stronger than the initial hydrogel. Among storage conditions of 4 °C, 30 °C and 40 °C, the DG/PVA hydrogel kept at 40 °C was significantly stronger than 4 °C, whereas DG/PVA hydrogel kept at 30 °C was not different to other conditions.

Elongations of the DG/PVA and PVA hydrogels before and after keeping 1-3 months are shown in Figure 63. The PVA hydrogels kept at all temperature conditions after 3 months stretched more than initial hydrogel ($p<0.05$). Among the groups of PVA hydrogels kept at 4 °C, 30 °C and 40 °C were not different.

Elongations of the DG/PVA hydrogels kept at 4 °C and 30 °C were slightly lower than the initial while the DG/PVA hydrogel kept at 40 °C elongated more than the initial hydrogel. But after 3 months, elongations of all DG/PVA hydrogels were not different between before and after storage.

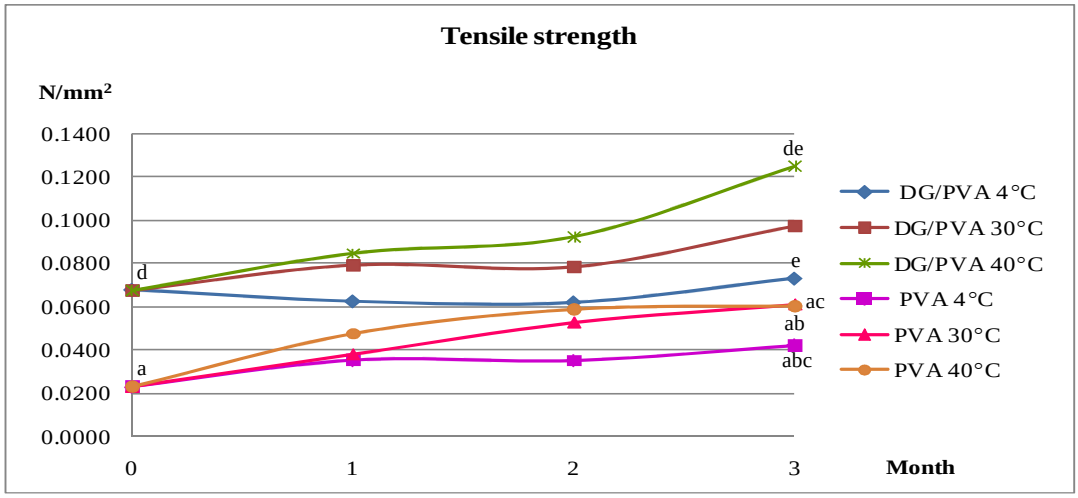



Figure 62. Tensile strength of PVA and DG/PVA hydrogels after keeping 1-3 months

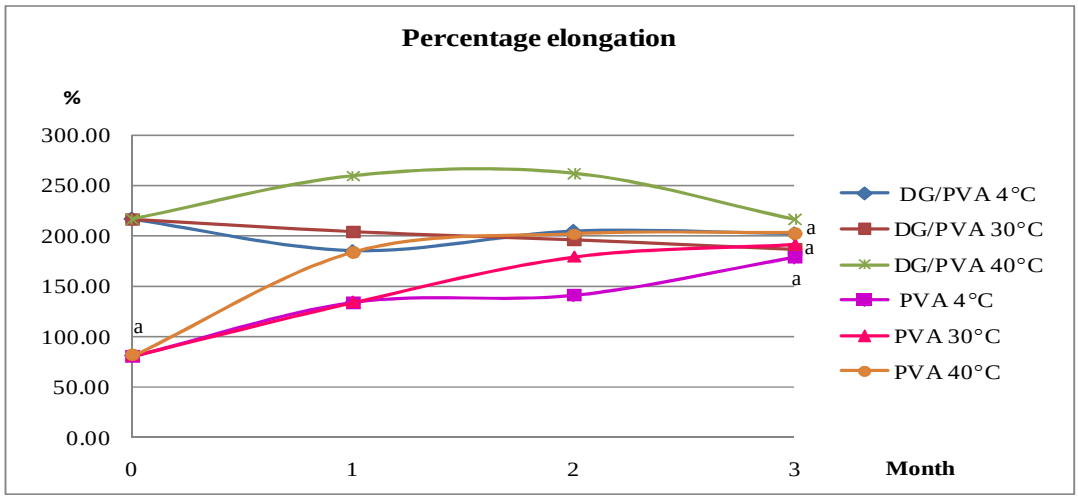



Figure 63. Percent elongation of PVA and DG/PVA hydrogels after keeping 1-3 months

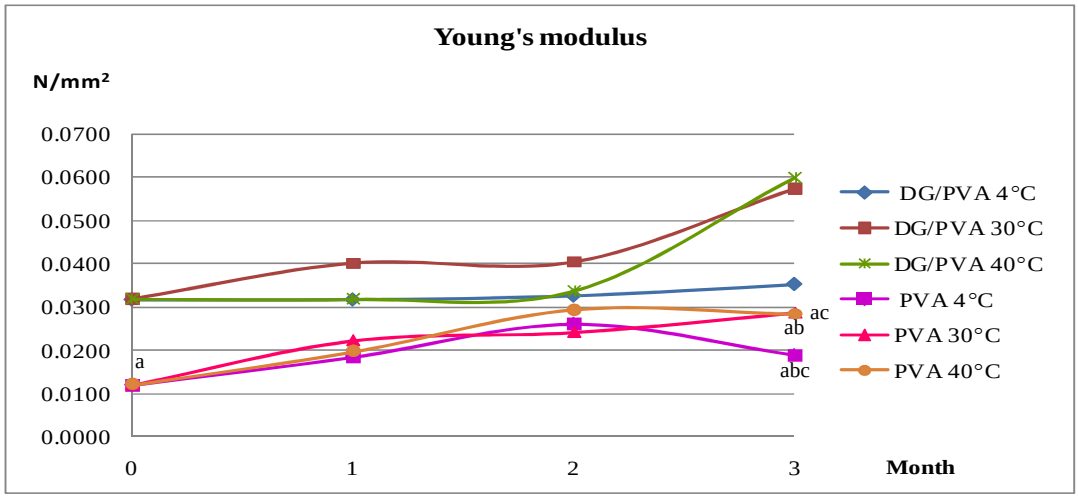



Figure 64. Young's modulus of PVA and DG/PVA hydrogels after keeping 1-3 months

a, b, c, d and e are significant differences between groups ($p < 0.05$)

Stiffness of the PVA hydrogels after keeping for 3 months was changed as same as the strength. All storage PVA hydrogels were stiffer than initial hydrogel. Only PVA hydrogel kept in refrigerator was more softness than keeping at 30 °C and 40 °C ($p<0.05$). Hardness of the DG/PVA hydrogels kept for 3 months corresponded to the PVA hydrogels. The DG/PVA hydrogel kept at 4 °C was softer than the DG/PVA hydrogels kept at higher temperatures (Figure 64).

In conclusion, PVA hydrogels kept at all temperatures were stronger, more expandable, and stiffer than the initial. While the PVA hydrogel kept at 4 °C in refrigerator was weak and softer than keeping at other temperatures. Among DG/PVA hydrogels, the results did not resemble to the PVA hydrogels. Mechanical properties of the DG/PVA hydrogels kept at 4 °C and 30 °C were not different to initial hydrogels whereas keeping at 40 °C increased strength of the DG/PVA hydrogel. From the results of both hydrogels, storage at 4 °C changed hydrogel properties less than other temperatures. Mechanical properties of PVA hydrogels incorporated with DG changed from initial less than PVA hydrogel without DG. Thus DG/PVA hydrogels kept for 3 months could preserved their mechanical properties better than PVA hydrogels.

### 4.6 Scanning Electron Microscopy (SEM)

Morphology of the PVA hydrogels kept at 4 °C, 30 °C and 40 °C for 3 months are shown in Figure 65. The PVA hydrogels kept at all temperatures appeared to have less pores and lobes of network comparing to initial PVA hydrogel. Morphology of the PVA hydrogel kept at 4 °C in refrigerator for 3 months was more similarity to the initial hydrogel. While pores of hydrogel kept at 40 °C 75 %RH was smallest than any temperatures.

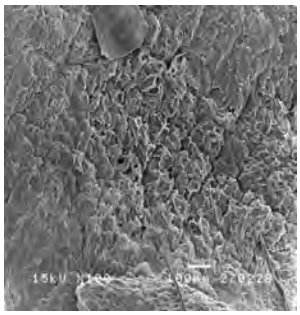
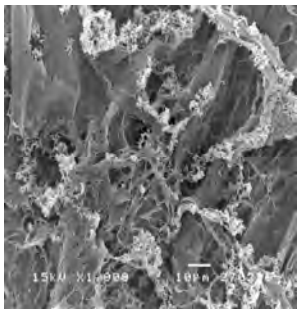
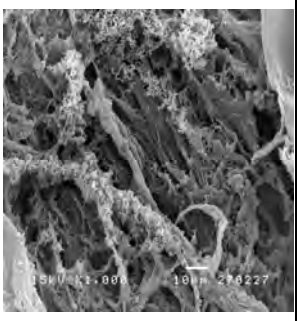
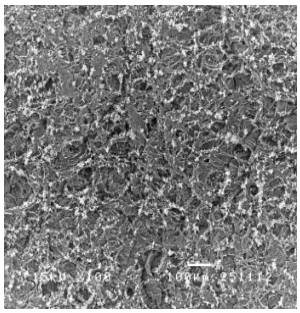
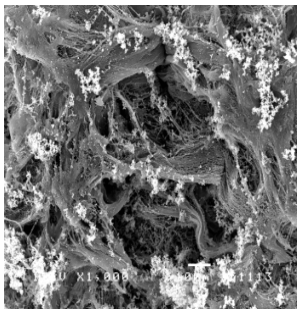
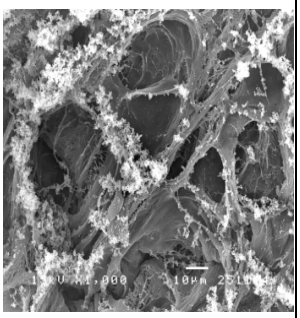
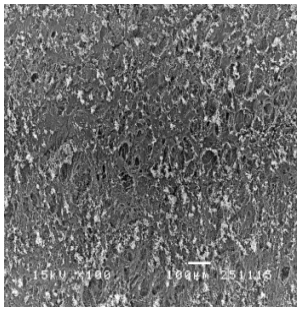
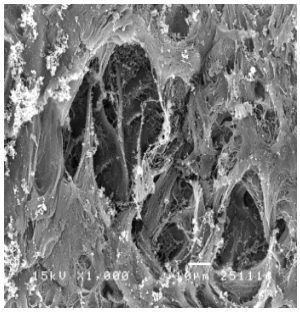
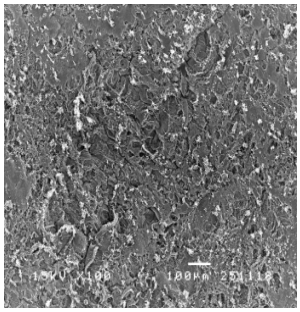
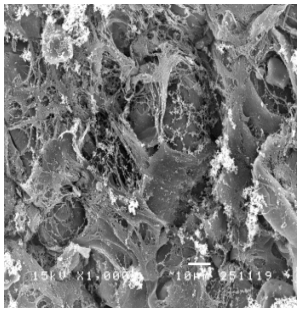
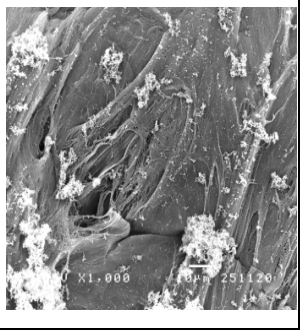
| Hydrogel | Surface (x 100) | Surface (x 1000) | Cross-section (x 1000) |
|---|---|---|---|
| Initial |  |  |  |
| 4 °C |  |  |  |
| 30 °C 75 %RH |  |  |  |
| 40 °C 75 %RH |  |  |  |

Figure 65. Scanning electron micrographs of the PVA hydrogels before and after keeping at 4 °C, 30 °C and 40 °C for 3 months

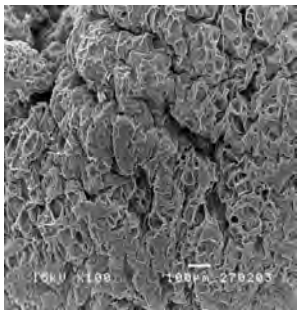
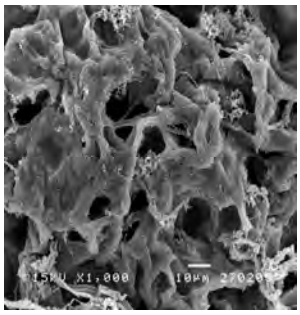
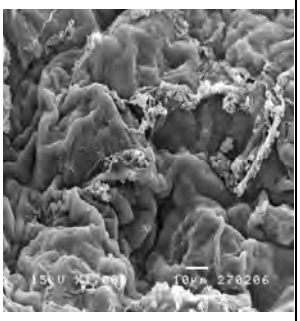
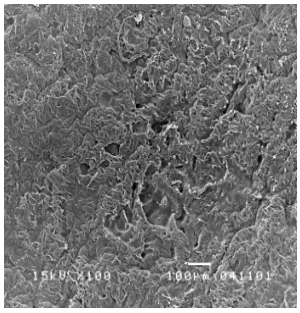
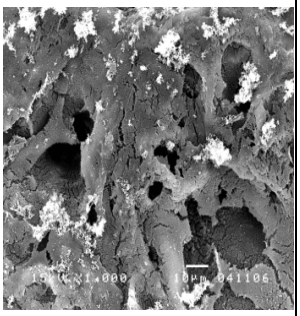
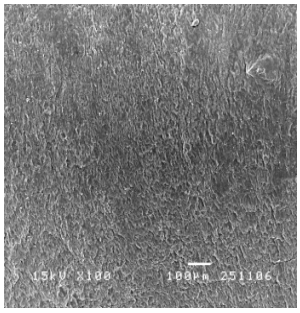
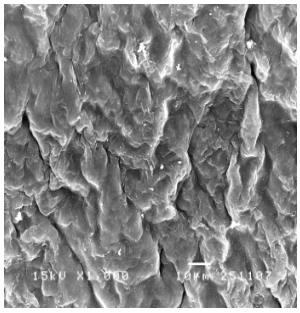
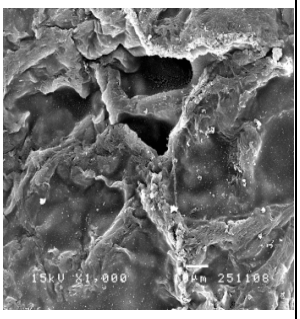
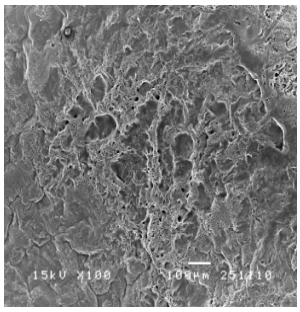
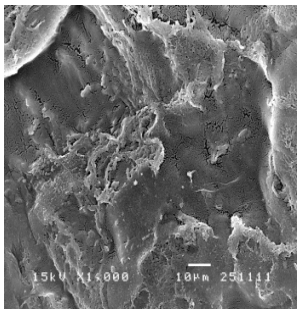
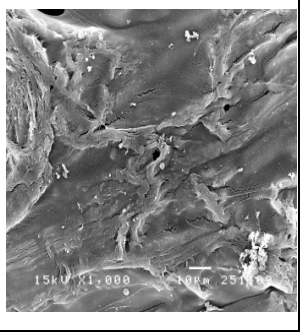
| Hydrogel | Surface (x 100) | Surface (x 1000) | Cross-section (x 1000) |
|---|---|---|---|
| Initial |  |  |  |
| 4 °C |  |  |  |
| 30 °C 75 %RH |  |  |  |
| 40 °C 75 %RH |  |  |  |

Figure 66. Scanning electron micrographs of the DG/PVA hydrogels before and after keeping at 4 °C, 30 °C and 40 °C for 3 months

Morphology of the DG/PVA hydrogels kept for 3 months were not corresponding to the PVA hydrogels (Figure 66). The DG/PVA hydrogel kept at 4 °C in refrigerator was ruptured in their structure due to more bonding. While hydrogels kept at high temperatures i.e. 30 °C and 40 °C collapsed and contracted due to the reduction of water in their structures. Storage temperature at 40 °C affected collapse of hydrogel more than 30 °C.

In conclusion, temperature of keeping affected network structure of both DG/PVA and PVA hydrogels. Cold temperature affected fracture of the DG/PVA structure but it was not clear in PVA hydrogel. While storage at warm temperatures showed the structures with partial melt and collapse.

### 4.7 Water vapor transmission rate (WVTR)

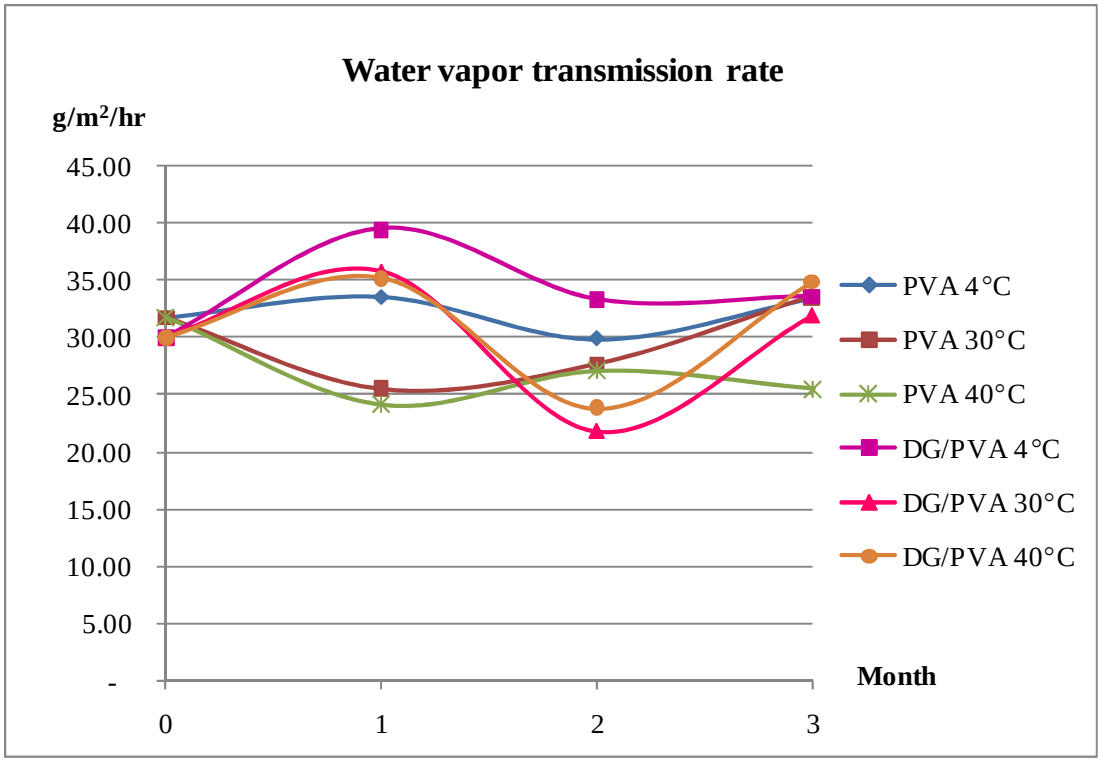



Figure 67. Water vapor transmission rates of the DG/PVA and PVA hydrogels before and after 1-3 months

Water vapor transmission rates (WVTR) of the DG/PVA and PVA hydrogels are shown in Figure 67. WVTR of the PVA hydrogels kept at 4 °C, 30 °C and 40 °C

were different. After 1 month, WVTR of PVA hydrogel kept at 4 °C slightly increased while WVTR of PVA hydrogels kept at 30 °C and 40 °C decreased. But after 3 months, WVTR of the PVA hydrogels before and after keeping at all temperatures were not different ($p>0.05$).

WVTR patterns of the DG/PVA hydrogels kept at all temperatures were similar. After keeping 1 month, increase of WVTR occurred then it decreased after 2 months. After 3 months, WVTR of all DG/PVA hydrogels were not significantly different from initial same to PVA hydrogels. Thus storage conditions did not change WVTR of both hydrogels.

### 4.8 Water evaporation rate from the hydrogels

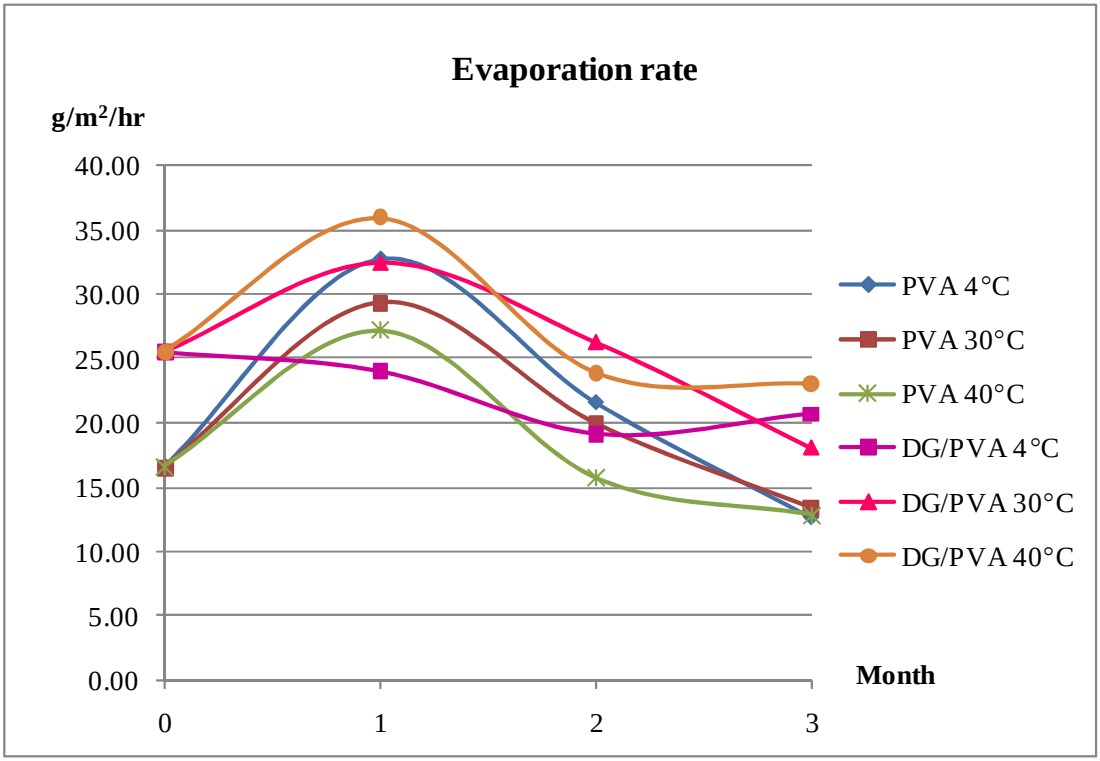



Figure 68. Water evaporation rate of the DG/PVA and PVA hydrogels before and after 1-3 months

Evaporations of water from DG/PVA and PVA hydrogels after keeping 1-3 months are shown in Figure 68. Water of the PVA hydrogels kept at all temperatures evaporated similarly, evaporation increased after 1 month and then decrease occurred.

Water evaporations of all PVA hydrogels after keeping 3 months were not different to initial ($p>0.05$).

Water evaporated from DG/PVA hydrogels kept at 30 °C and 40 °C similarly to PVA hydrogels, increase occurred after 1 month and then evaporation decreased. While DG/PVA hydrogel kept at 4 °C was different to other temperatures. The decreasing rates occurred in 1-2 months after that it increased slightly. Evaporation of water of all DG/PVA hydrogels after keeping 3 months were not different from before keeping same to PVA hydrogels ($p>0.05$).

Storage temperatures did not affect evaporation of both DG/PVA and PVA hydrogels. Between DG/PVA and PVA hydrogels before and after keeping for 3 months, only PVA hydrogel kept in refrigerator for 3 months and initial DG/PVA hydrogel were different.

### 4.9 Adhesion properties

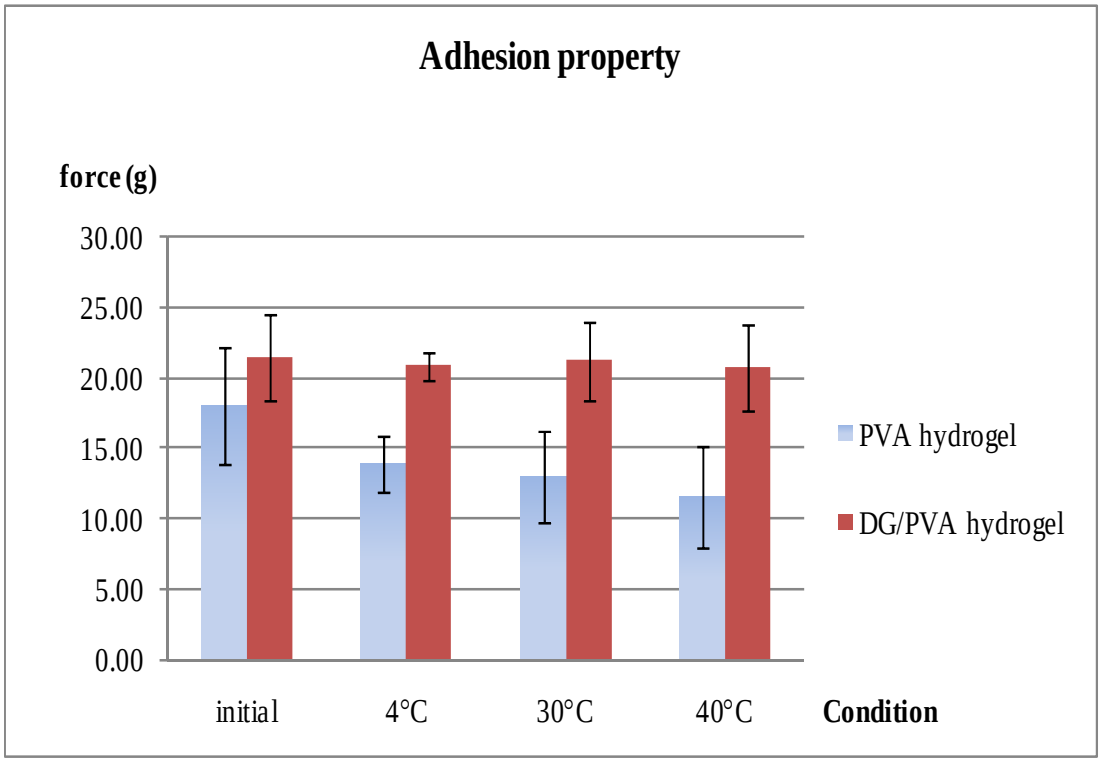



Figure 69. Adhesion property of the DG/PVA and PVA hydrogels before and after 3 months stability studies at various conditions (4 °C, 30 °C 75 %RH, 40 °C 75 %RH)

Adhesion properties of the DG/PVA and PVA hydrogels before and after 3 months are shown in Figure 69. Adhesiveness of the PVA hydrogel kept at 40 °C 75 %RH was significantly lower than the initial, while PVA hydrogels kept at 30 °C 75 %RH and 4°C were not different to the initial hydrogel. The DG/PVA hydrogels before and after kept at all conditions were not different. In conclusion, high temperature changed adhesion of the PVA hydrogel, while temperature did not affect the adhesiveness of DG/PVA hydrogel.

In summary, cold temperature (4 °C) did not affect physical appearances of both DG/PVA and PVA hydrogels while warm temperature (30 °C 75 %RH and 40 °C 75 %RH) affected dark color and contraction of both hydrogels. Thickness and weights of both storage hydrogels were changed. The DG/PVA hydrogels could retain their thickness and weights better than PVA hydrogel. Storage temperature at 4 °C showed less decrease than other temperatures. All temperatures changed amount of water in hydrogel networks, both hydrogel kept at warm temperatures lost much more water than 4 °C. Water absorptions of all PVA hydrogels kept for 3 months were higher than the initial. All DG/PVA hydrogels kept for 3 months absorbed less water than initial but DG/PVA hydrogels kept at 4 °C could absorb much more water than other temperatures.

Furthermore, all temperatures increased crosslink of both hydrogels, storage at 40 °C affected gel fractions more than other temperatures. Storage temperatures did not affect WVTR and WER of both hydrogels kept for 3 months. All mechanical properties of PVA hydrogels kept for 3 months differed from initial, while only strength of DG/PVA hydrogel kept at 40 °C changed from initial. Adhesion of all DG/PVA hydrogels after keeping for 3 months was not different from initial while adhesiveness of PVA hydrogel kept at 40 °C was lower than other temperatures. Thus keeping at 4 °C in refrigerator was the best storage condition for DG/PVA and PVA hydrogels.

## 5. Microbial testing of hydrogels

The DG/PVA (3.5 : 5) hydrogel with the thickness of 3.50 mm prepared by F24T24, 3 cycles were used in antimicrobial study in comparison to PVA hydrogel.

### 5.1 Characterization of hydrogels

The hydrogels should be sterilized before use to test antimicrobial activity. Autoclave was not able to use to sterilize because the PVA hydrogel prepared by freeze-thaw cycle reversed to solution when temperature increased over 55 °C (Yoshii et al., 1995).

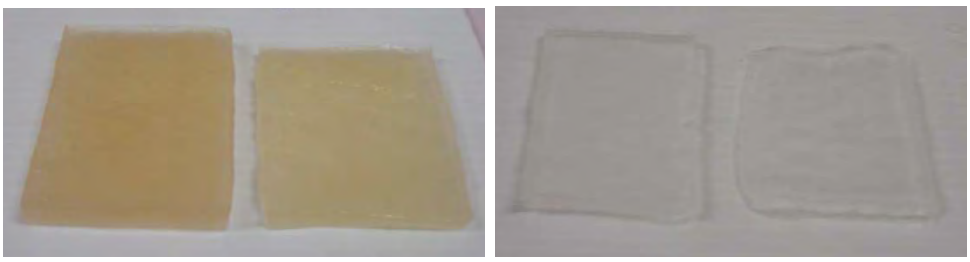
A   B

Figure 70. The PVA hydrogel (A) and DG/PVA hydrogel (B) after gas sterilization

Gas sterilization was investigated by using ethylene oxide at 37°C for 12 hrs (Figure 70). It was found that the DG/PVA (3.5 : 5) and PVA hydrogels changed to hard film due to evaporation of water in network. Thus gamma ray was used to sterilize hydrogels before antimicrobial testing.

#### 5.1.1 Hydrogel appearances

The DG/PVA and PVA hydrogels were sterilized by gamma ray with dose 25 kGy. Both hydrogels contracted and changed their size. Color of the DG/PVA hydrogel faded (Figures 71A-B), in contrast to the PVA hydrogel which did not change (Figures 71C-D).



A B C D

Figure 71. Photographs of hydrogels (A and B): DG/PVA hydrogels before and after gamma sterilization, (C and D): PVA hydrogels before and after gamma sterilization

Weight and thickness of the DG/PVA hydrogel decreased after sterilization similarly to PVA hydrogel. The DG/PVA hydrogel decreased their weight and thickness 19.51 ± 1.13 % and 5.72 ± 0.89 %, respectively. After sterilization, weight of PVA hydrogel changed 11.35 ± 0.59 %, lower than DG/PVA hydrogel. PVA hydrogel thickness changed nearly DG/PVA hydrogel 5.10 ± 0.67 %.

### 5.1.2 Water content

Water contents of the DG/PVA and PVA hydrogels slightly changed, decreasing occurred after gamma sterilization (Table 11).

Table 11. Water contents of the DG/PVA and PVA hydrogels before and after sterilization

| **Hydrogels** | **Water content (%w/w)** | |
|---|---|---|
| | **Before sterilization** | **After sterilization** |
| PVA | 93.17 ± 0.04 | 92.17 ± 0.43 |
| DG/PVA | 89.52 ± 0.09 | 86.14 ± 0.82 |

### 5.1.3 Water absorption capacity

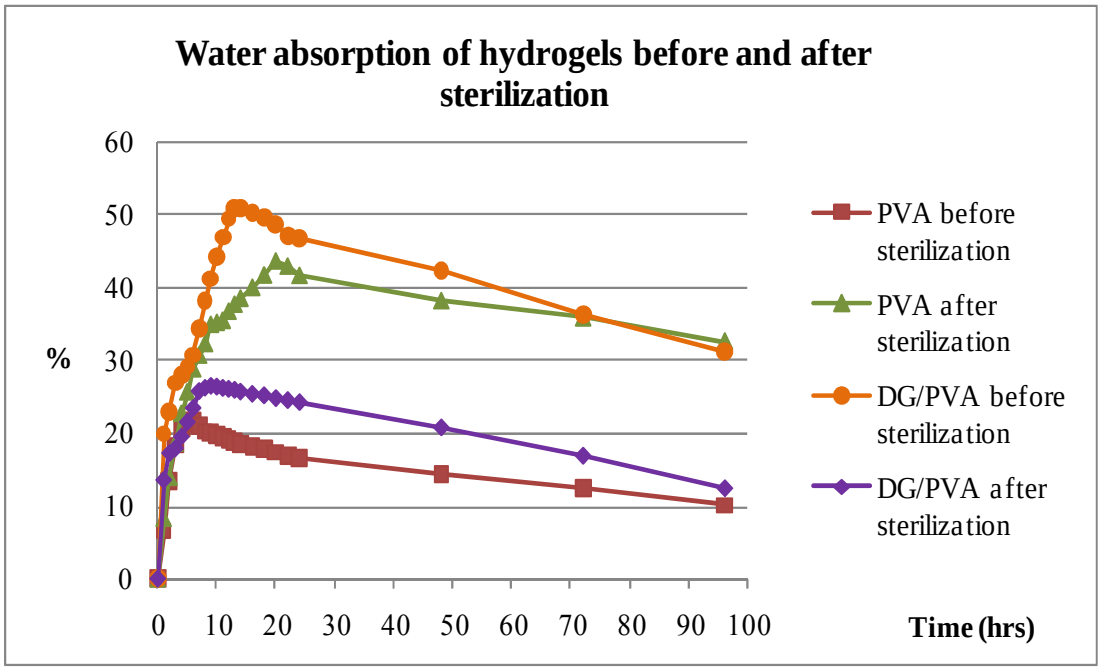



Figure 72. Water absorption capacity of DG/PVA and PVA hydrogels before and after sterilization

Water absorption characters of the DG/PVA before and after sterilization differed to the PVA hydrogel. The PVA hydrogel absorbed much more water after sterilization while lower absorption of the DG/PVA hydrogel occurred after sterilization (Figure 72).

### 5.1.4 Gel fraction

Gel fractions of the DG/PVA and PVA hydrogels changed after sterilization (Table 12). The PVA hydrogel crosslink was higher significantly after sterilization ($p<0.05$). In contrast to DG/PVA hydrogel, gel fractions before and after sterilization were not statistical different. Thus gamma ray affected only network structure of PVA hydrogel but it did not increase DG crosslink.

Table 12. Gel fraction of the DG/PVA and PVA hydrogels before and after sterilization

| **Hydrogels** | **Gel fraction (%)** | |
|---|---|---|
| | **Before sterilization** | **After sterilization** |
| PVA | 53.56 ± 1.56* | 70.88 ± 3.99* |
| DG/PVA | 42.40 ± 2.52 | 46.21 ± 0.98 |

* significant difference between groups ($p < 0.05$)

### 5.1.5 Mechanical properties

Table 13. Mechanical properties of the DG/PVA and PVA hydrogels before and after gamma sterilization

| **Hydrogel** | **Tensile strength** | **Percentage elongation** | **Young's modulus** |
|---|---|---|---|
| PVA before sterilization | 0.0270 ± 0.0031* | 122.35 ± 11.27 | 0.0154 ± 0.0017 |
| PVA after sterilization | 0.0337 ± 0.0028* | 128.79 ± 2.34 | 0.0145 ± 0.0007 |
| DG/PVA before sterilization | 0.0354 ± 0.0050 | 128.70 ± 16.81 | 0.0213 ± 0.0025 |
| DG/PVA after sterilization | 0.0397 ± 0.0050 | 120.29 ± 9.87 | 0.0241 ± 0.0048 |

* significant difference between groups ($p < 0.05$)

The PVA hydrogel after sterilization was stronger than initial hydrogel significantly ($p<0.05$). Ductility and stiffness of PVA hydrogel between sterilization and initial hydrogels were not different. However, strength, elongation and hardness

of the DG/PVA hydrogels before and after sterilization were similar (Table 13). From the data obtained, it was found that strength of the PVA hydrogel increased due to its dense structure (high gel fraction) from further cross-link by gamma ray. While the DG/PVA hydrogel after sterilization did not change its mechanical properties and gel fraction.

### 5.1.6 Scanning Electron Microscopy (SEM)

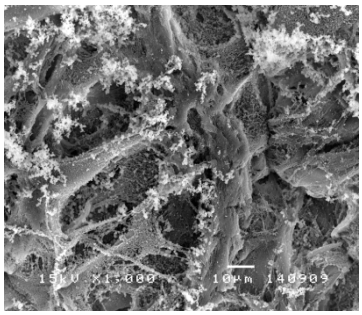
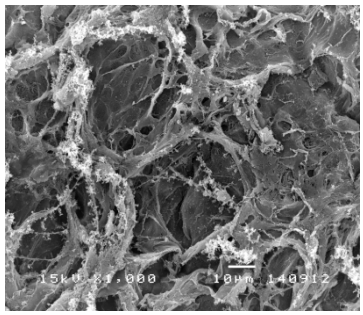
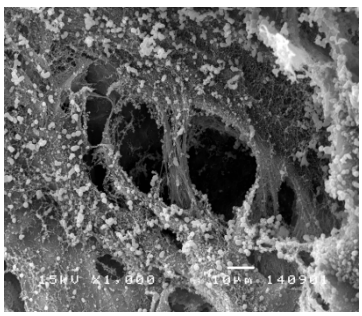
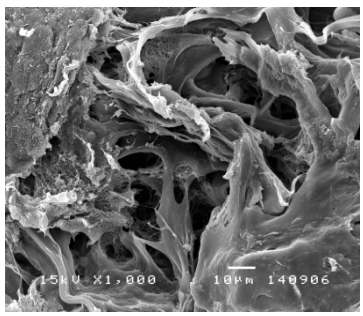
| **Hydrogel** | **Before sterilization (x1000)** | **After sterilization (x1000)** |
|---|---|---|
| PVA |  |  |
| DG/PVA (3.5 : 5) |  |  |

Figure 73. Cross section morphology of the DG/PVA and PVA hydogels before and after gamma sterilization

Gamma ray was used for sterilization of the DG/PVA and PVA hydrogels before antimicrobial testing. Both DG/PVA and PVA hydrogel networks were dense, white dots of free polymer disappeared and bonded together (Figure 73). Furthermore, the dense bonding affected contraction of the hydrogels which show in Figures 71B and 71D.

### 5.1.7 Water vapor transmission rate (WVTR)

Table 14. WVTR of the DG/PVA and PVA hydrogels before and after sterilization

| Hydrogels | WVTR ($g/m^2/h$) |
|---|---|
| PVA before sterilization | 38.78 ± 1.72 |
| PVA after sterilization | 36.35 ± 1.25 |
| DG/PVA before sterilization | 25.14 ± 2.95 |
| DG/PVA after sterilization | 29.80 ± 0.26 |

Table 14 shows WVTR of the DG/PVA and PVA hydrogels before and after sterilization. In statistical view, WVTR of the DG/PVA and PVA hydrogels after sterilization were not different to initial hydrogels. Sterilization by gamma ray did not change WVTR of both hydrogels.

### 5.1.8 Water evaporation rate from the hydrogel

Evaporation rates of water from surface of DG/PVA and PVA hydrogels slightly changed after sterilization (Table 15). But there was no statistical difference between before and after sterilization of both hydrogels.

Table 15. WER of the DG/PVA and PVA hydrogels before and after sterilization

| **Hydrogels** | **Evaporation ($g/m^2/h$)** |
|---|---|
| PVA before sterilization | 14.29 ± 2.40 |
| PVA after sterilization | 16.60 ± 1.08 |
| DG/PVA before sterilization | 20.17 ± 2.74 |
| DG/PVA after sterilization | 15.77 ± 0.55 |

In conclusion, the DG/PVA and PVA hydrogels were sterilized before utilization in antimicrobial study. Gamma sterilization was the best method between 3 methods described in this research. Physical appearance, weight and thickness of both hydrogels changed after gamma sterilization. Water contents of two hydrogels after sterilization were slightly lower. After sterilization, PVA hydrogel absorbed much more water than initial in contrast to DG/PVA hydrogel. Gamma ray affected the higher crosslink of PVA in hydrogel due to high gel fraction occurred, while it did not correlate to DG/PVA hydrogel. Strength of the PVA hydrogel increased after sterilization but mechanical properties of the DG/PVA hydrogel did not change. WVTR and WER of both hydrogel did not change significantly after sterilization.

### 5.2 Antimicrobial activity test

Two types of bacteria were selected for antimicrobial testing i.e. *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 2738 as representatives of gram positive and gram negative bacteria, respectively. *S. aureus* is a common microorganism which causes wound infections (Bracciano, 2008: 54). *E. coli*, a normal flora of bowel, can be contaminated by hand to the wound (Bale and Jones, 1997: 22).

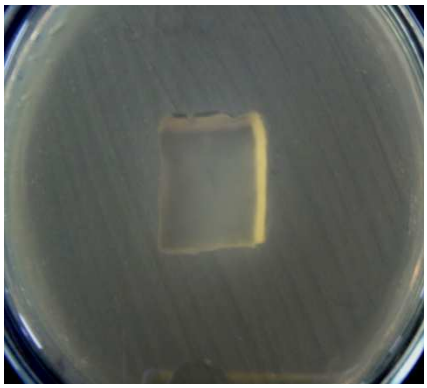
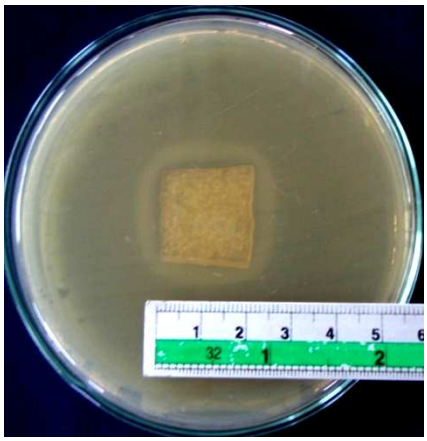
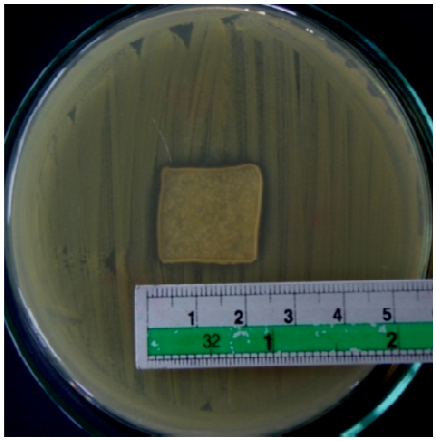
| Samples | *E.coli* | *S.aureus* |
|---|---|---|
| **PVA hydrogel** |  |  |
| **DG/PVA hydrogel** |  |  |

Figure 74. Antimicrobial property of DG/PVA and PVA hydrogels against *E.coli* and *S.aureus* at concentration of Mcfarland No. 0.5

The DG/PVA hydrogel was used to assay an antimicrobial activity compared to the PVA hydrogel. Inhibition activity of hydrogel was illustrated by observation of clear area around hydrogels covered on the surface of Mueller Hinton Agar (MHA) against *E. coli* and *S. aurues* which are representatives of gram negative and gram positive microorganisms. Figure 74 shows antimicrobial test of the hydrogels cut into size 2.0 x 2.0 $cm^2$ against microbes at concentration of Mcfarland No. 0.5 (1.5 x $10^8$ CFU/ml) by agar diffusion method. MHA plates covered with the PVA hydrogel did not have inhibition zone in both types of bacteria. However, agar plates covered with the DG/PVA hydrogel had clear zone (23.3 ± 0.6 $mm^2$) and translucent zone (31.3 ±

1.2 $mm^2$) against *S. aureus* and *E. coli*, respectively. This study found that PVA hydrogel couldn't decrease amount of *E. coli* and *S. aurues*. Whereas DG/PVA hydrogel was able to inhibit growth of *S. aureus* and it reduced amount of *E. coli*. In agreement with Nantawanit (2001), the current study found that antimicrobial property of the DG/PVA hydrogel was important factor for wound healing process improvement due to prevention of bacteria inflammation from environment.

## 5.3 Microbial penetration test

### 5.3.1 Survival growth of bacteria under the hydrogels

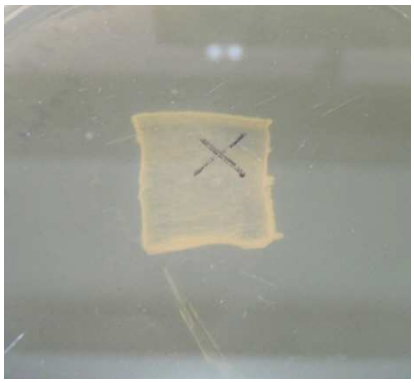
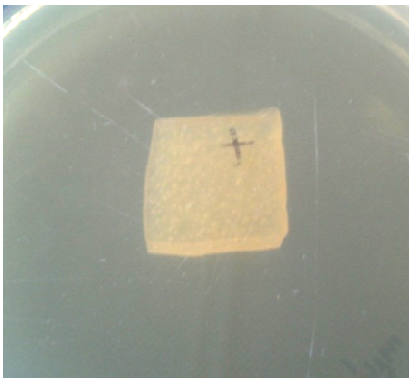
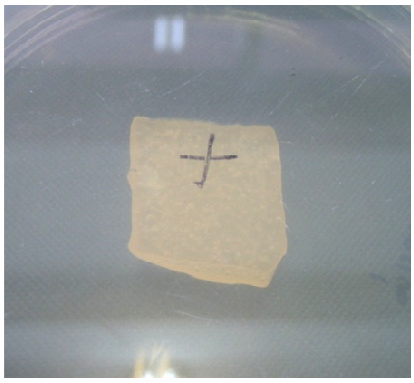
| **Samples** | ***E. coli*** | ***S. aureus*** |
|---|---|---|
| **PVA hydrogel** |  |  |
| **DG/PVA Hydrogel** |  |  |

Figure 75. Growth of *E.coli* and *S.aureus* under the PVA and DG/PVA hydrogels

*E. coli* and *S. aureus* fully grew under the PVA hydrogel which was used as control. Many colonies of bacteria appeared under the PVA hydrogel. It was found that *E. coli* and *S. aureus* were able to survive without oxygen under the PVA hydrogel. However, *E. coli* and *S. aureus* were completely inhibited under the DG/PVA hydrogel (Figure 75). Thus, DG/PVA hydrogel was suitable for use as dressing because it could inhibit growth of bacteria which contaminated in wound.

### 5.3.2 Bacteria penetration through the hydrogels

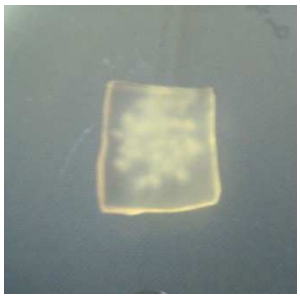
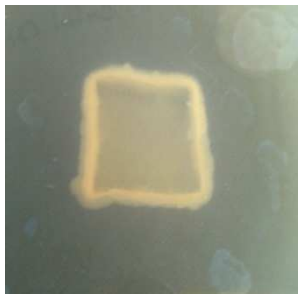
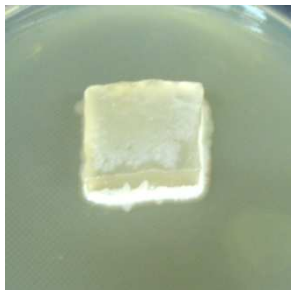
| Samples | 1 day | 2 days | |
|---|---|---|---|
| | Below | Below | Top |
| PVA hydrogel |  |  |  |
| DG/PVA hydrogel |  |  |  |

Figure 76. Penetration of *E. coli* through the DG/PVA and PVA hydrogels

Bacteria penetration test was used to investigate hydrogel barrier against microbe from environment. Figure 76 shows ability of *E. coli* passed through the DG/PVA and PVA hydrogels. After incubation 1 day, *E. coli* grew on the top surface of the PVA hydrogel but it could not pass through MHA below. White colonies of

*E. coli* expanded to edge of patch and dropped onto agar surface in 2 days, thus the penetration test finished. Whereas *E. coli* was not able to grow on the DG/PVA hydrogel surface after incubate 2 days.

*S. aureus* grew on the top surface of the PVA hydrogel well and could not pass through agar like *E. coli*. Yellow colonies enlarged to rim of hydrogel in 2 days same to *E. coli*. While the DG/PVA hydrogel also inhibited growth of *S. aureus* and did not allow *S. aureus* pass through agar (Figure 77).

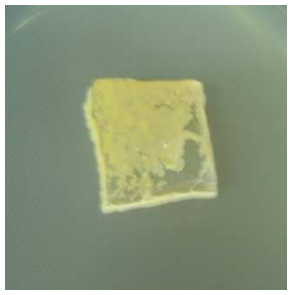
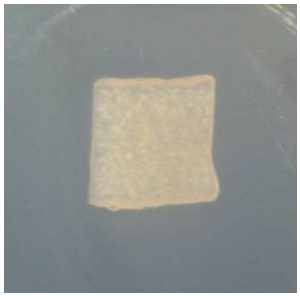
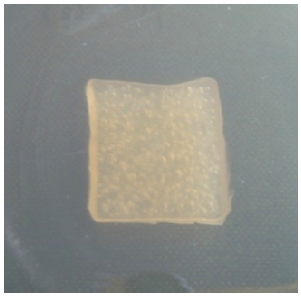
| **Samples** | **1 day** | **2 days** | |
|---|---|---|---|
| | **Below** | **Below** | **Top** |
| **PVA hydrogel** |  |  |  |
| **DG/PVA hydrogel** |  |  |  |

Figure 77. Penetration of *S. aureus* through the DG/PVA and PVA hydrogels

From the data obtained, it was found that the DG/PVA and PVA hydrogels could protect wound from further infection due to their barrier against *E. coli* and *S. aureus* from environment. Moreover, DG/PVA hydrogel also inhibited growth of bacteria on the top of wound surface, but PVA hydrogel could not do.

# CHAPTER V

# CONCLUSIONS

Polysaccharide gel extracted from Durian fruit-hulls (DG) was blended with polyvinyl alcohol (PVA) to form physically cross-linked hydrogels by freeze-thaw technique. Process parameters, including freeze-thaw duration, mixing temperature, thickness level, cycle of freeze-thaw process and concentration of DG, were investigated. Hydrogels prepared by freeze-thaw process for 1-2 cycles were not strong enough for handling and testing. Freeze-thaw condition of freezing at -20 °C for 24 hrs and thawing at 30 °C for 24 hrs (F24T24), 3 cycles was better than the condition of freezing at -20 °C for 18 hrs and thawing at 30 °C for 6 hrs (F18T6), 3 cycles. By F24T24, DG/PVA hydrogel membranes absorbed much more water than by F18T6. After swelling for 4 days, their strength properties were also stronger than F18T6 hydrogels which had lower gel fraction. Mixing at room temperature, DG/PVA hydrogels had better membrane properties than mixing at higher temperatures. They could absorb much more water due to their low gel fraction. Variation of hydrogel thickness levels affected on water absorption capacity and gel fraction. DG/PVA hydrogel with the thickness of 1.00 mm absorbed much more water than thicker hydrogels (2.00 and 3.50 mm). But after maximum water swelling, the membrane with lower gel fraction was resulted in immediately decreasing in its weight. High cycles of freeze-thaw process, the membrane had high gel fraction and high mechanical properties but reduced in water absorption capacity. High concentration of DG affected on several membrane properties. With high concentration of DG (3.5 %w/w) in hydrogel, the membrane showed better properties in gel fraction, water absorption capacity and all mechanical properties. Thus F24T24, 3 cycles condition, initial mixing at room temperature, DG concentration of 3.5 %w/w and the membrane thickness of 3.50 mm were the most suitable factors for preparation of DG/PVA hydrogel. The DG/PVA hydrogel, prepared using the suitable condition, was transparent and light-tan color while PVA hydrogel was transparent with colorless. Comparison of DG/PVA hydrogel to PVA hydrogel showed that water absorption

capacity of DG/PVA hydrogel was approximate 8 times higher than PVA hydrogel. All mechanical properties of DG/PVA hydrogel were better than PVA hydrogel. No differences of water content (slightly higher in PVA hydrogel), gel fraction (slightly higher in DG/PVA hydrogel), water vapor transmission rate (WVTR), water evaporation rate (WER) and adhesion property occurred between DG/PVA and PVA hydrogels. Gamma irradiation was the optimum method for sterilization of hydrogels before antimicrobial testing. From agar diffusion test, DG/PVA hydrogel was able to inhibit growth of bacteria, *Escherichia coli* and *Staphylococcus aureus*. DG/PVA exhibited clear zone 23.3 ± 0.6 $mm^2$ and translucent zone 31.3 ± 1.2 $mm^2$ against *S. aureus* and *E. coli*, respectively.

Storage condition of the DG/PVA hydrogel before using was one factor affected hydrogel properties. Keeping at 4 °C was the suitable storage condition as the membrane appearance was not changed while the membranes were changed in storage at other temperatures. Thickness decreasing, weight decreasing, water content, gel fraction and water absorption capacity were changed less than keeping at higher temperatures. In addition, WVTR, WER, mechanical properties and adhesion of DG/PVA hydrogel kept at 4 °C were not changed from the initial membrane. Thus storage at 4°C preserved hydrogel properties better than keeping at higher temperatures.

The addition of polysaccharide gel extracted from durian fruit-hulls into polyvinyl alcohol hydrogel resulted in better membrane properties for biomedical applications.

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# APPENDICES

# APPENDIX A

# MEDIUM AGAR FOR ANTIMICROBIAL TEST

**Mueller Hinton Agar**

Approximate formula per liter:

| | | |
|---|---|---|
| Beef extract powder | 2.0 | g |
| Acid digest of casein | 17.5 | g |
| Starch | 1.5 | g |
| Agar | 17.0 | g |
| Final pH 7.3± 0.1 | | |

Preparation:

Agar 38 g was dispensed in 1 L of purified water and mixed throughly. Medium was heated with frequent agitation and boiled for 1 min to achieve complete dissolution. Then, agar solution was autoclaved at 121 °C for 15 minutes.

# APPENDIX B

# STATISTICAL ANALYSIS

**Table 1B. One-Way ANOVA of tensile strength of the PVA and DG/PVA (2 : 5), (3 : 5) and (3.5 : 5) hydrogels prepared by F24T24 for 3, 4 and 5 cycles**

## 1.1 Test of assumption

### 1.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .066 | 51 | .200(*) | .989 | 51 | .903 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

### 1.1.2 Test of homogeneity of variances

Tensile strength

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.067 | 11 | 39 | .411 |

## 1.2 Hypothesis test

**ANOVA**

Tensile strength

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .027 | 11 | .002 | 14.841 | .000 |
| Within Groups | .006 | 39 | .000 | | |
| Total | .033 | 50 | | | |

**Multiple Comparisons**

Dependent Variable: Tensile strength

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA 3cycle | PVA 4cycle | -.0240417 | .0097901 | .858 | -.070466 | .022383 |
| | PVA 5cycle | -.0580667 (*) | .0090638 | .001 | -.101048 | -.015086 |
| | DG/PVA (2 : 5)3cycle | -.0296667 | .0104660 | .703 | -.079297 | .019963 |
| | DG/PVA (3 : 5)3cycle | -.0283417 | .0097901 | .674 | -.074766 | .018083 |
| | DG/PVA (3.5:5) 3cycle | -.0496667 (*) | .0097901 | .025 | -.096091 | -.003242 |
| PVA 4cycle | PVA 5cycle | -.0340250 | .0082741 | .157 | -.073261 | .005211 |
| | DG/PVA (2 : 5)4cycle | -.0227917 | .0097901 | .896 | -.069216 | .023633 |
| | DG/PVA (3 : 5)4cycle | -.0227450 | .0085987 | .787 | -.063520 | .018030 |
| | DG/PVA (3.5:5) 4cycle | -.0447250 (*) | .0085987 | .019 | -.085500 | -.003950 |
| PVA 5cycle | DG/PVA (2 : 5)5cycle | -.0075833 | .0074006 | 1.000 | -.042677 | .027510 |
| | DG/PVA (3 : 5)5cycle | -.0006000 | .0090638 | 1.000 | -.043581 | .042381 |
| | DG/PVA (3.5:5) 5cycle | -.0358400 | .0077618 | .064 | -.072647 | .000967 |
| DG/PVA (2:5)3cycle | DG/PVA (2 : 5)4cycle | -.0171667 | .0104660 | .992 | -.066797 | .032463 |
| | DG/PVA (2 : 5)5cycle | -.0359833 | .0090638 | .197 | -.078964 | .006998 |
| | DG/PVA (3 : 5)3cycle | .0013250 | .0097901 | 1.000 | -.045100 | .047750 |
| | DG/PVA (3.5:5) 3cycle | -.0200000 | .0097901 | .956 | -.066425 | .026425 |
| DG/PVA (2:5) 4cycle | DG/PVA (2 : 5)5cycle | -.0188167 | .0090638 | .951 | -.061798 | .024164 |
| | DG/PVA (3 : 5)4cycle | .0000467 | .0093611 | 1.000 | -.044344 | .044437 |
| | DG/PVA (3.5:5) 4cycle | -.0219333 | .0093611 | .892 | -.066324 | .022457 |
| DG/PVA (2:5) 5cycle | DG/PVA (3 : 5)5cycle | .0069833 | .0090638 | 1.000 | -.035998 | .049964 |
| | DG/PVA (3.5:5) 5cycle | -.0282567 | .0077618 | .316 | -.065063 | .008550 |

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| DG/PVA (3:5) 3cycle | DG/PVA (3 : 5)4cycle | -.0184450 | .0085987 | .939 | -.059220 | .022330 |
| | DG/PVA (3 : 5)5cycle | -.0303250 | .0097901 | .574 | -.076750 | .016100 |
| | DG/PVA (3.5:5) 4cycle | -.0404250 | .0085987 | .054 | -.081200 | .000350 |
| DG/PVA (3:5) 4cycle | DG/PVA (3 : 5)5cycle | -.0118800 | .0093611 | .999 | -.056271 | .032511 |
| | DG/PVA (3.5:5) 4cycle | -.0219800 | .0081069 | .759 | -.060423 | .016463 |
| DG/PVA (3:5) 5cycle | DG/PVA (3.5:5) 5cycle | -.0352400 | .0093611 | .267 | -.079631 | .009151 |
| DG/PVA (3.5 : 5) 3cycle | DG/PVA (3.5:5) 4cycle | -.0191000 | .0085987 | .923 | -.059875 | .021675 |
| | DG/PVA (3.5:5) 5cycle | -.0442400 (*) | .0085987 | .022 | -.085015 | -.003465 |
| DG/PVA (3.5 : 5) 4cycle | DG/PVA (3.5:5) 5cycle | -.0251400 | .0081069 | .572 | -.063583 | .013303 |

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

**Table 2B. One-Way ANOVA of percentage elongation of the PVA and DG/PVA (2 : 5), (3 : 5) and (3.5 : 5) hydrogels prepared by F24T24 for 3, 4 and 5 cycles**

### 2.1 Test of assumption

#### 2.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Percent elongation | .085 | 51 | .200(*) | .945 | 51 | .019 |

* This is a lower bound of the true significance.

A Lilliefors Significance Correction

#### 2.1.2 Test of homogeneity of variances

Percent elongation

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.613 | 11 | 39 | .133 |

## 2.2 Hypothesis test

**ANOVA**

Percent elongation

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 87911.819 | 11 | 7991.984 | 7.859 | .000 |
| Within Groups | 39658.952 | 39 | 1016.896 | | |
| Total | 127570.771 | 50 | | | |

**Multiple Comparisons**

Dependent Variable: Percent elongation

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA 3cycle | PVA 4cycle | -97.05333 | 24.35548 | .193 | -212.5476 | 18.4409 |
| | PVA 5cycle | -140.46667 (*) | 22.54879 | .002 | -247.3936 | -33.5398 |
| | DG/PVA (2:5) 3cycle | -83.78333 | 26.03710 | .513 | -207.2519 | 39.6852 |
| | DG/PVA (3:5) 3cycle | -115.59583 (*) | 24.35548 | .050 | -231.0901 | -.1016 |
| | DG/PVA (3.5 : 5) 3cycle | -156.37833 (*) | 24.35548 | .001 | -271.8726 | -40.8841 |
| PVA 4cycle | PVA 5cycle | -43.41333 | 20.58414 | .946 | -141.0238 | 54.1971 |
| | DG/PVA (2:5) 4cycle | -28.42333 | 24.35548 | 1.000 | -143.9176 | 87.0709 |
| | DG/PVA (3:5) 4cycle | -25.16800 | 21.39166 | 1.000 | -126.6078 | 76.2718 |
| | DG/PVA (3.5 : 5) 4cycle | -73.16800 | 21.39166 | .414 | -174.6078 | 28.2718 |
| PVA 5cycle | DG/PVA (2:5) 5cycle | -4.32833 | 18.41101 | 1.000 | -91.6338 | 82.9771 |
| | DG/PVA (3:5) 5cycle | 17.82333 | 22.54879 | 1.000 | -89.1036 | 124.7502 |
| | DG/PVA (3.5 : 5) 5cycle | -39.34467 | 19.30963 | .957 | -130.9114 | 52.2221 |

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| DG/PVA (2 : 5) 3cycle | DG/PVA (2:5) 4cycle | -41.69333 | 26.03710 | .994 | -165.1619 | 81.7752 |
| | DG/PVA (2:5) 5cycle | -61.01167 | 22.54879 | .761 | -167.9386 | 45.9152 |
| | DG/PVA (3:5) 3cycle | -31.81250 | 24.35548 | .999 | -147.3068 | 83.6818 |
| | DG/PVA (3.5 : 5) 3cycle | -72.59500 | 24.35548 | .632 | -188.0893 | 42.8993 |
| DG/PVA (2 : 5) 4cycle | DG/PVA (2:5) 5cycle | -19.31833 | 22.54879 | 1.000 | -126.2452 | 87.6086 |
| | DG/PVA (3:5) 4cycle | 3.25533 | 23.28829 | 1.000 | -107.1783 | 113.6890 |
| | DG/PVA (3.5 : 5) 4cycle | -44.74467 | 23.28829 | .972 | -155.1783 | 65.6890 |
| DG/PVA (2 : 5) 5cycle | DG/PVA (3:5) 5cycle | 22.15167 | 22.54879 | 1.000 | -84.7752 | 129.0786 |
| | DG/PVA (3.5 : 5) 5cycle | -35.01633 | 19.30963 | .982 | -126.5831 | 56.5504 |
| DG/PVA (3 : 5) 3cycle | DG/PVA (3:5) 4cycle | -6.62550 | 21.39166 | 1.000 | -108.0653 | 94.8143 |
| | DG/PVA (3:5) 5cycle | -7.04750 | 24.35548 | 1.000 | -122.5418 | 108.4468 |
| | DG/PVA (3.5 : 5) 3cycle | -40.78250 | 22.54879 | .982 | -147.7094 | 66.1444 |
| DG/PVA (3 : 5) 4cycle | DG/PVA (3:5) 5cycle | -.42200 | 23.28829 | 1.000 | -110.8556 | 110.0116 |
| | DG/PVA (3.5 : 5) 4cycle | -48.00000 | 20.16825 | .881 | -143.6383 | 47.6383 |
| DG/PVA (3 : 5) 5cycle | DG/PVA (3.5 : 5) 5cycle | -57.16800 | 23.28829 | .858 | -167.6016 | 53.2656 |
| DG/PVA (3.5 : 5) 3cycle | DG/PVA (3.5 : 5) 4cycle | -13.84300 | 21.39166 | 1.000 | -115.2828 | 87.5968 |
| | DG/PVA (3.5 : 5) 5cycle | -23.43300 | 21.39166 | 1.000 | -124.8728 | 78.0068 |

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| DG/PVA (3.5 : 5) 4cycle | DG/PVA (3.5 : 5) 5cycle | -9.59000 | 20.16825 | 1.000 | -105.2283 | 86.0483 |

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

**Table 3B. One-Way ANOVA of Young's modulus of the PVA and DG/PVA (2 : 5), (3 : 5) and (3.5 : 5) hydrogels prepared by F24T24 for 3, 4 and 5 cycles**

### 3.1 Test of assumption

#### 3.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Young's modulus | .087 | 51 | .200(*) | .970 | 51 | .220 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

#### 3.1.2 Test of homogeneity of variances

Young's modulus

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.333 | 11 | 39 | .244 |

### 3.2 Hypothesis test

**ANOVA**

Young's modulus

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .004 | 11 | .000 | 13.749 | .000 |
| Within Groups | .001 | 39 | .000 | | |
| Total | .005 | 50 | | | |

**Multiple Comparisons**

Dependent Variable: Young's modulus

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA 3cycle | PVA 4cycle | -.0148193 | .0038632 | .241 | -.033139 | .003500 |
| | PVA 5cycle | -.0282337 (*) | .0035766 | .000 | -.045194 | -.011273 |
| | DG/PVA (2 : 5) 3cycle | -.0215433 (*) | .0041299 | .018 | -.041127 | -.001959 |
| | DG/PVA (3 : 5) 3cycle | -.0147051 | .0038632 | .252 | -.033024 | .003614 |
| | DG/PVA (3.5 : 5) 3cycle | -.0206136 (*) | .0038632 | .014 | -.038933 | -.002294 |
| PVA 4cycle | PVA 5cycle | -.0134143 | .0032650 | .158 | -.028897 | .002068 |
| | DG/PVA (2 : 5) 4cycle | -.0067560 | .0038632 | .987 | -.025075 | .011563 |
| | DG/PVA (3 : 5) 4cycle | -.0078032 | .0033931 | .904 | -.023893 | .008287 |
| | DG/PVA (3.5 : 5) 4cycle | -.0127654 | .0033931 | .268 | -.028855 | .003325 |
| PVA 5cycle | DG/PVA (2 : 5) 5cycle | .0000650 | .0029203 | 1.000 | -.013783 | .013913 |
| | DG/PVA (3 : 5) 5cycle | -.0006783 | .0035766 | 1.000 | -.017639 | .016282 |
| | DG/PVA (3.5 : 5) 5cycle | -.0101357 | .0030628 | .467 | -.024660 | .004388 |
| DG/PVA (2 : 5) 3cycle | DG/PVA (2 : 5) 4cycle | -.0000320 | .0041299 | 1.000 | -.019616 | .019552 |
| | DG/PVA (2 : 5) 5cycle | -.0066253 | .0035766 | .979 | -.023586 | .010335 |
| DG/PVA (2 : 5) 3cycle | DG/PVA (3 : 5) 3cycle | .0068383 | .0038632 | .985 | -.011481 | .025158 |
| | DG/PVA (3.5 : 5) 3cycle | .0009298 | .0038632 | 1.000 | -.017390 | .019249 |
| DG/PVA (2 : 5) 4cycle | DG/PVA (2 : 5) 5cycle | -.0065933 | .0035766 | .980 | -.023554 | .010367 |
| | DG/PVA (3 : 5) 4cycle | -.0010472 | .0036939 | 1.000 | -.018564 | .016469 |
| | DG/PVA (3.5 : 5) 4cycle | -.0060094 | .0036939 | .993 | -.023526 | .011507 |

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| DG/PVA (2 : 5) 5cycle | DG/PVA (3 : 5) 5cycle | -.0007433 | .0035766 | 1.000 | -.017704 | .016217 |
| | DG/PVA (3.5 : 5) 5cycle | -.0102007 | .0030628 | .457 | -.024725 | .004323 |
| DG/PVA (3 : 5) 3cycle | DG/PVA (3 : 5) 4cycle | -.0079175 | .0033931 | .895 | -.024007 | .008173 |
| | DG/PVA (3 : 5) 5cycle | -.0142069 | .0038632 | .301 | -.032526 | .004112 |
| | DG/PVA (3.5 : 5) 3cycle | -.0059085 | .0035766 | .992 | -.022869 | .011052 |
| DG/PVA (3 : 5) 4cycle | DG/PVA (3 : 5) 5cycle | -.0062895 | .0036939 | .989 | -.023806 | .011227 |
| | DG/PVA (3.5 : 5) 4cycle | -.0049622 | .0031990 | .995 | -.020132 | .010208 |
| DG/PVA (3 : 5) 5cycle | DG/PVA (3.5 : 5) 5cycle | -.0094573 | .0036939 | .820 | -.026974 | .008059 |
| DG/PVA (3.5 : 5) 3cycle | DG/PVA (3.5 : 5) 4cycle | -.0069712 | .0033931 | .955 | -.023061 | .009119 |
| | DG/PVA (3.5 : 5) 5cycle | -.0177558 (*) | .0033931 | .018 | -.033846 | -.001666 |
| DG/PVA (3.5 : 5) 4cycle | DG/PVA (3.5 : 5) 5cycle | -.0107846 | .0031990 | .437 | -.025954 | .004385 |

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

**Table 4B. One-Way ANOVA of water vapor transmission rate of the DG/PVA (3.5 : 5) and PVA hydrogels prepared by F24T24 for 3 cycles before and after 3 months**

### 4.1 Test of assumption

#### 4.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| WVTR | .157 | 24 | .130 | .938 | 24 | .151 |

a Lilliefors Significance Correction

### 4.1.2 Test of homogeneity of variances

WVTR

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 2.441 | 7 | 16 | .066 |

## 4.2 Hypothesis test

**ANOVA**

WVTR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 187.005 | 7 | 26.715 | 1.625 | .199 |
| Within Groups | 263.024 | 16 | 16.439 | | |
| Total | 450.029 | 23 | | | |

**Table 5B. One-Way ANOVA of water evaporation rate of the DG/PVA (3.5 : 5) and PVA hydrogels prepared by F24T24 for , 3 cycles before and after 3 months**

## 5.1 Test of assumption

### 5.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| WER | .192 | 24 | .022 | .923 | 24 | .068 |

a Lilliefors Significance Correction

### 5.1.2 Test of homogeneity of variances

WER

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 2.217 | 7 | 16 | .089 |

### 5.2 Hypothesis test

**ANOVA**

WER

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 509.290 | 7 | 72.756 | 5.484 | .002 |
| Within Groups | 212.289 | 16 | 13.268 | | |
| Total | 721.579 | 23 | | | |

**Multiple Comparisons**

Dependent Variable: WER

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA 4°C 3mo | DG/PVA initial | -12.96333 (*) | 2.97412 | .047 | -25.7901 | -.1365 |

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

**Table 6B. One-Way ANOVA of adhesion property of the DG/PVA (3.5 : 5) and PVA hydrogels prepared by F24T24 for 3 cycles before and after 3 months**

### 6.1 Test of assumption

#### 6.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Adhesion property | .126 | 24 | .200(*) | .955 | 24 | .345 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 6.1.2 Test of homogeneity of variances

Adhesion property

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .613 | 7 | 16 | .737 |

## 6.2 Hypothesis test

**ANOVA**

Adhesion property

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 361.905 | 7 | 51.701 | 5.688 | .002 |
| Within Groups | 145.439 | 16 | 9.090 | | |
| Total | 507.344 | 23 | | | |

**Multiple Comparisons**

Dependent Variable: Adhesion property

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA initial | PVA 4°C 3mo | 4.16667 | 2.46170 | .110 | -1.0519 | 9.3852 |
| | PVA 30°C3mo | 5.10000 | 2.46170 | .055 | -.1186 | 10.3186 |
| | PVA 40°C3mo | 6.56333(*) | 2.46170 | .017 | 1.3448 | 11.7819 |
| | DG/PVA initial | -3.37333 | 2.46170 | .190 | -8.5919 | 1.8452 |
| PVA 4°C 3 mo | PVA 30°C3mo | .93333 | 2.46170 | .710 | -4.2852 | 6.1519 |
| | PVA 40°C3mo | 2.39667 | 2.46170 | .345 | -2.8219 | 7.6152 |
| PVA30°C 3mo | PVA 40°C3mo | 1.46333 | 2.46170 | .561 | -3.7552 | 6.6819 |
| DG/PVA initial | DG/PVA 4°C 3mo | .55333 | 2.46170 | .825 | -4.6652 | 5.7719 |
| | DG/PVA 30°C 3mo | .26667 | 2.46170 | .915 | -4.9519 | 5.4852 |
| | DG/PVA 40°C 3mo | .71333 | 2.46170 | .776 | -4.5052 | 5.9319 |
| DG/PVA 4°C 3mo | DG/PVA 30°C 3mo | -.28667 | 2.46170 | .909 | -5.5052 | 4.9319 |
| | DG 40°C 3mo | .16000 | 2.46170 | .949 | -5.0586 | 5.3786 |

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| DG/PVA 30°C 3mo | DG/PVA 40°C 3mo | .44667 | 2.46170 | .858 | -4.7719 | 5.6652 |

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

**Table 7B. Independent T-Test of tensile strength of the PVA hydrogels prepared by F24T24 for 3 cycles before and after swelling in water 13 hrs**

### 7.1 Test of assumption

**Tests of normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .142 | 7 | .200(*) | .983 | 7 | .971 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 7.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | .072 | .799 | -2.159 | 5 | .083 | -.0041417 | .0019181 | -.0090722 | .0007889 |
| Equal variances not assumed | | | -2.130 | 4.222 | .097 | -.0041417 | .0019447 | -.0094306 | .0011473 |

**Table 8B. Independent T-Test of percentage elongation of the PVA hydrogels prepared by F24T24 for 3 cycles before and after swelling in water 13 hrs**

### 8.1 Test of assumption

**Tests of normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Elongation | .190 | 7 | .200(*) | .948 | 7 | .708 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

### 8.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 4.563 | .086 | -.176 | 5 | .868 | -1.83833 | 10.46995 | -28.75219 | 25.07552 |
| Equal variances not assumed | | | -.153 | 2.345 | .891 | -1.83833 | 12.04858 | -47.01302 | 43.33635 |

**Table 9B. Independent T-Test of Young's modulus of the PVA hydrogels prepared by F24T24 for 3 cycles before and after swelling in water 13 hrs**

### 9.1 Test of assumption

**Tests of normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Young's modulus | .235 | 6 | .200(*) | .925 | 6 | .539 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

### 9.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.766 | .255 | -1.749 | 4 | .155 | -.0020130 | .0011511 | -.0052089 | .0011829 |
| Equal variances not assumed | | | -1.749 | 3.085 | .176 | -.0020130 | .0011511 | -.0056196 | .0015936 |

**Table 10B. Independent T-Test of tensile strength of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3 cycles before and after swelling in water 13 hrs**

### 10.1 Test of assumption

**Tests of normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .265 | 8 | .103 | .846 | 8 | .087 |

a Lilliefors Significance Correction

### 10.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.565 | .257 | 9.626 | 6 | .000 | .0556500 | .0057812 | .0415039 | .0697961 |
| Equal variances not assumed | | | 9.626 | 4.073 | .001 | .0556500 | .0057812 | .0397119 | .0715881 |

**Table 11B. Independent T-Test of percentage elongation of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3 cycles before and after swelling in water 13 hrs**

### 11.1 Test of assumption

**Tests of normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Elongation | .246 | 8 | .167 | .909 | 8 | .345 |

a Lilliefors Significance Correction

### 11.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.908 | .216 | 6.401 | 6 | .001 | 174.11500 | 27.20222 | 107.55355 | 240.67645 |
| Equal variances not assumed | | | 6.401 | 4.140 | .003 | 174.11500 | 27.20222 | 99.58793 | 248.64207 |

**Table 12B. Independent T-Test of Young's modulus of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3 cycles before and after swelling in water 13 hrs**

### 12.1 Test of assumption

**Tests of normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Young's modulus | .244 | 8 | .175 | .868 | 8 | .143 |

a Lilliefors Significance Correction

### 12.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.099 | .335 | 6.917 | 6 | .000 | .0137868 | .0019931 | .0089099 | .0186636 |
| Equal variances not assumed | | | 6.917 | 4.652 | .001 | .0137868 | .0019931 | .0085458 | .0190277 |

**Table 13B. One-Way ANOVA of tensile strength of the PVA hydrogel prepared by F24T24 for 3 cycles before and after 3 months**

### 13.1 Test of assumption

#### 13.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .157 | 12 | .200(*) | .910 | 12 | .216 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

#### 13.1.2 Test of homogeneity of variances

Tensile strength

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .906 | 3 | 8 | .480 |

### 13.2 Hypothesis test

**ANOVA**

Tensile strength

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .003 | 3 | .001 | 41.493 | .000 |
| Within Groups | .000 | 8 | .000 | | |
| Total | .003 | 11 | | | |

**Multiple Comparisons**

Dependent Variable: Tensile strength

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA initial | PVA 4°C 3mo | -.0192000(*) | .0039512 | .009 | -.033000 | -.005400 |
| | PVA 30°C 3mo | -.0381667(*) | .0039512 | .000 | -.051967 | -.024367 |
| | PVA 40°C 3mo | -.0371333(*) | .0039512 | .000 | -.050933 | -.023333 |
| PVA 4°C 3mo | PVA 30°C 3mo | -.0189667(*) | .0039512 | .010 | -.032767 | -.005167 |
| | PVA 40°C 3mo | -.0179333(*) | .0039512 | .013 | -.031733 | -.004133 |
| PVA 30°C 3mo | PVA 40°C 3mo | .0010333 | .0039512 | .995 | -.012767 | .014833 |

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

**Table 14B. One-Way ANOVA of percentage elongation of the PVA hydrogel prepared by F24T24 for 3 cycles before and after 3 months**

### 14.1 Test of assumption

#### 14.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Percent elongation | .190 | 12 | .200(*) | .878 | 12 | .083 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 14.1.2 Test of homogeneity of variances

Percent elongation

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .632 | 3 | 8 | .615 |

## 14.2 Hypothesis test

**ANOVA**

Percent elongation

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 27933.700 | 3 | 9311.233 | 14.206 | .001 |
| Within Groups | 5243.462 | 8 | 655.433 | | |
| Total | 33177.162 | 11 | | | |

**Multiple Comparisons**

Dependent Variable: Percent elongation

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA initial | PVA 4°C3mo | -97.44667(*) | 20.90347 | .011 | -170.4550 | -24.4383 |
| | PVA 30°C 3mo | -110.27000(*) | 20.90347 | .006 | -183.2783 | -37.2617 |
| | PVA 40°C 3mo | -121.36667(*) | 20.90347 | .003 | -194.3750 | -48.3583 |
| PVA 4°C 3mo | PVA 30°C 3mo | -12.82333 | 20.90347 | .942 | -85.8317 | 60.1850 |
| | PVA 40°C 3mo | -23.92000 | 20.90347 | .733 | -96.9283 | 49.0883 |
| PVA 30C 3mo | PVA 40°C 3mo | -11.09667 | 20.90347 | .961 | -84.1050 | 61.9117 |

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

**Table 15B. One-Way ANOVA of Yong's modulus of the PVA hydrogel prepared by F24T24 for 3 cycles before and after 3 months**

### 15.1 Test of assumption

#### 15.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Young's modulus | .188 | 12 | .200(*) | .891 | 12 | .122 |

* This is a lower bound of the true significance.
A Lilliefors Significance Correction

#### 15.1.2 Test of homogeneity of variances

Young's modulus

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.984 | 3 | 8 | .195 |

### 15.2 Hypothesis test

**ANOVA**

Young's modulus

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .001 | 3 | .000 | 44.547 | .000 |
| Within Groups | .000 | 8 | .000 | | |
| Total | .001 | 11 | | | |

**Multiple Comparisons**

Dependent Variable: Young's modulus

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| PVA initial | PVA 4°C 3mo | -.0069400(*) | .0017134 | .024 | -.012924 | -.000956 |
| | PVA 30°C3mo | -.0167563(*) | .0017134 | .000 | -.022741 | -.010772 |
| | PVA 40°C3mo | -.0164153(*) | .0017134 | .000 | -.022400 | -.010431 |
| PVA 4°C 3mo | PVA 30°C3mo | -.0098163(*) | .0017134 | .003 | -.015801 | -.003832 |
| | PVA 40°C3mo | -.0094753(*) | .0017134 | .004 | -.015460 | -.003491 |
| PVA 30°C 3mo | PVA 40°C3mo | .0003410 | .0017134 | .998 | -.005643 | .006325 |

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

**Table 16B. One-Way ANOVA of tensile strength of the DG/PVA (3.5 : 5) hydrogel prepared by F24T24 for 3 cycles before and after 3 months**

### 16.1 Test of assumption

#### 16.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .180 | 12 | .200(*) | .882 | 12 | .094 |

* This is a lower bound of the true significance.

A Lilliefors Significance Correction

#### 16.1.2 Test of homogeneity of variances

Tensile strength

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 2.262 | 3 | 8 | .158 |

## 16.2 Hypothesis test

**ANOVA**

Tensile strength

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .006 | 3 | .002 | 10.993 | .003 |
| Within Groups | .002 | 8 | .000 | | |
| Total | .008 | 11 | | | |

**Multiple Comparisons**

Dependent Variable: Tensile strength

| (I) Hydrogel | (J) Hydrogel | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| DG/PVA initial | DG/PVA 4°C 3mo | -.0053333 | .0111843 | .971 | -.044396 | .033729 |
| | DG/PVA 30°C 3mo | -.0295333 | .0111843 | .151 | -.068596 | .009529 |
| | DG/PVA 40°C 3mo | -.0573333(*) | .0111843 | .007 | -.096396 | -.018271 |
| DG/PVA 4°C 3mo | DG/PVA 30°C 3mo | -.0242000 | .0111843 | .273 | -.063263 | .014863 |
| | DG/PVA 40°C 3mo | -.0520000(*) | .0111843 | .012 | -.091063 | -.012937 |
| DG/PVA 30°C 3mo | DG/PVA 40°C 3mo | -.0278000 | .0111843 | .184 | -.066863 | .011263 |

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

**Table 17B. One-Way ANOVA of percentage elongation of the DG/PVA (3.5 : 5) hydrogel prepared by F24T24 for 3 cycles before and after 3 months**

### 17.1 Test of assumption

#### 17.1.1 Test of normality

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Percent elongation | .153 | 12 | .200(*) | .973 | 12 | .936 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

#### 17.1.2 Test of homogeneity of variances

Percent elongation

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .186 | 3 | 8 | .903 |

### 17.2 Hypothesis test

**ANOVA**

Percent elongation

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1735.553 | 3 | 578.518 | .789 | .533 |
| Within Groups | 5868.907 | 8 | 733.613 | | |
| Total | 7604.460 | 11 | | | |

**Table 18B. Independent T-Test of gel fraction of the PVA hydrogels prepared by F24T24 for 3 cycles before and after sterilization**

### 18.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Gel fraction | .257 | 6 | .200(*) | .853 | 6 | .167 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

### 18.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 2.265 | .207 | -7.010 | 4 | .002 | -17.32000 | 2.47076 | -24.17993 | -10.46007 |
| Equal variances not assumed | | | -7.010 | 2.603 | .009 | -17.32000 | 2.47076 | -25.90735 | -8.73265 |

**Table 19B. Independent T-Test of gel fraction of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 19.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Gel fraction | .295 | 6 | .111 | .895 | 6 | .346 |

a Lilliefors Significance Correction

### 19.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 2.851 | .167 | -2.435 | 4 | .072 | -3.80667 | 1.56338 | -8.14729 | .53396 |
| Equal variances not assumed | | | -2.435 | 2.585 | .107 | -3.80667 | 1.56338 | -9.26551 | 1.65217 |

**Table 20B. Independent T-Test of tensile strength of the PVA hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 20.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .236 | 7 | .200(*) | .889 | 7 | .272 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 20.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | .031 | .866 | -2.936 | 5 | .032 | -.0067500 | .0022987 | -.0126589 | -.0008411 |
| Equal variances not assumed | | | -2.995 | 4.742 | .032 | -.0067500 | .0022541 | -.0126405 | -.0008595 |

**Table 21B. Independent T-Test of percentage elongation of the PVA hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 21.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Percent elongation | .123 | 7 | .200(*) | .987 | 7 | .987 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 21.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.952 | .221 | -.953 | 5 | .384 | -6.44333 | 6.76165 | -23.82471 | 10.93804 |
| Equal variances not assumed | | | -1.112 | 3.338 | .340 | -6.44333 | 5.79382 | -23.86977 | 10.98311 |

**Table 22B. Independent T-Test of Young's modulus of the PVA hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 22.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Young's modulus | .283 | 7 | .096 | .862 | 7 | .157 |

a Lilliefors Significance Correction

### 22.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 4.827 | .079 | .821 | 5 | .449 | .0008895 | .0010839 | -.0018968 | .0036758 |
| Equal variances not assumed | | | .925 | 4.184 | .405 | .0008895 | .0009613 | -.0017338 | .0035128 |

**Table 23B. Independent T-Test of tensile strength of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 23.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Tensile strength | .221 | 6 | .200(*) | .956 | 6 | .786 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 23.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | .028 | .876 | -1.048 | 4 | .354 | -.0042667 | .0040695 | -.0155655 | .0070322 |
| Equal variances not assumed | | | -1.048 | 4.000 | .354 | -.0042667 | .0040695 | -.0155655 | .0070322 |

**Table 24B. Independent T-Test of percentage elongation of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3cycles before and after sterilization**

**24.1 Test of assumption**

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Percent elongation | .267 | 6 | .200(*) | .862 | 6 | .196 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

**24.2 Hypothesis test**

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 1.960 | .234 | .747 | 4 | .497 | 8.40333 | 11.25175 | -22.83653 | 39.64319 |
| Equal variances not assumed | | | .747 | 3.232 | .506 | 8.40333 | 11.25175 | -25.99315 | 42.79982 |

**Table 25B. Independent T-Test of Young's modulus of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3cycles before and after sterilization**

**25.1 Test of assumption**

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Young's modulus | .255 | 6 | .200(*) | .868 | 6 | .217 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

### 25.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 2.783 | .171 | -.883 | 4 | .427 | -.0027820 | .0031495 | -.0115264 | .0059624 |
| Equal variances not assumed | | | -.883 | 2.994 | .442 | -.0027820 | .0031495 | -.0128171 | .0072531 |

**Table 26B. Independent T-Test of water vapor transmission rate of the PVA hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 26.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| WVTR | .276 | 6 | .170 | .876 | 6 | .253 |

a Lilliefors Significance Correction

### 26.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | .750 | .435 | 1.976 | 4 | .119 | 2.42333 | 1.22668 | -.98248 | 5.82914 |
| Equal variances not assumed | | | 1.976 | 3.662 | .126 | 2.42333 | 1.22668 | -1.11009 | 5.95676 |

**Table 27B. Independent T-Test of water vapor transmission rate of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3cycles before and after sterilization**

**27.1 Test of assumption**

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| WVTR | .292 | 6 | .120 | .794 | 6 | .052 |

a Lilliefors Significance Correction

**27.2 Hypothesis test**

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 9.985 | .034 | -2.727 | 4 | .053 | -4.65667 | 1.70741 | -9.39719 | .08386 |
| Equal variances not assumed | | | -2.727 | 2.030 | .110 | -4.65667 | 1.70741 | -11.89948 | 2.58615 |

**Table 28B. Independent T-Test of water evaporation rate of the PVA hydrogels prepared by F24T24 for 3cycles before and after sterilization**

**28.1 Test of assumption**

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| WER | .217 | 6 | .200(*) | .901 | 6 | .382 |

* This is a lower bound of the true significance.

a Lilliefors Significance Correction

### 28.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 2.007 | .230 | -1.518 | 4 | .204 | -2.30667 | 1.51999 | -6.52684 | 1.91351 |
| Equal variances not assumed | | | -1.518 | 2.779 | .233 | -2.30667 | 1.51999 | -7.36847 | 2.75514 |

**Table 29B. Independent T-Test of water evaporation rate of the DG/PVA (3.5 : 5) hydrogels prepared by F24T24 for 3cycles before and after sterilization**

### 29.1 Test of assumption

**Tests of Normality**

| | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| WER | .256 | 6 | .200(*) | .873 | 6 | .239 |

* This is a lower bound of the true significance.
a Lilliefors Significance Correction

### 29.2 Hypothesis test

**Independent Samples Test**

| | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | Lower | Upper |
| Equal variances assumed | 3.301 | .143 | 2.729 | 4 | .053 | 4.40333 | 1.61358 | -.07669 | 8.88335 |
| Equal variances not assumed | | | 2.729 | 2.158 | .103 | 4.40333 | 1.61358 | -2.07550 | 10.88217 |

# BIOGRAPHY

Miss Pattaranut Eakwaropas was born in Phuket, Thailand, on June 9th, 1981. She received her Bachelor of Science in Pharmacy degree in 2003 from the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. After graduation, she has worked at Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Pathumthani, Thailand since 2003. She entered the Master's Program in Industrial Pharmacy at Chulalongkorn University in 2006.